Global Academic Journal of Pharmacy and Drug Research

Available online at https://www.gajrc.com
DOI: https://doi.org/10.36348/gajpdr.2026.v08i04.001

OPEN ACCESS

ISSN (P): 2706-9044
ISSN (O): 2707-255X

Original Research Article

Formulation and *In Vitro* Characterization of Flucytosin Loaded Transethosomal Gel

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Article History
Received: 28.05.2026
Accepted: 22.07.2026
Published: 24.07.2026

Abstract: Fungal infections have emerged as a major global health concern due to their increasing prevalence, high recurrence rate, and growing resistance to conventional antifungal therapies. These infections, commonly known as mycoses, are caused by pathogenic fungi and are broadly classified into superficial, subcutaneous, and systemic infections depending on the depth and site of infection. Superficial fungal infections of the skin, hair, and nails are among the most common infectious diseases worldwide, affecting nearly one-fourth of the global population. Individuals with weakened immune systems, diabetes, prolonged antibiotic therapy, or poor hygiene are at a significantly higher risk of developing fungal infections. Although several antifungal agents are available, conventional topical formulations often fail to provide adequate therapeutic outcomes because of poor drug penetration through the stratum corneum, limited drug retention at the infected site, and the need for repeated application. These limitations necessitate the development of novel drug delivery systems capable of improving skin penetration and enhancing therapeutic efficacy. Flucytosine (5-fluorocytosine) is a well-established antifungal agent with potent activity against Candida and Cryptococcus species. It exerts its antifungal action by inhibiting fungal DNA and RNA synthesis after its intracellular conversion into 5-fluorouracil. Despite its effectiveness, the clinical use of flucytosine is limited due to rapid renal elimination, systemic toxicity, and the possibility of developing drug resistance during prolonged therapy.

Keywords: Flucytosin, Antifungal Activity, Topical Formulation, and Transethosomal Gel.

INTRODUCTION

Fungal Infection

Often referred to as mycosis, a fungal infection is a disease caused by fungi. Various kinds are conventionally categorised as superficial, subcutaneous, or systemic, depending on the afflicted body portion. (Kyle and Dahl, 2004). Pityriasis versicolour, a yeast infection, and common tinea of the skin, which can affect the body, groin, hands, feet, and beard, are a few of instances of superficial fungal infections. Chromoblastomycosis and eumycetoma are examples of subcutaneous forms that usually affect tissues in and under the skin. Mucormycosis, aspergillosis, pneumonia, pneumocystis, histoplasmosis, and cryptococcosis are among the most serious systemic fungal diseases. There are many different indications and symptoms. For a surface infection, a rash is usually seen. Lumpiness and changes in the skin might be signs of an undersurface fungal infection. Meningitis or

symptoms similar to pneumonia may manifest with a more severe or systemic infection.

It's not always the case that all fungi are bad. Fungal illnesses can develop from spores entering the body through the skin, such as by an injection, cut, or wound, or from spores being inhaled and coming into contact with the skin. It is more common in people with weakened immune systems. This covers individuals undergoing steroids or cancer treatments, as well as those afflicted with illnesses like HIV/AIDS.

Moulds and yeasts are two examples of fungi that may infect people, as can yeasts and moulds, which may coexist. Yeast In healthy people, Candida albicans can cause superficial mild candidiasis, including vaginal yeast infections or oral thrush, as well as severe systemic candidiasis in those who are unable to fend off infection. People might have Candida albicans and not show any symptoms [1].

MATERIALS AND METHOD

Material

The formulation of the flucytosine-loaded Transethosomal gel was developed using pharmaceutical-grade ingredients sourced from reputed suppliers. Flucytosine was obtained as a gift sample from a pharmaceutical company, while cholesterol was procured from Ash Chemie India, Thane. Analyticalgrade disodium hydrogen phosphate, dipotassium hydrogen orthophosphate, sodium chloride, carbopol 934P, methyl paraben, propyl paraben, and propylene glycol were supplied by S. D. Fine Chem. Ltd., Mumbai. Methanol, ethanol, and chloroform of analytical grade were obtained from Qualigens Fine Chemicals, Mumbai. All chemicals and reagents used were of analytical or pharmaceutical grade and were employed without further purification to ensure formulation quality and reproducibility.

Method

Preformlation Study of Flucytosine

UV-Spectroscopic Study

a. Preparation of Stock Solution in Methanol

50 mg of flucytosine was added to a 50 ml volumetric flask to create the stock solution. Methanol was added to the flask, resulting in a solution with 1000 µg/ml [2].

Preparation of sub-stock solution in methanol

To get the desired concentration of 100 µg/ml, add methanol to a 10-milliliter volumetric flask containing 1 millilitre of the stock solution.

b. Preparation of Calibration Curve

Samples of 1, 2, 3, 4, and 5 ml were added to a 10 ml volumetric flask to produce 10, 20, 30, 40, and 50 µg/ml solutions. A working standard of 100 µg/ml was made using one millilitre (1000 µg/ml) of the stock solution mentioned above. A 10 ml volumetric flask was then filled with the designated amount of methanol until it reached the mark, creating the calibration curve. Each answer was then examined independently [3, 4].

Determination of λ Max in Methanol

By proper dilution of standard drug solution with methanol, a solution containing 50 µg/ml of concentration opposed to a suitable solvent for the identification of maximal absorption for the drug in both the solvent [5].

Determination of Melting Point

The melting point of flucytosine was ascertained via the capillary technique, which entailed storing melting point samples in capillary melting point tubes with thin walls. One end of this tube must be sealed. To pack the tube, a small amount of the flucytosine sample was gently squeezed into the open end. To move the crystals from the open end to the bottom, gently tap the tube's bottom on the benchtop. In the tube, a densely packed column of crystals, about 3 mm high, was formed and placed on the capillary hole in the device that held the common mercury thermometer. The glass pane of the device, which also recorded the temperature, was used to measure the melting point time [6].

Solubility Determination

The straightforward approach was used to ascertain the solubility of flucytosine in methanol. In a stoppered flask or vial, an excess of medicine was typically dissolved in 5 ml of solvent to create the sample. The vials were sealed and shaken for ten minutes. They were then agitated at 37°C for 24 hours using a magnetic stirrer. An additional quantity was added to the vial once the medication had been dissolved. Until the existence of an undissolved material demonstrated saturation solubility, this procedure was repeated. A UV-visible spectrophotometer was used to measure the drug's methanol content at 274 nm [8, 6].

Determination of Partition Coefficient

Three different funnels were employed, each holding 25 mg of octanol and flucytosine in aqueous phase. A wrist motion shaker was used to shake the separating funnels for two hours in order to equilibrate them. Following the separation of the two phases, spectrophotometry was used to determine the drug's concentration in the aqueous phase. The drug partition coefficient was calculated phase-by-phase [7].

Drug-Excipients Compatibility Study Using FT-IR

Both the generated formulations and the drug's soylecithin FT-IR spectra were recorded. After the samples were scanned between 4000 and 500 cm-1, the peaks were compared to the IR databases to control for the different functional groups that were present.

Physical Appearance

Flucytosine transethosomal gel's (flucytosine) organoleptic properties, including its liquefaction, colour, appearance, and odour, were evaluated.

Analysis of pH

The pH of the Transethosomal Gel was measured using a digital pH meter. One gramme of flucytosine was diluted in 100 millilitres of distilled water to make a 1% aqueous solution of Transethosomal Gel. After that, the mixture was agitated to create a uniform suspension [8].

Viscosity Analysis

A Brookfield viscometer equipped with a spindle 92 was used to test the viscosity of transethosomal gel. Formulations were put in a beaker with a thermostatic jacket to test their viscosity. Three duplicates of the spindle were allowed to enter the formulation, and the average outcomes were noted [9].

Spreadability

Spreadability was evaluated using wooden blocks and glass slides. One gramme of extra transethosomal gel was present between the two slides. To eliminate any air pockets between the slides and produce a consistent coating of transethosomal gel, a 100-gram weight was placed on the slide for five minutes. The edges of the slides were carefully cleaned of extra gel. The bottom slide was securely fastened, and the top slide was polled using a 10-gram weight. recorded, in seconds, the time it took for the top slide to move 5 cm. A shorter period indicates more spreadability. The transethosomal gel's spreadability was estimated using the formula below [10].

$S = M \times L/T$

Where,

S = gel spreadability

M = pan weight fastened to the top slide

L = length that the glass slide moved

T = the amount of time in seconds needed to fully separate the slide

Formulation of Flucytosine Loaded Transethosomes

FLUCYTOSINE-loaded TEs were prepared using the cold technique. The precise amount of ethanol needed to dissolve the following materials is displayed in Table 1: Tween 80, cholesterol, soybean soylecithin, and flucytosine. The aqueous phase was introduced dropwise while being continuously swirled at 750 rpm the flucytosine-loaded TE suspension was subjected to sonication in order to reduce the particle size of the TEs. For additional analysis, the flucytosine-loaded TE preparations were kept in airtight containers at 4°C. As shown in table 2, several experiments were conducted using different amounts of soylecithin and ethanol to produce the optimal vesicle size and high entrapment efficiency transethosomal formulation.

Table 1: Selection of independent variables

Coded Values Level	Independent Variables (%w/v)	
	X1, Soylecithin	X2, Ethanol
-1	2.5	20
+1	3.5	60

Table 2: Composition of different FLUCYTOSINE-loaded transethosomal formulations

Code	Soylecithin (%w/v)	Ethanol (%v/v)	Tween 80 (%w/v of Soylecithin)	Cholesterol (%w/v)	Drug (%w/v)
F1	3.71	60	25	0.5	1
F2	2.29	40	25	0.5	1
F3	3.5	40	25	0.5	1
F4	3	68.28	25	0.5	1
F5	3	11.72	25	0.5	1
F6	2.5	60	25	0.5	1
F7	2.5	20	25	0.5	1
F8	3	40	25	0.5	1
F9	3.5	20	25	0.5	1

Evaluation of Flucytosine Loaded Transethosomes
Vesicular Size, Poly-Dispersity Index (PDI) and Zeta Potential

Electrostatic charge potential in suspended transethosomes (TEs) and the average particle size (PS) were assessed as two essential properties for stability studies. Two measures of polydispersity are distribution width and uniformity, or how symmetrical the distribution is around the median point. Small PDI values of 0.1 imply a homogenous dispersion, whereas large values >0.3 imply significant heterogeneity. The charged particles were tested for mutual attraction or repellence using zeta potential, or ZP. The Zetasizer ZS90 (Malvern, UK) was used to measure the mean PS, PDI, and ZP of the prepared flucytosine-loaded transethosomes. Following the instructions, the samples were scanned at 25 °C and a 90° scattering angle after being diluted with deionized water [11].

Entrapment Efficiency

An indirect method was used to calculate the transethosomal suspensions' entrapment efficiency (% EE) Clear supernatants were obtained after a 30-minute ultracentrifugation at 12,000× g of transethosomal suspensions. The concentration of flucytosine in transethosomal formulations was ascertained by spectrophotometric analysis of the collected supernatants. At λmax = 272 nm, the concentrations of the drug, both free and total, were measured. A calibration curve was used to determine the drug's concentration [12]. The amount of free drug in the supernatant was calculated using the standard curve calibration equation (R2 = 0.998). The percentage of EE was computed following the acquisition of readings in triplicate.

$$\%\text{Entrapment Efficiency} = \frac{\text{Total drug amount in formulation} - \text{Free drug amount}}{\text{Total drug amount in formulation}} \times 100$$

Optimum Formula Selection

The optimal formulation for additional study was determined to be the one with the smallest particle size and the highest entrapment efficacy percentage. The needed factor that was closest to one was used to determine the optimal formula.

Transmission Electron Microscopy

An FEI Tecnai G2 F20 X-Twin transmission electron microscope was used to examine the optimized formulation's morphology. To enhance the nanovesicles' adsorption onto the carbon layer, a tiny quantity of the solution was put to a grid covered with carbon and left undisturbed for five minutes. Prior to analysis, the material was treated with tiny doses of phosphoric tungstic acid.

Stability Studies of Optimized Transethosomal Formulation

Short-term stability studies were conducted in accordance with ICH Q1A (R2) recommendations in order to determine the stability of the improved transethosomal formulation that had been developed. The prepared formulation was placed in glass vials and placed in different storage condition at 40°C ± 2°C/75% RH ± 5% RH and 5°C ± 3°C (refrigerated conditions). For analysis, a sample was taken every 0, 30 and 60 days.

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

UV- Spectroscopic Study

a. Determination of Wavelength by using UV-Spectroscopic Study

The absorption maxima of Flucytosine in methanol were found to be 284.80 nm which matched the reported wavelength as mentioned in figure 1.

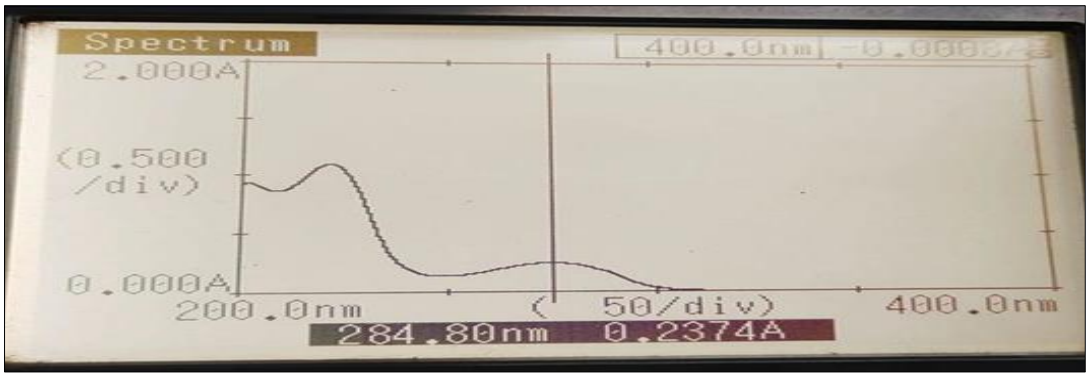

Figure 1: UV-spectrum of flucytosine in methanol

b. Preparation of Calibration Curve in Methanol

The calibration curves of Flucytosine in methanol were prepared in shown in Figure 2.

Table 3: Absorbance data of Flucytosine in methanol at 274nm

S. No.	Concentration (µg/ml)	Absorbance (mean ± SEM) (n=3)
1	0	0
2	10	0.238 ± 0.002
3	20	0.484 ±0.0016
4	30	0.649 ± 0.0024
5	40	0.852 ± 0.00207
6	50	1.028 ± 0.132

c. Preparation of Calibration Curve in Methanol

Table 4: Absorbance data of Flucytosine in PBS pH 7.4 at 284nm

S. No.	Concentration (µg/ml)	Absorbance (mean ± SEM) (n=3)
1	0	0
2	10	0.062 ±0.010
3	20	0.120 ±0.009
4	30	0.137 ±0.005
5	40	0.209 ±0.002
6	50	0.219 ±0.008

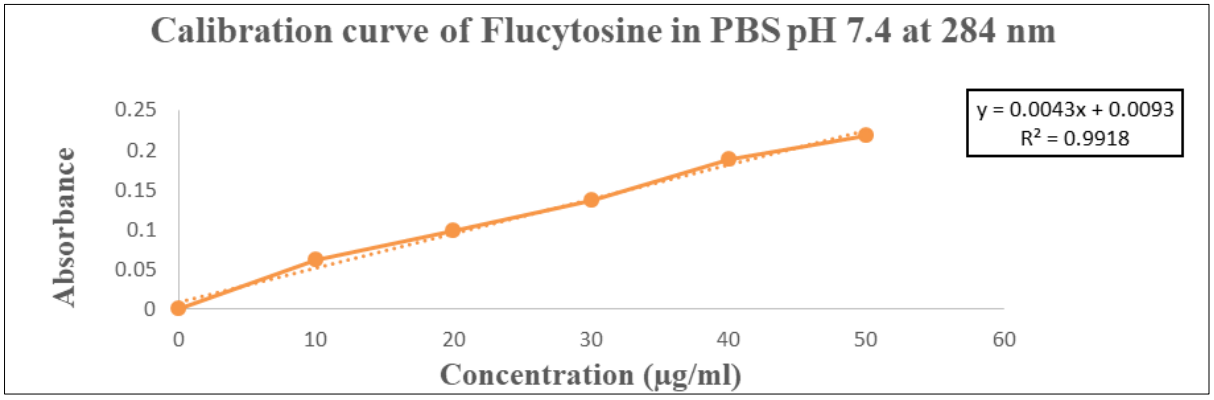

Figure 2: Calibration curve of Flucytosine in PBS pH 7.4 at 284nm

Drug Characterization

a. Melting Point Determination:

The melting point of Flucytosine was found to 296-298°C which was found reported in literature and table shows the melting point Flucytosine.

Table 5: Melting point of Flucytosine

S. No	Reported Melting point of drug (°C)	Melting point of Sample drug (°C)	Average melting point of Sample Drug (°C)
1	295-300	294-296	296-298
2		296-298	
3		296-298	

b. Determination of Solubility of Flucytosine:

The solubility of Flucytosine in methanol was found 2.2 mg/ml, and in Phosphate buffer pH 6.8 was found 0.026 mg/ml.

c. Determination of Partition Coefficient

In the n-octanol and water system, the flucytosine partition coefficient value was discovered to be -0.57. Log P values >5 indicate high lipophilicity (non-polar), while values <1 suggest high hydrophilicity (polar).

d. Drug-Excipients Compatibility Study Using FTIR Method

FTIR analyses were carried out on the pure FLUCYTOSINE, Soya soylecithin and optimized formulation in the range of 500-4000cm^{-1}, as demonstrate in figure 3 (a, b).

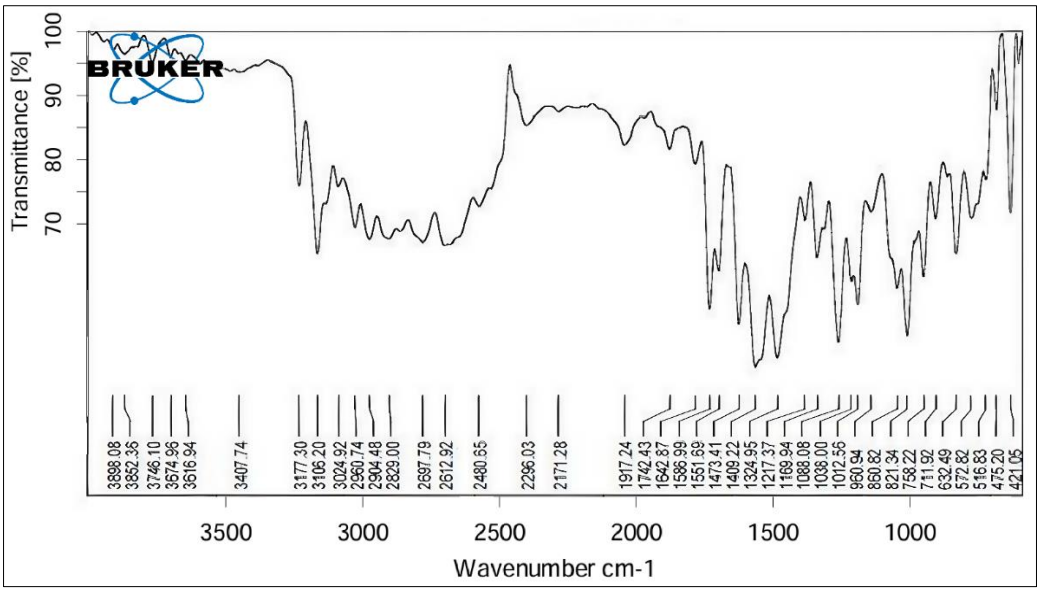

Figure 3(a): FTIR spectra of Flucytosine

Table 6: Interpretation of FTIR spectra of Flucytosine

Group	Standard Frequency (cm^{-1})	Observation Frequency (cm^{-1})
O–H stretch	3640–3610	3616.94
O–H stretch	3500–3200	3407.74
C-H stretch	3100–3000	3024.92
C–H stretch (Alkyl)	3000–2850	2960.74, 2904.48
H–C=O: C–H stretch	2830–2695	2697.79
C=O stretch (esters)	1750–1735	1742.42
C=O stretch (ketones)	1715	1715.22
N–H bend	1650–1580	1642
C–C stretch	1500–1400	1473.41, 1409.22
C-N stretch	1335–1250	1324.95, 1217.37
C-O stretch (Alkyl/Arylether)	1100-1275	1169.94
C–N stretch (aliphatic amines	1250–1020	1088.08, 1038
=C–H bend (alkenes)	1000–650	711.92- 960.42
C–Cl stretch	850–550	821.34
–C (triple bond) C–H: C–H bend	700–610	632.59

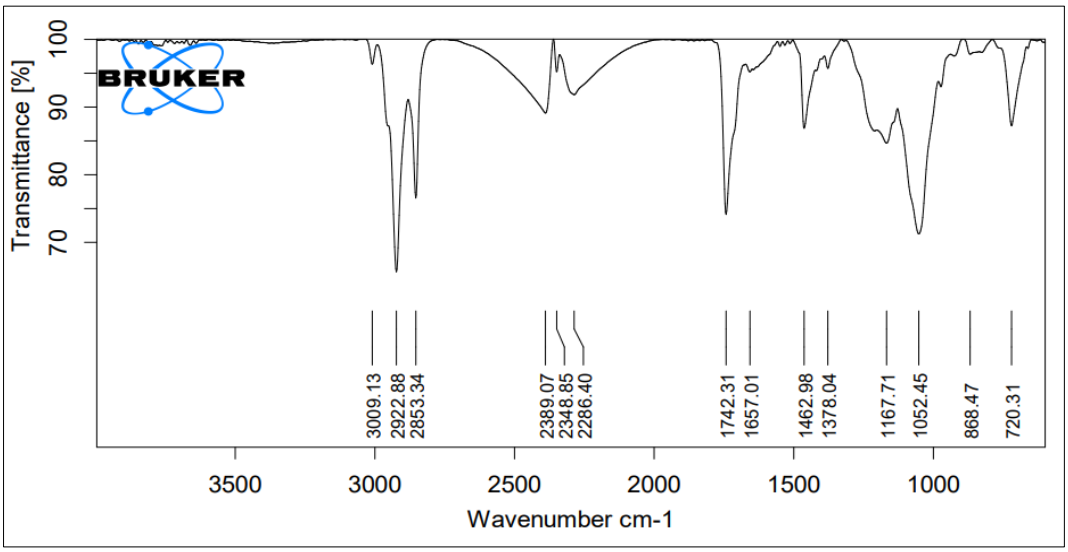

Figure 3(b): FTIR spectra of soylecithin

The results showed that all of the characteristics peaks in the optimized formulation were the same as those in the flucytosine alone, indicating that the drug and excipient work well together and that the drug is stable in the presence of the excipient.

Evaluation of Flucytosine Transethosomes
Optimization of Formulation

To identify the appropriate variables, the central composite design, or CCD, was employed. Table 6 lists the independent variables that were chosen. Nine experimental runs were conducted using the chosen levels, and Table 6 shows the recorded results. A total of nine formulations were produced as a result of applying the CCD to determine the relationship between the independent and dependent variables and to optimize the formulation; the results are compiled in Table 7.

Table 7: Entrapment efficiency and particle size of all formulations

Formulation Code	X_1, soylecithin (%w/v)	X_2, Ethanol (%w/v)	Y_1, Entrapment Efficiency (%)	Y_2, Particle Size (nm)
F1	3.71	60	70.62	898.15
F2	2.29	40	62.61	1132.6
F3	3.5	40	81.56	360.15
F4	3	68.28	56.75	872.32
F5	3	11.72	79.82	820.7
F6	2.5	60	60.54	740.32
F7	2.5	20	68.72	912.13
F8	3	40	72.51	715.2
F9	3.5	20	82.64	401.5

Response Analysis through Polynomial Equation
a. Effect of Variables on Entrapment Efficiency

In order to correlate the various studied responses with the investigated variables, data were analysed to fit complete linear polynomial equations with additional interaction terms. Figure 4 (a, b), 2D and 3D graphs demonstrate a positive correlation between the drug's % entrapment efficiency and the concentrations of X1, soylecithin, and X2, ethanol.

$$\% EE\ Y1 = 45.74 + 12.69 * X1 - 0.33 * X2 \quad (1)$$

The linear model Equation (1) indicates that the Soylecithin positively affected the % EE and ethanol negatively affected the % EE of flucytosine-loaded TE formulations.

The entrapment efficiencies of the nine flucytosinet-loaded TE formulations ranged from 56.75 to 82.64 nm. Formulation F3, whose lipid concentration was 3.5% w/v and ethanol concentration were 40% v/v, showed the highest percentage of EE (81.56%). Since %EE has a strong correlation with ethanol and lipid concentrations, these substances can be blamed for it. Particle size increases in tandem with %EE when lipid concentrations are higher. According to earlier research, an increase in ethanol concentration results in leaky vesicles with a lower percentage of ethylene ester (%EE), which in turn reduces vesicular size and increases entrapment efficiency, albeit only slightly. This phenomenon occurs even at the same level of lipid content [14].

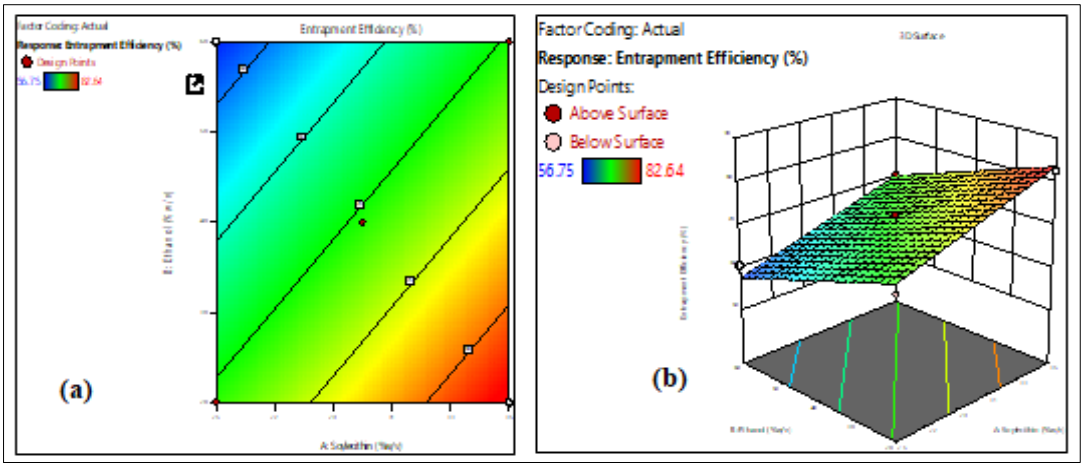

Figure 4: 2D contour plots (a) and 3D response surface plots (b) for evaluating the influence of Soylecithin (X1) and ethanol (X2) on entrapment efficiency (Y1)

P-values less than 0.0500 suggest that model terms are significant, according to the built-in ANOVA result. Model F-value of 47.14 indicates that the model is significant, and model terms X1 and X2 are significant model terms. The adjusted R2 value was 0.9202 and the predicted determination coefficient (R2) was 0.8592; the differences between the two values were less than 0.2. With a signal-to-noise ratio of 16.814, the signal is deemed adequate; a ratio of more than 4 is preferred.

b. Effect of Variables on Particle Size

The nine TE formulations loaded with flucytosine had particle sizes ranging from 360.15 to 1132.6 nm. The smallest particle size, 360.15 nm, is displayed in F3. The results of the study clearly showed a relationship between particle size and lipid concentration, as Table 6 demonstrates. It appears that an increase in ethanol concentration had a beneficial effect on particle size at lower soylecithin levels. Increasing the amounts of soylecithin at constant ethanol concentration also resulted in an increase in particle size, as shown by the 2D contour plot and 3D response surface plot (Figure 5 a, b). The final mathematical model, as calculated by the Design-Expert software, is shown in terms of coded factors in equation (2) for particle size below.

Particle Size (nm) = 3751.27 -1029.76 * X1 -47.64 * X2 + 16.71 * X1X2 (2)

It was discovered that the particle size increased as the formulation's lipid content concentration increased. In all formulations, there was an inverse relationship (up to 40%) between the ethanol content and the mean particle size. Formulations with 40% ethanol content had smaller particle sizes than those with 20% ethanol content. The interpermeating of the ethanol hydrocarbon chain causes the vesicular membrane to thin, and as was previously mentioned, a rise in ethanol concentration causes a decrease in particle size.

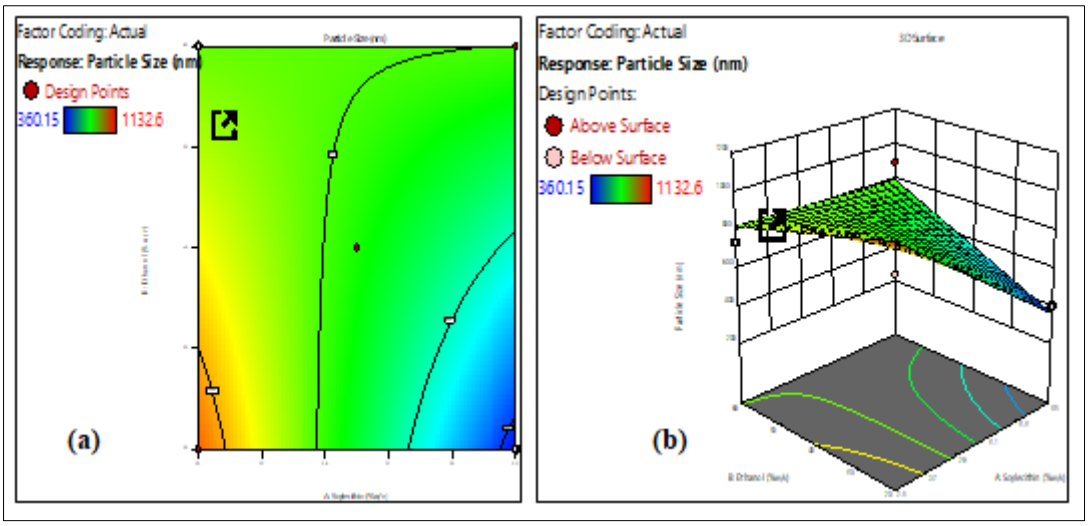

Figure 5: 2D contour plots (a) and 3D response surface plots (b) for evaluating the influence of soylecithin (X1) and ethanol (X2) on particle size (Y2)

The built-in ANOVA result indicates that model terms are significant if P-values are less than 0.0500. Model terms X1 and 2 are important model terms, and the model's F-value of 6.85 suggests that the model is significant. The anticipated determination coefficient (R2) value of 0.1815 and the corrected R2 value of 0.6868 did not differ by less than 0.2. This can indicate a sizable block effect or a possible problem with the model or data. It is important to take into account outliers, response transformation, model reduction, and other problems. Any empirical model should be tested using confirmation runs. The signal is deemed satisfactory with a signal-to-noise ratio of 7.546. This model can be used to navigate the design space.

Optimum Formula Selection

Discussion of particle size and entrapment efficiency formulation based on the results above Because F3 had the lowest particle size, the program deemed it optimised; this might be because it had the proper process variables. The chosen batch was manufactured and analysed three times to validate the recipe, and the results produced with slide modifications were comparable.

Poly-Dispersity Index (PDI)

The formulation's PDI is a crucial metric for evaluating the homogeneity of nanoparticles in the dispersed solution. The ideal transethosomes PDI of 0.6 indicates that a homogeneous transethosomal solution has been created. When the PDI is less than 0.5, the system is considered monodisperse and

meets the drug delivery requirements. A large size distribution is indicated by a PDI score higher than 0.7. Lipids and edge activators are crucial elements of transethosomal formulations that influence the PDI. Transethosomes with higher lipid concentrations tend to have larger vesicles

Zeta Potential

An essential feature of a colloidal dispersion system that demonstrates the electrostatic stability of the charge on the drug nanoparticles is the zeta potential. The optimised transethosome formulation F3 has a zeta potential of -28.65 mV due to the negative charge of the phospholipids. Transethosome stability and penetration are greatly enhanced by the net negative charge that ethanol produces in the vesicular system [17].

Transmission Electron Microscopy

Using a FEI Tecnai G2 F20 X-Twin transmission electron microscope (TEM), the shape of the optimized FLUCYTOSINE-loaded optimized formulation was evaluated in its initial suspension phase. Figure 6 displays the TEM images of the optimized formulation with spherical FLUCYTOSINE loaded.

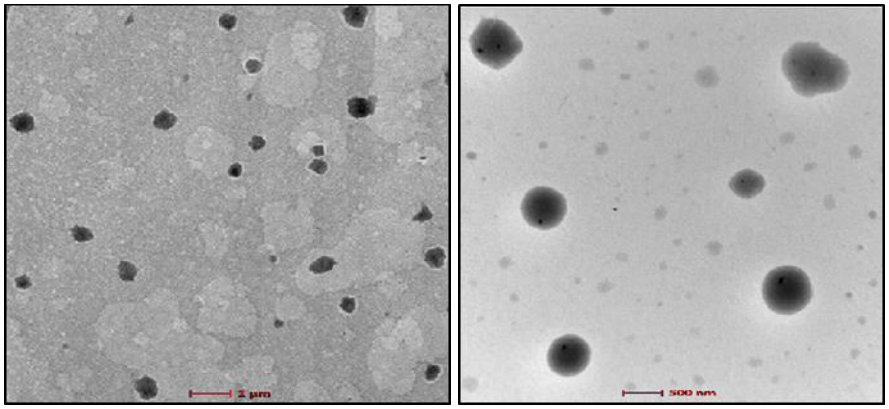

Figure 6: TEM image of flucytosine-loaded optimized formulation.

Stability Studies of Flucytosine Optimized Transethosomes

Table 8: Stability of optimized FLUCYTOSINE -Loaded TE at different temperature

Parameter	Times (days)	5℃	40℃
Appearance	0	Milky	Milky
	30	Milky	Milky
	60	Milky	Milky
Color	0	Pale yellow-white	Pale yellow-white
	30	Pale yellow-white	Pale yellow-white
	60	Pale yellow-white	Pale yellow-white
%EE	0	80.81	80.81
	30	80.24	79.52
	60	78.42	75.82

Evaluation of Flucytosine Loaded Transethosomal Gel

Physical Appearance

The flucytosinet-loaded transethosomal gel were visually consistent in texture, smooth, and milky.

Analysis of pH

When it comes to the longevity and effectiveness of skin treatments, pH is a critical factor. Numerous publications state that the pH range of the skin varies, roughly between 4.0 and 7.0. It typically falls between 4.5 and 6.0. Nonetheless, it is believed that the skin's natural pH is 5.5. The flucytosineTG had a pH of 5.82 ± 0.13.

Viscosity Analysis

A gel's viscosity, which indicates the formulation's physical stability for topical application, gives a good description of its flow properties. Viscosity has a direct impact on the drug release profile, and higher viscosity can cause the structure to become more rigid, which slows down the rate at which the drug penetrates the structure [18]. The optimized flucytosineTG that was prepared, and the viscosity observed 11120 cps at 20 rpm.

Spreadability

One important component that affects how uniformly the formulation is applied to the skin is the gel's spreadability. A gel's effectiveness is influenced by how easily it spreads; a high spreadability gel

spreads quickly, increasing patient compliance. 5.6 gm.cm/sec was the spreadability optimized TEG.

Determination of Drug Content

The content of prepared transethosomal preparation was determined and it was investigated the 95.10 % means the 19.02 mg drug present in the 1 gm of transethosomal gel. Which shows the prepared gel exhibited the 2% w/w solution of drug.

***In Vitro* Drug Permeation Study**

At pH 7.4, in vitro tests were performed to examine the release patterns of the market formulation Micogel (2%w/w) and the optimized formulation (F3) loaded transethosomal gel (flucytosineTG). Table 9 and figure 7 presents the findings. It was observed that 61.77% of the flucytosine was released from the commercial formulation Micogel (2%w/w) and 73.66% from the (F3) loaded transethosomal gel (flucytosineTG) within 24 hr. The study's findings showed that the medication was first burst out of (F3) loaded transethosomal gel (flucytosineTG), most likely as a result of drug release and desorption from the vesicle surface. Subsequently, there was a gradual release of the drug, suggesting a controlled release pattern. This demonstrated that the transethosomes that were developed could release medications continuously and for a long time. Transethosomal gel (flucytosineTG) has a higher release than the commercially available formulation.

Table 9: % drug release of marketed gel and optimized transethosomal gel

Sr.no	Time (hours)	Marketed formulation	Optimized TEG
1	0.25	0.75	5.57
2	0.5	3.55	7.24
3	1	7.24	17.24
4	2	11.55	29.48
5	3	18.31	37.46
6	4	18.85	41.43
7	5	18.99	44.58
8	6	18.62	55.39
9	7	18.57	65.98
10	8	18.85	73.66
11	12	31.21	73.72
12	24	61.77	73.74

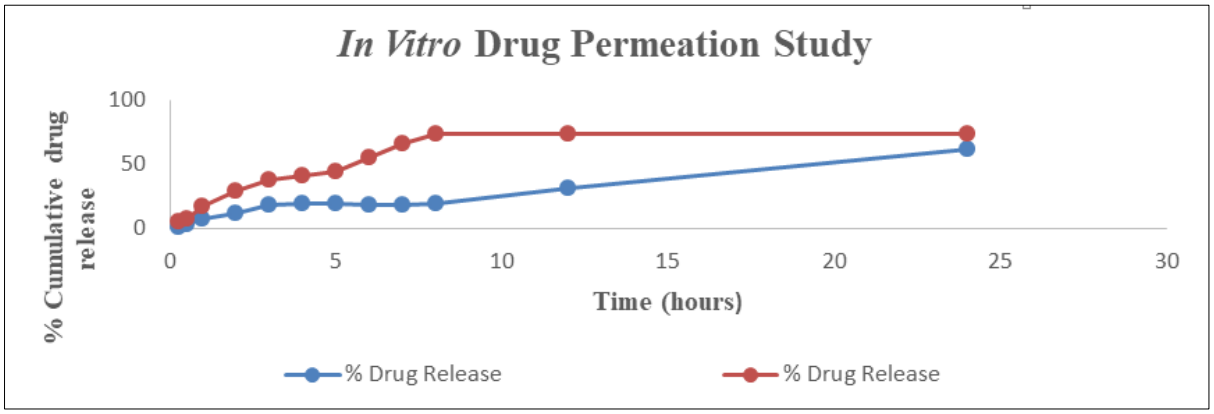

Figure 7: In vitro permeation pattern of optimized transethosomal gel (flucytosineTG) and marketed formulation micogel (2% w/w)

***In Vitro* Antifungal Activity**

Using the disc diffusion method, the antifungal activity of the manufactured formulation and the prepared flucytosine transethosomal gel against Candida albicans was assessed. The zone of inhibition was measured in orde r to ascertain the antifungal activity. The simple Flucytosine gel (c) had a zone of inhibition of 9.10 ± 0.01 mm, and flucytosine transethosomal gel (b) had a zone of inhibition of 12.50 ± 0.02 mm.

While the standard drug Clotrimazole (a) had a zone of inhibition of 14.40 ± 0.02 mm as shown in figure. According to the findings, the marketed cream lacked the flucytosine transethosomal gel antifungal activity. The remarkable pliability of transethosomes, which enables them to break through the fungal cell walls of Candida albicans, and their inhibition of ergosterol synthesis, which results in the lysis and death of fungal cell membranes, may account for this

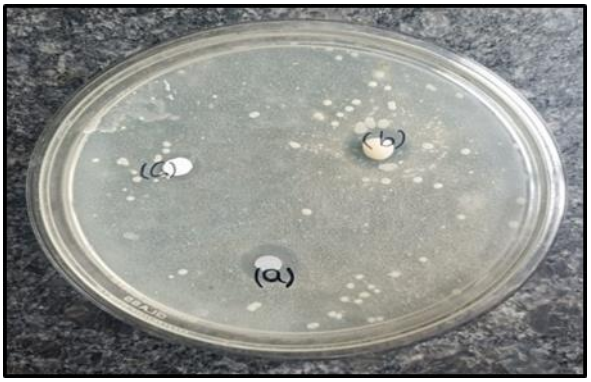

Figure 8: Zone of inhibition of different formulation of drug

Stability Studies

Optimized formulation was examined up to 3 months for any physical appearance, change in pH, rheological property and spreadability, displays in table 10. The stability study reveals that the optimized formulation of transethosomal gel shows less deviation, so it can be concluded that it showed acceptable stability on given temperature and humidity

Table 10: Stability studies of prepared transethosomal gel

Parameter	Times (days)	5℃	40℃
Appearance	0	Yellowish white	Yellowish white
	30	Yellowish white	Yellowish white
	60	Yellowish white	Light brownish white
Spreadability	0	5.6	5.6
	30	5.6	5.8
	60	5.8	6.0
pH	0	5.8	5.8
	30	5.8	5.8
	60	5.4	5.2
Viscosity (20 rpm)	0	11120	11120
	30	11095	11015
	60	10912	10816

SUMMARY

Present study focused on the development and evaluation of a flucytosine-loaded transethosomal gel for the topical treatment of fungal infections. Transethosomes, a lipid-based vesicular carrier system, were formulated to enhance drug delivery through the skin and improve therapeutic efficacy.

Nine transethosomal formulations were prepared and evaluated for vesicle size, zeta potential, particle size distribution, transmission electron microscopy, and entrapment efficiency. Among all formulations, F3 was selected as the optimized formulation due to its highest entrapment efficiency (81.56% ± 1.32) and lowest particle size (360.15 nm ± 1.14). optimized transethosomal formulation was incorporated into Carbopol-934 gel to prepare the transethosomal gel (Flucytosine TG). The prepared gel was evaluated for pH, viscosity, spreadability, in vitro permeation, antifungal activity against Candida albicans, skin irritation potential, and skin penetration studies using CLSM. The transethosomal gel demonstrated superior drug permeation, enhanced antifungal activity, better skin penetration, and lower skin irritation compared with the commercial formulation (Micogel 2% w/w) and flucytosine plain gel. Stability studies confirmed that the formulation remained stable under accelerated conditions for two months.

CONCLUSION

The study successfully developed a flucytosine-loaded transethosomal gel as an effective topical drug delivery system for fungal infections. The optimized formulation (F3) exhibited desirable physicochemical characteristics, high entrapment efficiency, and nanosized vesicles, contributing to enhanced drug delivery. The transethosomal gel showed improved skin permeation, deeper skin penetration, stronger antifungal activity against Candida albicans, and reduced skin irritation compared to the commercial formulation and plain gel. These findings suggest that the developed transethosomal gel has significant potential as an advanced topical therapeutic system for the

treatment of fungal infections. The formulation offers enhanced efficacy, improved patient compliance, and promising stability, making it a suitable candidate for further clinical investigation and potential commercialization.

REFERENCES

- Sawyer, P.R., Brogden, R.N., Pinder, R.M., Speight, T.M., Avery, G.S. Miconazole: A Review of its Antifungal Activity and Therapeutic Efficacy. Drugs 9, 406–423. https://doi.org/10.2165/00003495-197509060-00002
- Sguizzato, M., Ferrara, F., Hallan, S.S., Baldisserotto, A., Drechsler, M., Malatesta, M., Costanzo, M., Cortesi, R., Puglia, C., Valacchi, G.. Ethosomes and transethosomes for mangiferin transdermal delivery. Antioxidants 10, 768.
- Sharma, B., Singh, L.R., Pradesh, U., Pradesh, U. Pharmaceutical gels for topical drug delivery: An overview. Int J Pharm Pharm Sci 2, 19–24.
- Shende, M.A., Sayyad, H.G., n.d. Development and Validation of Spectroscopic Analytical Method for Simultaneous Estimation of Mupirocin and Satranidazole in Bulk and Topical Formulation 10.
- Singh, M., Lee, K.E., Vinayagam, R., Kang, S.G. Synthesis and Assessment of Lipid Nanovesicles for Efficient Transdermal Delivery of Hydrophilic Molecules. Nano 17, 2250031. https://doi.org/10.1142/S179329202250031X
- Song, C.K., Balakrishnan, P., Shim, C.-K., Chung, S.-J., Chong, S., Kim, D.-D.. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: characterization and in vitro/in vivo evaluation. Colloids Surf. B Biointerfaces 92, 299–304.
- Verma, A., Singh, S., Kaur, R., Jain, U.K. Topical gels as drug delivery systems: A review. Int J Pharm Sci Rev Res 23, 374–382.
- Verma, N., Jain, S., Gupta, N., Kapoor, V., Rajput, D. formulation, development and evaluation of transfersomal gel of amphotericin b. j. adv. sci. Res. 10, 254–260.
- Verma, P., Pathak, K. Therapeutic and cosmeceutical potential of ethosomes: An overview. J. Adv. Pharm. Technol. Res. 1, 274–282.
- Suvarnalata, S., Chaudhari, R.Y.. Transdermal Gel: As a Novel Drug Delivery Sysytem. Int. J. Pharm. Life Sci. 7.
- Shende, M.A., Sayyad, H.G., n.d. Development and Validation of Spectroscopic Analytical Method for Simultaneous Estimation of Mupirocin and Satranidazole in Bulk and Topical Formulation 10.
- Salim, M.W., Shabbir, K., Yousaf, A.M., Choi, H.-G., Khan, G.M. Preparation, in-vitro and in-vivo evaluation of Rifampicin and Vancomycin Co-loaded transfersomal gel for the treatment of cutaneous leishmaniasis. J. Drug Deliv. Sci. Technol. 60, 101996.
- Wang, W., Tham, P.H., Ding, C., Huang, P., Li, T., Luo, J., Xiang, H., Zeng, X., Chen, H., Zhao, Y. Transethosome-Based Topical Administration Systems with Enhanced Penetration and Dual Actions for Treating EGFR-Overexpressed Cutaneous Squamous Cell Carcinoma. Adv. Funct. Mater. 2312838.
- Westcott, C. pH measurements. Elsevier.
- Yoshida, K., Hirai, K., Ara, T., Ito, M., Wang, P. -L., Igarashi, Y.. Feasibility of using collagen as the base of the antifungal drug, miconazole. J. Oral Rehabil. 33, 363–367. https://doi.org/10.1111/j.1365-2842.2005.01568.x
- Young, J.C. True melting point determination. Chem Educ. 18, 203–208.
- Zahid, S.R., Upmanyu, N., Dangi, S., Ray, S.K., Jain, P., Parkhe, G. Ethosome: a novel vesicular carrier for transdermal drug delivery. J. Drug Deliv. Ther. 8, 318–326.
- Zaid Alkilani, A., McCrudden, M.T., Donnelly, R.F. Transdermal drug delivery: innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. Pharmaceutics 7, 438–470.
- Zan, P., Than, A., Duong, P.K., Song, J., Xu, C., Chen, P. Antimicrobial Microneedle Patch for Treating Deep Cutaneous Fungal Infection. Adv. Ther. 2, 1900064. https://doi.org/10.1002/adtp.201900064
- Zhou, Y., Wei, Y., Liu, H., Zhang, G., Wu, X.. Preparation and In vitro Evaluation of Ethosomal Total Alkaloids of Sophora alopecuroides Loaded by a Transmembrane pH-Gradient Method. AAPS PharmSciTech.