RESEARCH ARTICLE

Formulation and Characterization of Ethosomal Drug Delivery System for Antifungal Therapy

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ABSTRACT

The present study focuses on the formulation and evaluation of ethosomal gel for the transdermal delivery of fluconazole, a widely used antifungal drug. Ethosomes, phospholipid-based nanocarriers, enhance drug penetration through the skin, improving bioavailability and therapeutic efficacy. The ethosomes were prepared using the hot process. The optimized formulation was incorporated into a gel base for enhanced stability and ease of application. Various in-vitro evaluations such as determination of pH, spreadability, measurement of viscosity, drug content, Transmission Electron Microscopy (TEM): In-vitro diffusion study and stability testing, were conducted. The results demonstrated that the ethosomal formulation provided sustained drug release, improved skin permeation, and enhanced antifungal activity compared to conventional formulations. The study concludes that ethosomal gels are a promising approach for the effective transdermal delivery of antifungal agents.

Keywords: Ethosomes, Fluconazole, Antifungal Drug, Transdermal Delivery, TEM, Nanocarriers, Drug Permeation.

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INTRODUCTION

Fungal infections pose a significant health burden, necessitating the development of effective and patient-friendly drug delivery systems. Conventional antifungal treatments, such as creams and oral formulations, often suffer from poor penetration and limited bioavailability. Ethosomes, which are composed of phospholipids, ethanol, and water, offer an improved alternative due to their ability to penetrate deep into the skin and deliver the drug effectively. [1]

Ethosomes are lipid-based nanocarriers that enhance the permeability of drugs across biological membranes. Their structure and composition enable better skin penetration compared to traditional liposomes.

The presence of ethanol fluidizes the lipid bilayer, improving drug absorption. [2,3]

The objective of this work was to apply statistical methods to optimize the vesicular formulations (ethosomes and liposomes) in order to improve the administration of fluconazole, a model medicine used to treat fungal infections, to the skin.

Fluconazole is a strong candidate for liposomal and ethosomal preparation due to its half-life of 2.7 to 3.4 hours, with over 80% of the drug remaining in the circulation and 60 to 70% being excreted in the urine. Additionally, only 10% of fluconazole is bound to proteins. These characteristics make it an ideal choice for the development of controlled release formulations that can maintain a sufficient concentration of the drug in the body. [4,5]

Preparation of Ethosomes

Ethosomes were synthesized utilizing a modified version of the hot process described in US patent 5,540,934(1995), employing a magnetic stirrer, probe sonicator, and high pressure homogenizer. The ratio of Fluconazole (FLZ), Soya phosphatidyl choline (SPC), Ethanol, and Propylene glycol was modified, and the impact on drug entrapment efficiency and particle size was investigated. The constructed ethosomal system consists of 10mg of medication, SPC at concentrations of 1-5% (w/v), ethanol at concentrations of 20-50% (w/v), propylene glycol at concentrations of 10-20% (w/v), and water to make up 100% of the system. The drug was dissolved in a mixture of ethanol and propylene glycol and then added to a phospholipid dispersion in water at a temperature of 40°C. The mixture was stirred using a magnetic stirrer and subjected to 5 minutes of mixing between each cycle using a probe sonicator. The formulation underwent homogenization at a pressure of 15,000 psi in 3 cycles using a high-pressure homogenizer in order to obtain ethosomes with a nano-sized structure.

Preparation of Gels

Preparation of carbopol gel base

A quantity of 0.5 grams of Carbopol 934 was measured and mixed with water while gently stirring. The mixture was then left undisturbed for 24 hours to allow the Carbopol to absorb water and form a gel with a concentration of 0.5%. Subsequently, 2 cc of glycerin was used to get a gel-like texture. Additionally, gels containing 1% and 2% carbopol were made. The composition is shown in table 1.

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Table 1: Composition of different gel base

Formulation	Carbopol (%)
EF1	0.5
EF2	1.0
EF3	2.0

Table 2: Composition of optimized Ethosome formulation

Formulation	SPC (%w/v)	Ethanol (%w/v)	Propylene Glycol (%w/v)
EF1	3	43	10

Preparation of Ethosomal gels

One gram of ethosome formulation was diluted in 10 milliliters of ethanol and subjected to centrifugation at a speed of 6000 revolutions per minute for duration of 20 minutes in order to eliminate the drug that was not encapsulated. The supernatant was separated and the sediment was mixed with the gel vehicle. The composition is shown in table 2.

The integration of the ethosomes into gels was accomplished through a gradual process of mechanical mixing at a speed of 25 revolutions per minute for a duration of 10 minutes. The optimized formulation was included in three distinct gel concentrations: 0.5%, 1%, and 2% w/w.

Evaluation of Gels

Determination of pH

50 grams of each gel formulation were placed into a 10 ml beaker and tested using a digital pH meter. The optimal pH range for the topical gel formulation to effectively treat skin infections is 3–9. [6]

Spreadability

An customized apparatus was utilized to determine spreadability. The spreadability of the gels was assessed based on their slip and drag properties. The revised apparatus was constructed using two glass slides, with the lower slide securely fastened to a wooden plate and the upper slide connected to a balance via a hook. The spreadability was assessed using the formula: S=ml/t, where S represents spreadability, m denotes the weight in the pan attached to the upper slide, t signifies the time taken to move a certain distance, and l represents the distance traveled. In order to ensure practicality, the mass and length were maintained at a constant value, and the variable 't' was determined. The spreadability of each formulation was measured in triplicate and the average data are provided. [7]

Measurement of viscosity

The gel's viscosity was measured using a Brook Field viscometer DV-II model. The viscosity was measured using T-Bar spindles in conjunction with a helipath stand, which provided precise and accurate data.

(a) Selection of spindle

The optimal approach for selecting the spindle involved a process of trial and error, beginning with the T91 spindle. The objective was to achieve a viscometer dial or display reading (% torque) ranging from 10 to 100. The accuracy of the measurement increases as the reading approaches 100. The viscosity of all the gels was measured using Spindle T 95.

(b) Spindle Immersion

The T-bar spindle (T95) was vertically lowered in the center, ensuring that the spindle does not make contact with the bottom of the jar.

(c) Measurement of Viscosity

The T-bar spindle (T95) was employed to measure the viscosity of the gels. The variables such as temperature, pressure, and sample size that influence viscosity were controlled throughout the process. The helipath T-bar spindle was vertically displaced, resulting in viscosity measurements at various locations along the path. The torque value consistently exceeded 10%. The viscosity was determined by calculating the average of five observations collected during a 60-second period. [8,9]

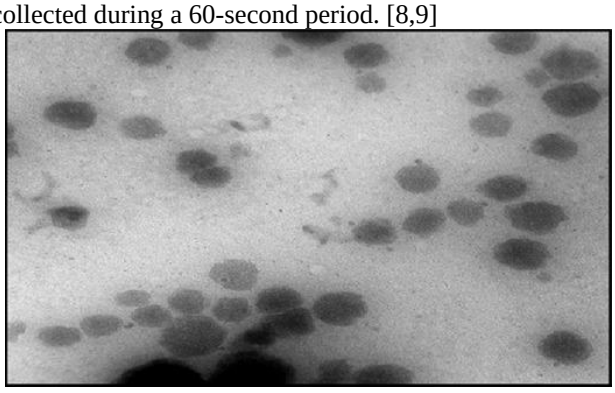

Figure 1: TEM image of Ethosome

Table 3: *In-vitro* drug release data for F1 (Marketed Fluconazole gel, Zocon Transgel^{R})

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	3.629±0.072	0.559	96.371	1.983
2	1.414	0.301	6.314±0.101	0.801	93.686	1.971
3	1.732	0.477	13.914±0.271	1.143	86.086	1.934
4	2.000	0.602	19.857±0.289	1.297	80.143	1.903
6	2.449	0.778	30.657±0.459	1.486	69.343	1.841
8	2.828	0.903	45.687±0.514	1.659	54.313	1.734
12	3.464	1.079	56.712±0.915	1.753	43.288	1.636
14	3.742	1.146	62.783±0.961	1.797	37.217	1.570

*Average of three readings

Table 4: *In-vitro* drug release data for EF1

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	8.229±0.095	0.915	91.771	1.963
2	1.414	0.301	15.086±0.203	1.179	84.914	1.929
3	1.732	0.477	26.971±0.439	1.431	73.029	1.863
4	2.000	0.602	34.286±0.586	1.535	65.714	1.818
6	2.449	0.778	44.800±0.896	1.651	55.200	1.742
8	2.828	0.903	52.800±0.956	1.723	47.200	1.674
12	3.464	1.079	64.320±1.154	1.808	35.680	1.552
14	3.742	1.146	76.800±1.342	1.885	23.200	1.365

*Average of three readings

Table 5: *In-vitro* drug release data for EF2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	9.600±0.112	0.982	90.400	1.956
2	1.414	0.301	22.857±0.413	1.359	77.143	1.887
3	1.732	0.477	32.000±0.625	1.505	68.000	1.833
4	2.000	0.602	43.429±0.756	1.638	56.571	1.753
6	2.449	0.778	50.286±0.915	1.701	49.714	1.696
8	2.828	0.903	69.600±1.114	1.843	30.400	1.483
12	3.464	1.079	80.160±1.213	1.904	19.840	1.298
14	3.742	1.146	94.080±1.765	1.973	5.920	0.772

*Average of three readings

Table 6: *In-vitro* drug release data for EF3

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	9.143±0.115	0.961	90.857	1.958
2	1.414	0.301	15.920±0.296	1.283	98.080	1.992
3	1.732	0.477	28.800±0.456	1.459	71.200	1.852
4	2.000	0.602	37.029±0.512	1.569	62.971	1.799
6	2.449	0.778	48.457±0.812	1.685	51.543	1.712
8	2.828	0.903	63.840±1.146	1.805	36.160	1.558
12	3.464	1.079	76.320±1.214	1.883	23.680	1.374
14	3.742	1.146	87.840±1.412	1.944	12.160	1.085

Drug content

The produced gel was combined with 100 ml of ethyl alcohol, with a ratio of 1 gram of gel per 100 milliliters of alcohol. Various concentrations were created by diluting the stock solution and filtering it. The absorbance was then measured at a wavelength of 260 nm. The drug content was determined using linear regression analysis of the calibration curve. [10]

***In-vitro* diffusion study**

Drug release research was conducted utilizing a modified Franz diffusion cell in a laboratory setting. A dialysis membrane with a molecular weight cutoff of 5000 Daltons (Hi Media) was positioned between the receptor and donor compartments. A gel containing ethosomes and liposomes, which is equal to 500 mg of fluconazole, was placed in the donor compartment. The receptor compartment was filled with 24 cc of phosphate buffer at pH 7.4. The diffusion cells were kept at a temperature of 37±0.5°C and were continuously stirred at a rate of 50 rpm during the whole experiment. At various time intervals, 5 ml samples were taken from the receiver compartment via a side tube and tested for the concentration of the medication using a UV-visible spectrophotometer. [11]

Transmission Electron Microscopy (TEM)

TEM is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

Stability studies

The optimized formulations of fluconazole loaded ethosome and liposomes gel were tested for stability under accelerated storage conditions at 4 ± 1°C and at room temperature (28 ± 1°C). The two formulations were stored in tiny glass bottles with screw caps. The bottles were amber colored and kept at temperatures of 4 ± 1°C and 28 ± 1°C. The samples were analyzed to determine the size of the vesicles and the amount of medication present after 7, 14, 21, and 28 days.

(a) Effect of storage temperature on vesicle size

The change in vesicle size of the formulations held at temperatures of 4±1°C and 28 ± 1°C was measured using a Zetasizer (Malvern Instrument, UK) after 7, 14, 21, and 28 days. [12,13]

(b) Effect of storage temperature on drug content

The medication content of both formulations was evaluated after being stored for certain durations of 7, 14, 21, and 28 days. The drug content in ethosome and liposomes gel was measured using spectrophotometry to indirectly assess the quantity of drug trapped in the gel.

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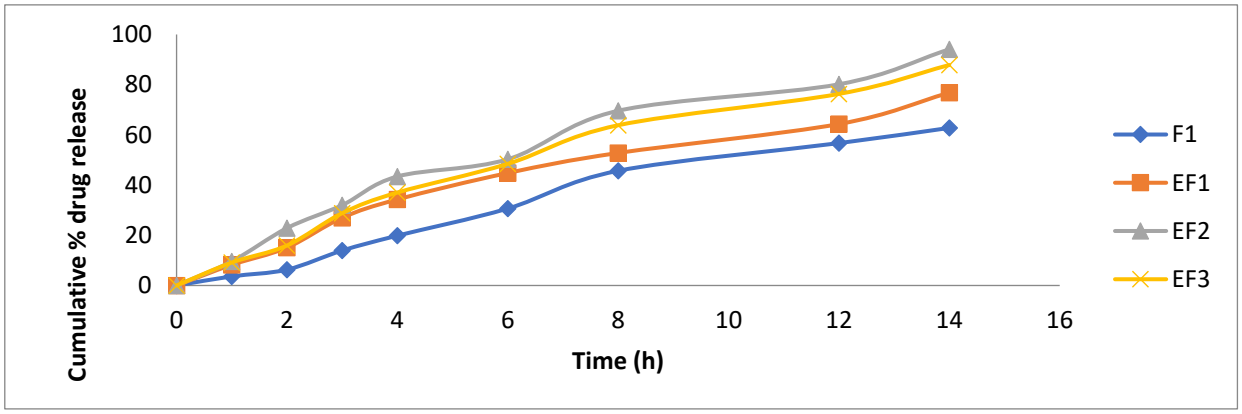

Figure 2: Cumulative %t drug released Vs Time

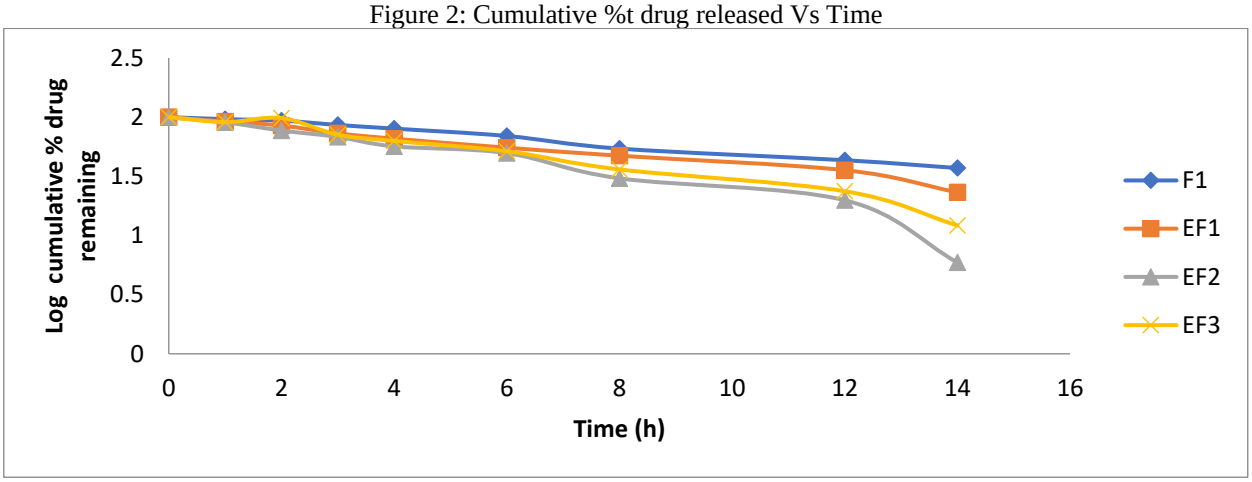

Figure 3: Log cumulative % drug remaining Vs Time

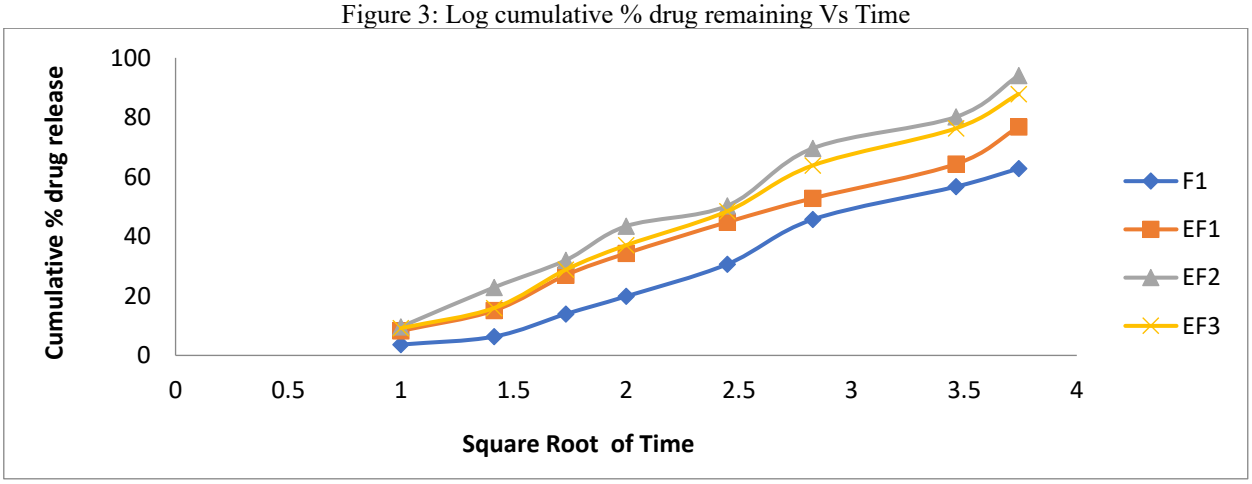

Figure 4: Cumulative % drug released Vs Square root of Time

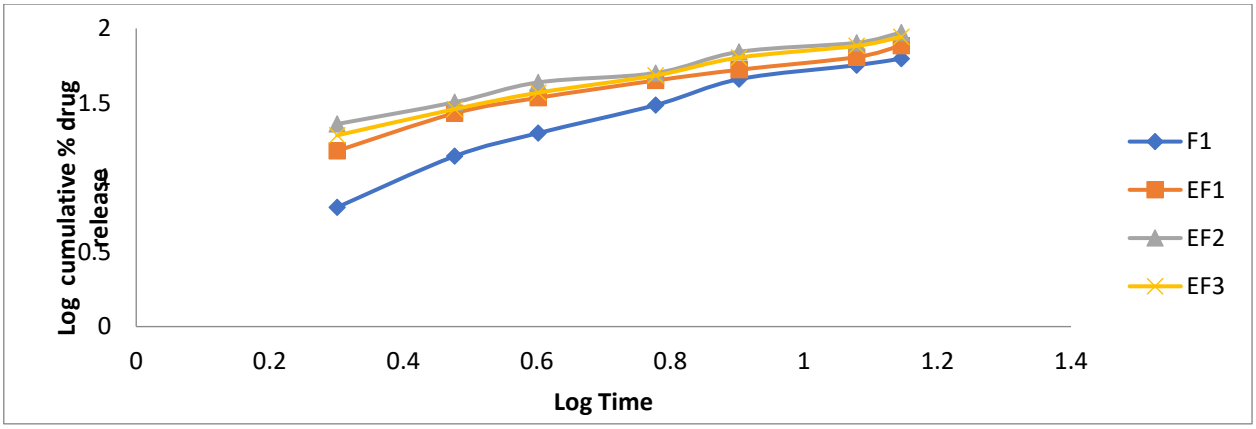

Figure 5: Log cumulative % drug released Vs log Time

RESULTS AND DISCUSSION

Evaluation of Ethosomal Gel

Drug content

Drug content is most important in ethosomal formulation and the data found are satisfactory. It was found to be 97.5 to 98.25% which shows the good capacity of formulation to hold the drug.

pH

In transdermal drug delivery system pH plays an important role, the result of ethosomal formulation shows that all the formulations are suitable for skin delivery. The pH values of the prepared ethosomal gels were within acceptable limits of 6.9-7.3.

Spreadibility

A modified apparatus was used for determining spreadability. The spreadability was measured on the basis of slip and drag characteristics of the gels and was in the range of 10.75 - 11.75 gms. cm. /sec. The gels should have optimum spreadability because very high and very low spreadability values indicate that the application of the gel to the site is difficult. [14]

Viscosity measurements

The viscosity of gels was determined by using a Brookfield viscometer DV-II model. T-Bar spindle in combination with a helipath stand was used to measure the viscosity and have accurate readings. The factors like temperature, pressure, sample size etc. which affect the rheological properties of gels were kept under control in the working area. The temperature, pressure, sample size etc were also kept constant. Some materials are quite sensitive to temperature a relatively small variation will result in a significant change in viscosity while others are insensitive. The temperature which alters the viscosity was maintained at 25ºC because the increase of temperature decreases the viscosity of gels and vice-versa. The shear rate (rpm) and time were variable factors. Before the measurement of viscosity the gels were removed from the freezer and were brought to normal by placing them at room temperature. This was necessary as there was a rise in viscosity in the gels which were not normalized.

The best method for the selection of spindle was trial and error starting from T91 spindle. Spindles in increasing number were used depending on the % torque and error. The goal is to obtain a viscometer dial or display (% torque) reading between 10 & 100, the relative error of measurement improves as the reading approaches 100. Spindle T 95 was found to be suitable and was used for the measurement of viscosity of all the gels. The Helipath T-Bar spindles were rotated up and down in the sample giving variable viscosities at a number of points programmed over the time. Five readings taken over a period of 60 seconds were averaged to obtain viscosity.

The results show that the viscosity of the gels increased with an increase in polymer concentration. The increase in viscosity with the polymer concentration may be due to increase in bonds between the polymer molecules which lead to formation of a hard and dense compact mass. This may also be due to less amount of liquid in gels with high polymer concentration as compared to gels of low polymer concentration or in other words it can be said the higher the polymer concentration more shear stress if required to produce a specified rate of shear. [15]

Transmission Electron Microscopy (TEM)

TEM revealed that ethosomes are multilamellar vesicles with mean size of 100 to 300 nm.It also suggests that ethosomes vesicles seems to be very malleable, as evident by imperfect round shape and this characteristics can be explained by the fluiding effect of ethanol on Fluconazole ethosomes as shown in figure 1***In-vitro*** **drug release study**

In-vitro diffusion study of the optimized Ethosomal gel (EF1, EF2, and EF3) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffer pH 7.4 for a period of 14 hours. The data obtained from diffusion studies are summarized in Table 3-6. The release rate of Fluconazole from Ethosome formulation over dialysis membrane was significantly higher than its transport across skin, indicating the barrier properties of skin for drugs. Ethosomal gel released drug in controlled release manner in 14 hour but in case of marketed formulation there is no controlled release of drug from gel. The release of the drug from ethosomal gel was found to follow the order: EF2> EF3> EF1. . [16]

In-vitro **drug release data of ethosomal gel formulation**

The cumulative percent drug release with time of the optimized Ethosomal gel (EF1, EF2, and EF3) are given in figure 2. Figure 3 contains the Log cumulative % drug

Table 7: Effect of storage temperature on the particle size of drug loaded Ethosomal gel

Time (Days)	Average Vesicle Size (nm) 4.0 ± 0.5°C	28 ± 0.5ºC
0	367.537 ± 3.138	367.537 ± 3.138
7	366.141± 3.364	364.672 ± 3.107
14	365.234 ± 4.106	365.132 ± 5.713
21	364.892 ± 3.441	366.248 ± 6.284
28	364.326 ± 4.383	366.421 ± 4.153

*Average of 03 readings

Table 8: Effect of storage temperature on the drug content of loaded Ethosomal gel EF2

Time (Days)	Drug Content (%) 4.0 ±0.5°C	28 ± 0.5ºC
0	98.120 ± 0.021	98.120 ± 0.021
7	97.917± 0.575	98.083± 0.158
14	97.265 ±0.279	97.692 ± 0.573
21	97.119 ± 0.265	97.096 ± 0.875
28	97.086 ± 0.887	96.852 ± 0.745

*Average of 03 readings

remaining versus time of Ethosomal gel (EF1, EF2, and EF3). Cumulative % drug released Vs Square root of Time is displayed in figure 4 and Log cumulative % drug released Vs log Time for the optimized formulations are given in figure 5.

Stability studies

Stability studies for optimized formulations were carried out at 4.0 ± 0.5°C and 28 ± 0.5ºC for a period of four weeks. There was no significant variation found in physical appearance, average particle size and % drug content of the Ethosomal gel EF2. No visible changes in the appearance of the gel formulation were observed at the end of the storage period as shown in Table 7-8.

CONCLUSION

The study successfully developed a fluconazole-loaded ethosomal gel with enhanced skin permeation and antifungal efficacy. Ethosomal formulations significantly improved drug penetration, providing sustained release and superior antifungal activity compared to conventional gels. The findings suggest that ethosomal technology is a promising approach for the effective transdermal delivery of antifungal drugs, offering potential advantages for clinical applications.

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