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Original article

Design and Optimization of Ketoconazole-Loaded Ethosomal Gel for Enhanced Topical Antifungal Delivery: A Box–Behnken Approach

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ABSTRACT:

The present study aimed to develop Ketoconazole -loaded ethosomal gel intended to be applied topically for treating skin infections. Ethosomes were prepared using the cold method. The formulation variables were optimized using3 factorial design and Design Expert® software for analyzing the data statistically and graphically using response surface plots. Phospholipid (X1) and ethanol (X2) and propyleneglycol (X3) were chosen as the independent variables, while the dependent variables comprised entrapment efficiency (Y1), vesicles size (Y2) and zeta potential (Y3). Ultra- centrifugation was used to assess the encapsulated medication after confirming the presence and size of vesicles. There was a greater increase in value (79.62%) in sonicated particles containing 30% w/w ethanol. The optimized ethosomes were subsequently incorporated into Carbopol® 940 gel and characterized for rheological behaviour, in-vitro release, ex-vivo skin permeation and deposition. Morphologically, the produced ethosome formulations were consistent when examined by SEM. All of the vesicles met or exceeded the criteria for nanotechnology in terms of size (less than 200 nm), polydispersity index (PDI), and entrapment efficiency (of the intended medication). The percentage of Ketoconazole released after 24hours was significantly decreased (p0.05) when the ethosomes were included into a variety of gel bases. By contrast, ethosomal gel showed considerably greater anthralin penetration than the other tested preparations ($p<0.05$). Compared to the ethosomal gel, the drug solution in receptor medium, and the drug hydroalcoholic solution, the total quantity of drug penetrated from the ethosomal gel was around 2.5-, 3.5-, and 4.5-fold greater ($p<0.05$).Stability studies displayed that after 2 months, all of the gels' physicochemical characteristics, including viscosity and color, remained unchanged, passing the tests.

Keywords: Topical application; ethosomes; Ketoconazole, ex-vivo permeation, stabilitystudies.

1. INTRODUCTION

Modern drug delivery research has increasingly focused on alternative routes of administration to overcome the limitations associated with conventional oral and parenteral dosage forms. Among these, transdermal drug delivery systems (TDDS) have emerged as one of the most promising approaches, gaining recognition as a potential alternative to oral drug administration. Transdermal delivery involves the administration of drugs through the skin to achieve systemic therapeutic effects, offering several pharmacokinetic and therapeutic advantages. Over the past two decades, transdermal drug delivery technology has attracted significant attention from pharmaceutical researchers and industries worldwide. During the 1980s and 1990s, extensive research and development led to the commercialization of various transdermal patches, and since then, more than 35 transdermal products covering over 13 active pharmaceutical ingredients have been approved by the U.S. Food and Drug Administration (FDA). The first transdermal system, developed for motion sickness in 1981, marked the beginning of a new era in controlled drug delivery. The skin, being the largest organ of the human body, plays a crucial role in transdermal drug administration. Structurally, it consists of three major layers: the epidermis, dermis, and subcutaneous tissue. The outermost layer of the epidermis, known as the stratum corneum, acts as the primary barrier to drug penetration. This layer is composed of dead, flattened keratinized cells (corneocytes) embedded in a lipid matrix of ceramides, cholesterol, and fatty acids, forming a highly organized “brick and mortar” structure. Due to this barrier function, only drugs with suitable physicochemical properties—typically low molecular weight (less than 500 Da), adequate lipophilicity, and sufficient potency—can effectively permeate through the skin [1].

Despite these limitations, transdermal drug delivery offers several advantages over oral administration, including

avoidance of first-pass hepatic metabolism, reduction of gastrointestinal side effects, maintenance of steady plasma drug levels, and improved patient compliance. Additionally, TDDS allows for controlled and sustained drug release, making it particularly useful for drugs with short biological half-lives and narrow therapeutic windows. However, the low permeability of the stratum corneum remains the major challenge in achieving effective transdermal drug delivery.To overcome this barrier, various strategies have been developed, such as chemical penetration enhancers, physical techniques (iontophoresis, sonophoresis, electroporation), and novel carrier systems. Among these approaches, vesicular drug delivery systems have shown considerable promise. Vesicular carriers such as liposomes, niosomes, transfersomes, and ethosomes are designed to enhance drug penetration by interacting with the skin barrier and improving drug solubilization and transport. Ethosomes represent a novel and advanced vesicular system specifically developed to enhance transdermal drug delivery. First introduced by Touitou in 1996, ethosomes are soft, malleable lipid vesicles composed mainly of phospholipids, a high concentration of ethanol (20–45%), and water. The presence of ethanol distinguishes ethosomes from conventional liposomes and plays a crucial role in improving skin permeation. Ethanol disrupts the lipid structure of the stratum corneum, increases membrane fluidity, and enhances the deformability of the vesicles, allowing them to penetrate deeper into the skin layers. Ethosomes have demonstrated superior skin permeation properties compared to traditional vesicular systems, enabling the delivery of both hydrophilic and lipophilic drugs, as well as molecules with larger molecular sizes. Once applied to the skin, ethosomes can penetrate through the disrupted stratum corneum, reach the deeper layers of the epidermis and dermis, and release the encapsulated drug in a controlled manner. This makes them particularly suitable for transdermal and topical drug delivery applications.

In recent years, ethosomal gels have gained significant interest as a topical dosage form combining the advantages of ethosomes with those of gel formulations. Gels are widely preferred for topical application due to their non-greasy nature, ease of application, better patient acceptability, and enhanced drug release characteristics. Incorporation of ethosomes into a gel base further improves formulation stability, spreadability, and residence time at the site of application.

Ethosomal gels offer an effective approach for enhancing transdermal drug permeation while minimizing systemic side effects and skin irritation. They are particularly beneficial for drugs that exhibit poor oral bioavailability, extensive first-pass metabolism, or gastrointestinal instability. Due to their ease of preparation, scalability, and improved therapeutic efficacy, ethosomal gels have emerged as a promising carrier system in modern transdermal drug delivery research [2].

2. MATERIALS AND METHODS

Rational dosage form development begins with a thorough preformulation analysis. Effective and stable dosage forms need examination of the physicochemical characteristics of both the drug molecule and any excipients used [1].

1) Solubility of Drug: An essential factor in the design of ethosomes is the drug's solubility. For the purpose of selecting an appropriate solvent system, the solubility of Ketoconazole was measured in a wide variety of solvents, including but not limited to distilled water, methanol, ethanol, chloroform, and certain buffer solutions. Five milligrams of ketoconazole were dissolved in ten milliliters of the aforementioned solvents and sonicated for an additional ten minutes. The solubility of the solutions was visually evaluated and compared to industry norms [67].

2) Melting Point Determination: Pure Ketoconazole's melting point was measured using a capillary technique. Capillary was loaded with a measured quantity of Ketoconazole powder, and the melting point was recorded digitally using a digital melting point device.

3) Determination of Absorption Maxima: The UV-visible spectrophotometer was used to scan a Ketoconazole solution in ethanol at 10 g/mL across the range of200 to 400 nm. The measured max was compared to the theoretical maximum value.

4) Calibration Curve of TH in PBS (pH 7.4): For the preparation of the standard stock solution, 10 mg of Ketoconazole was added to a 100 mL volumetric flask and the weights were checked. The stock concentration was brought up to 100 g/mL by diluting the sample to the appropriate amount with PBS (pH 7.4) [1].

Preparation of Sample: All of the diluted samples were made from the stock solution (100 g/mL). To achieve serial dilutions of 5, 10, 15, 20, and 25 g/mL, a UV-Visible spectrophotometer was used to record the absorbance at 223 nm when0.5,1.0,1.5,2.0,2.5,3.0,3.5,and4.0mL of the stock solution were transferred to a 10 mL volumetric flask [3].

Formulation of Ketoconazole-Loaded Ethosomes:

Ketoconazole-loaded ethosomes were prepared using a modified thin-film hydration method described by Touitou et al. Tosumup, PL80H, cholesterol, and ketoconazole were dissolved in ethanol and evaporated under reduced pressure to form a thin lipid film. The film was hydrated with a hydro-ethanolic solution above the lipid glass transition temperature to form multilamellar vesicles, which were converted into unilamellar vesicles by probe sonication. The prepared ethosomes were stored under refrigerated conditions [4].

Table 1: Ingredients In Test Batches

Formulation (F)	Lecithin (%)	Propylene Glycol (%)	Ethanol (%)	Cholesterol (mg)	Drug (mg)	Water
F1	2	10	20	0.05	100	Q.s
F2	3	10	20	0.05	100	Q.s
F3	4	10	20	0.05	100	Q.s
F4	3	10	30	0.05	100	Q.s
F5	3	10	40	0.05	100	Q.s
F6	3	10	50	0.05	100	Q.s
F7	-	10	30	0.05	100	Q.s

For X-ray diffraction (XRD) analysis, the ketoconazole ethosomal suspension was lyophilized to obtain a dry powder. The formulation was stored in an ultra-cold freezer (MDF-382E, SANYO, Japan) and lyophilized with mannitol as a cryoprotectant using a lyophilizer (Labconco, United Kingdom) at 0.1 mbar and −60 °C for 48 h. The lyophilized powder was stored in a desiccator for further studies.

The purity of ethanol-containing vesicles was evaluated using a dialysis membrane (MWCO 50,000; HiMedia). The cellulose acetate membrane was soaked in saline for 2 hours prior to use. Drug-loaded vesicles (1 mL) were diluted to 500 mL with phosphate-buffered saline (pH 6.8) and stirred at 500 rpm. Samples (10 mL) were withdrawn at 0.5, 1, 1.5, 2, 2.5, and 3 hours and replaced with fresh medium. The samples were diluted and analyzed by HPLC to determine drug content [5].

Formulation of Ketoconazole-Loaded Ethosomal Gel:

For in vitro, ex vivo, and in vivo studies, the optimized ethosomes were incorporated into a Carbopol gel base. Carbopol 934 was hydrated overnight in water and stirred to obtain a uniform dispersion. A 1% w/v ketoconazole ethosomal formulation was then incorporated, with benzyl alcohol added as a preservative. The pH was adjusted to 6.5–7.4 using triethanolamine.

Table-2: Composition of different gel formulation

Gel formulation	Ketoconazole suspension (ml)	Carbopol (%)	Tri ethanol amine (ml)	Water
G1	100	1	0.5	Q.s
G2	100	1.5	0.5	Q.s
G3	100	2	0.5	Q.s
*G4	100	1.5	0.5	Q.s

*G-4 free drug gel

Ethosomes were prepared using a three-factor, three-level factorial design to study the effects of phospholipid (X1), ethanol (X2), and propylene glycol (X3) on vesicle size (Y1), entrapment efficiency (EE), and zeta potential. Nine batches containing the same amount of Ketoconazole were evaluated using Design-Expert®. A Box-Behnken design and response surface analysis were applied to optimize formulation variables and assess their interactions. Quadratic polynomial models including interaction (X1X2) and non-linear ($X1^2$, $X2^2$) terms were fitted, and ANOVA confirmed model validity. Results were visualized using 2D contour plots and 3D response surfaces.

Table 3:Variables in the EZNSLN formulation.

Parameter	Low(-1)	Medium(0)	High(+1)
IndependentVariables			
LipidConcentration(X1)	2	3.5	5
Ethanol(X2)	20	30	40
PropyleneGlycol(X3)	7	8.5	10
Dependentvariables			
R1:EntrapmentEfficiency(%)		Maximize	
R2:VesicleSize (nm)		Minimize	
R3:Cumulative Drug Release(%)		Maximize	

Characterization of Ketoconazole-Loaded Vesicles Determination of Drug Encapsulation Efficiency

1. Centrifugation: Spin ethosomes at 36,670 × g, 4 °C, 30 min to separate supernatant (free drug) and pellet (encapsulated drug).

2. Measurement:

o Supernatant: dilute and measure absorbance at 257 nm.

o Pellet: wash, lyse in methanol with sonication, dilute, and measure absorbance.

3. Quantification: Use the calibration curve Y=0.0401X+0.0058Y = 0.0401X + 0.0058Y=0.0401X+0.0058 to calculate drug concentrations.

4. Encapsulation Efficiency:

EE%=T−CT×100

Where TTT = total drug, CCC = free drug in supernatant.

5. Reproducibility: Perform in three formulations to get mean ± SD.

Vesicle Size and Zeta Potential Determination

Zetasizer ver. 6.01 (Malvern Instruments Ltd, Malvern, Worcestershire, UK) wasused to assess vesicle size, zeta potential, and polydispersity index by diluting one drop of a composite ethosomal suspension with hydroethanolic solution at 25°C in disposable transparent zeta cells. Each sample was measured three times to ensure accuracy.

Vesicular Visualization by Scanning Electron Microscopy

The best ethosomal solution and gel were characterized using a scanning electron microscope (Philips, UK; CM 10 Scanning Electron Microscope, Mega View III FW 80 kV). One drop of each formulation was put on a copper grid for two to three minutes, and then the grid was negatively stained with phosphotungstic acid. At 4,500xmagnificationand80kVaccelerationvoltage,theair-driedsamplewasseenin a transmission electron microscope.

Evaluation of Gels Organoleptic Characters

The formulations were evaluated based on their psycho rheological characteristics. These included things like color, smell, texture, phase separation, and how they felt when applied (grittiness, greasiness)

Washability

Theskinwascoatedwithathinlayerofgel.Thegel'swashabilityafterbeingsubjected to a water wash was evaluated.

Spreadability

The answer was found using a wooden block and glass slide setup with a few tweaks. After placing a certain quantity of gel on the fixed glass slide, we sandwiched it between the two glass slides (one of which was linked to the moveable pan) for 5 minutes. The load was constantly being taken off. The following formula was used to ascertain the spreadability.

S=M/T

Where, S=Spreadabilitying/s, M=Massingrams, T=Time in seconds

pH and Viscosity Measurements

Gels' pH was measured using a Digital pH meter, model 111 E (HICON, New Delhi, India), and their viscosity was determined by spinning a T-spindle S-93 at 20 rpm (22.36 x 103 g) in a Brookfield viscometer R/S-CPS (Brookfield Engineering Lab, Inc, USA). The room temperature was maintained at 25 1 degrees Celsius [6].

Determination of Percent Drug Content

100 mg of the gel was diluted to 5 ml with methanolic HCl to evaluate the drug concentration. The drug's concentration was determined by spectrophotometry after it was shaken for 5 minutes in a vortex shaker (Vortex shaker, HICON, India) and brought to a final volume of 10 ml with methanolic HCl.

Thermal Analysis Studies

DSC (TA-60, Shimadzu, Kyoto, Japan) was used to determine the melting and solidification temperatures of ketoconazole, phospholipid, cholesterol, their physical mixture, ketoconazole-loaded ethosomes, and ethosomal gel. Samples (3–5 mg) were placed in 50 µL aluminum pans, sealed with lids, and empty pans were used as references. After equilibrating at 25 °C for 5 min, samples were heated to 300 °C at 10 °C/min under a nitrogen flow of 50 mL/min. Indium was used for instrument calibration.

Fourier-Transform Infrared Spectroscopy (FT-IR)Studies

Ketoconazole, phospholipids, cholesterol, a physical combination of these substances, an ethosome loaded with Ketoconazole, and an ethosomal gel were the substances analyzed. A JASCO FT-IR-4200 type A was used to evaluate samples that had been combined with potassium bromide (IR grade), compressed into disks under vacuum, and then read (JASCO Co., Tokyo, Japan). Scanning was performed at a resolution of 0.48-1.93 cm1 across the range of 4000-400 cm1.

In-Vitro Drug Release Studies

Ketoconazole-loaded ethosomal solution and gel were evaluated using the dialysis bag method (MWCO 6–8 kDa). Formulations (1 mL) were placed in dialysis membranes, with untreated drug solution as control. Release media included citrate buffer (10 mM, pH 3.3) with 30% methanol and 2% sodium lauryl sulfate. Tubes containing 25 mL medium were shaken at 37°C, 50 rpm. Samples (3 mL) were withdrawn at intervals and replaced with fresh medium. Ketoconazole was measured spectrophotometrically at 257 nm. Experiments were performed in triplicate, and cumulative drug release over time was analyzed [7].

Kinetic Analysis of Drug Release Data

Medication release kinetic models were used to Ketoconazole release data from a number of different formulations in order to better understand how the drug is released from its carriers. As a result, the release data were f it using the zero-order

Higuchi model, the first-order Baker-Lonsdale model, the Hixson-Crowell cube root equation, and the Korsmeyer-Peppas equation

ExVivoPermeability Studies

These experiments were performed on rat abdomen skin using the same procedures detailed in previous papers [29,37]. Male Wistar rats were obtained when they were about 6-8 weeks old and 90-100 g in weightfor their abdominal skin. In line with the Guide for the Care and Use of Laboratory Animals (8th ed.), National Academies Press, Washington, DC, all animal operations were approved by the Pharmacy Faculty. After the rats were killed, the region surrounding their bellies was shaved using an electric clipper. A portion of the abdominal skin has its connective tissue, fat, and/or subcutaneous tissue surgically removed. After being carefully examined for faults such microscopic holes or cracks, skin samples were washed in physiologic saline and dried in between two filter sheets. The skin may be used immediately; no preservatives were required. When the skin was placed in a test tube, the stratumcorneumside was facing up. The receiver compartment was maintained at 37 degrees Celsius with 100 milliliters of citrate buffer (10 mM, pH 6.8) containing 30% methanol and 2% sodium lauryl sulfate. Diffusion area via skin was determined to be 0.785 cm2. The donor area was prepared with ethmosomal gel and suspension gel containing 300 mg of Ketoconazole. Ketoconazole was included at a weight percentage of 1% (w/w) in all of the gels. Receptor medium and a 40:60 (v/v) ethanol/water pH 6.8 mixture were used to dilute ketoconazole solutions, which were then used as controls. A magnetic stir bar was rotated at 100 rpm to mix the receptor media. At 1, 2, 3, 4, 5, and 6 hour intervals, 5 mL samples of the receptor medium were obtained and then immediately replaced with fresh receptor medium. Ketoconazole concentration was calculated using 257 nm spectrophotometricanalysis. The amount of ketoconazole absorbed by the skin during both treatmentswas measured inmicrogramspersquarecentimeter.

Oncethepermeationstudieswere done, the amount of drug deposited in the skin could be determined. Three times, the skin was gently cleaned with water to remove any remaining formulation. The active component was extracted from skin scraps by soaking them in methanol for 24 hours. Drugcontent wasdeterminedbyspectrophotometricanalysis at 257nmafter filtration of the methanolic extract via a 0.2 m membrane. Three separate attempts were made to ensure reproducibility of the results. Calculate the steady-state flow(Js,g/cm2/h) as

the slope of the straight line that results when you plot the total amount of medicine penetrated (in g/cm2/h) against time. The apparent permeability coefficient (Papp, cmh1) may be calculated using this formula (2).

Papp=Jss/ C0, (2)

using C0 as the starting anthralin concentration in the donor medium and Js as the steady-state flow.

Antifungal activity

Cup plate technique testing was used to measure the antifungal efficacy against several strains of Candida albicans. The fungi were seeded into nutrient agar medium in a Petriplate, and the solution was poured over the top. 24 hours of incubation at 25 degrees Celsius was used on the plates. Antibiotic zone readers were used to examine the surrounding inhibition zones after incubation (Hicon Enterprises).

Stability studies

The vesicle stability was tested by keeping the prepared vesicular suspension at 250.5 degrees Celsius. After 180 days, the vesicle size, zeta potential, and EE were assessed using the aforementioned approach.

3. RESULT AND DISCUSSION

An ultraviolet spectrophotometer was used to scan pure ketoconazole in methanol between200and400 nanometers. Light absorbed by ketoconazole has a characteristic spectrum, with a maximum absorbance at 234 nm and a wavelength range of 220 to 360 nm. It has been determined that Ketoconazole is present because of the presence of a wide shoulder at about 257 nm. As a result of finding that ketoconazole had the largest peak at 257 nm, it was chosen for further testing (see statement number 36).

Calibration curve in water (make up with PH 6.8 phosphate buffer) Absorbance was measured at 257 nm from standard solutions of varying concentrations (Table 13). Drug concentrations were plotted against absorbance to generate a calibration curve, per the protocol (Figure.8). Beer's law was shown to be linear with a k2 value of 0.999Preformulation Study:

We performed pre-formulation research to predict the drug's physical and chemical characteristics in order to create a safe and effective dosage form that would be stable over time. The medication powder's integrity was confirmed by the preformulation research. The melting point, solubility, and standard plot ofthe medication are allpart of this

investigation. Capillary technique was used to measure the melting point of pure Ketoconazole [8].

Identification of Pure Drug:

Solubility of Drug:

Ketoconazole is lipophilic, as shown by its low water solubility. Drug was insolublein water but soluble in a wide variety of organic solvents (pH 7.4.).

Melting Point Determination:

It has been determined that 152 degrees Celsius is the melting point of Ketoconazole. Ketoconazole's melting point is often reported to fall between 148 and 152 degrees Celsius, a range consistent with its high level of purity.

Determination of Absorption Maxima:

An ultraviolet (UV) spectrophotometer was used to examine a Ketoconazole solution with a concentration of 10g/mL in the range of 200 to 400 nm. Absorbancemaximum (max) was observed at 257nm in the recorded spectra, as shown in Fig.9.1.

Optimization of Formulation:

This study evaluated a strategy to enhance transdermal delivery of ketoconazole by modifying lipid membrane composition and carrier efficiency. Seventeen formulations (F1–F17) were prepared based on preliminary trials optimizing phospholipid, ethanol, and propylene glycol levels. Soy lecithin below 2 mg resulted in low entrapment efficiency (45.34 ± 2.03), while amounts above 150 mg produced large, unstable vesicles (366.7 ± 0.32 nm). Ethanol concentration significantly affected vesicle size and drug entrapment: 20% ethanol formed large vesicles with poor entrapment, whereas concentrations above 40% caused leaky vesicles. Data from the 17 experiments were analyzed using ANOVA (Table X).

Table 4: Ketoconazole formulation batch composition and characterization

Run	X1	X2	X3	R1	R2	R3
1	5	30	10	65.51	750	78.56
2	2	20	8.5	71.72	112	90.23
3	3.5	30	8.5	73.94	520	68.73
4	3.5	20	10	88.89	98	96.49
5	3.5	40	7	50.82	235	69.41
6	3.5	40	10	55.69	490	60.28
7	3.5	30	8.5	75.93	450	68.72
8	3.5	30	8.5	73.84	521	70.62
9	5	40	8.5	46.19	759	70.27
10	2	40	8.5	45.76	1275	68.81
11	2	30	7	59.79	300	70.34
12	3.5	20	7	82.38	115	65.24
13	3.5	30	8.5	74.49	535	68.34
14	2	30	10	51.61	220	66.13
15	5	20	8.5	72.38	1025	88.69
16	3.5	30	8.5	73.49	495	69.76
17	5	30	7	50.25	285	48.98

Response1:Entrapment Efficiency:

EE=+74.34+0.6813A-14.61B+2.31C-0.0575AB+5.86AC-0.410BC-13.99A2- 1.34B2-3.56C2 (9.3)

The model showed statistical significance with an F-value of 80.54, indicating that such a result is unlikely due to random chance. Model terms with p-values below 0.0500 were considered significant, namely B, C, AC, A^2, and C^2, while terms with p-values above 0.1000 were deemed insignificant. The lack-of-fit was also significant (F = 8.52), occurring by chance only 3.28% of the time.

The effective extractable (EE) fraction represents the percentage of drug retained within the non-polar lipid chain and is critical for assessing transdermal drug encapsulation. Owing to the lipophilic nature of ketoconazole, %EE increased with higher phospholipid and ethanol concentrations (20–40%) due to improved drug solubility and membrane fluidity. However, ethanol levels above 40% caused vesicle leakage, reducing %EE and formulation stability.

Optimization using Design-Expert® identified phospholipid and hydroethanol concentration as key variables. Vesicle size, zeta potential, and %EE showed close agreement between predicted and observed values. Among all formulations, F8 exhibited the smallest vesicle size (127.39 ± 2.71 nm), highest zeta potential (−54.8 ± 4.25 mV), and greatest drug loading (87.55 ± 0.47%), and was therefore selected for further studies.

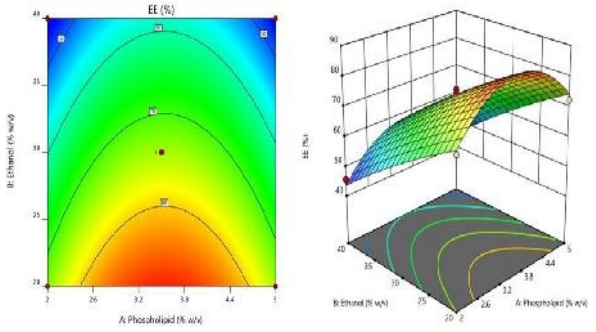

Fig 9: 3D Counter and RS Counter Effect of Independent Variables on Total Electrical Energy Consumption as a Percentage (%) Plot

Response2:VesicleSize

Size=+504.20+114.0A+176.13B+77.88C-357.25AB+136.25AC+68.0BC

+221.4 A2+67.15B2-336.5C2… (9.1)

The model was statistically significant with an F-value of 47.35, indicating that such a high value is unlikely due to random chance. Model terms A, B, C, AB, AC, A^2, and C^2 were significant ($p < 0.05$), while terms with $p > 0.10$ were not important. The Lack of Fit was also significant ($F = 6.80$), suggesting the model does not fully explain the variability.

Vesicle sizes ranged from 115 to 1275 nm and were strongly influenced by ethanol and phospholipid concentrations. Increasing ethanol (20–40%) and phospholipids significantly reduced vesicle size ($p < 0.05$), consistent with previous studies. Higher ethanol levels caused vesicle shrinkage due to phospholipid fluidization and reduced membrane thickness. All ketoconazole-loaded ethosomes were below 200 nm, indicating their potential for deep skin penetration and improved transdermal drug delivery.

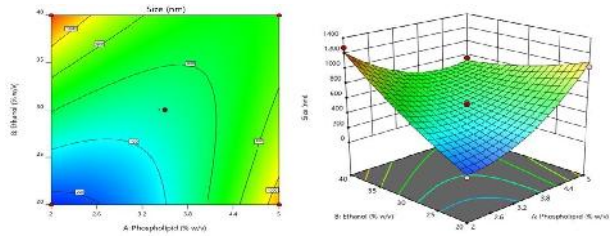

Fig 10: 3D counter and a response board Influence of Independent Variables on: Size, Location, and Time (3-D Plot)

Drug release from improved ethosomal formulations must be evaluated by the calculation of % CDR before pharmacological testing [27]. In this case, the % CDR varied from around 65% to 99% across all of the formulations (Table (Table4).4). It can be shown in Fig. that as the quantity of ethanol changed, so did the percentage CDR, as measured by the concentration of phospholipids. This pattern in percentCDR variation was quite similar to that seen in percent EE. It follows that the concentrations of phospholipids, ethanol, and PG have a major impact on ethosome vesicle size, EE, and CDR [9].

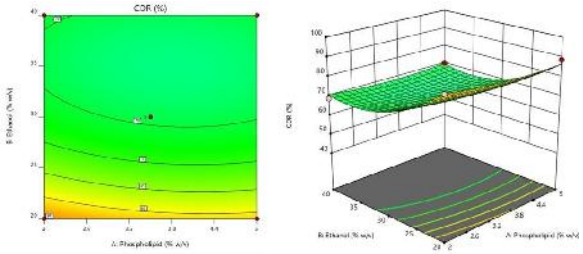

Fig 11: 3D Counter and RS Counter Diagrammatic Display of the Influence of Independent Factors on: CDR

Characterization of Ketoconazole Gel

The findings of the physical characterisationwerecompiledinonelocationbecauseof the importance of maintaining the physical integrity ofthe dosage form.

Size and shape analysis

Gel preparations were examined by microscope at several magnifications to see their vesicular structure, lamellarity, and size [10].

Scanning electron microscope (SEM)

Images taken at a high magnification (8–11inthefigures) revealed a sizer eduction. The size and form findings agree with the empirical evidence.

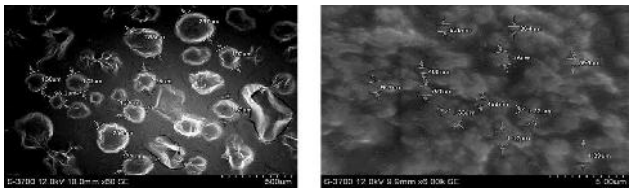

Figure-12 Scanning electron microscopy of ethosomal suspension and gel formul tion

Analysis of vesicle size revealed that ethanol concentration in fluencies vesicle size. Smaller vesicles were found at higher ethanol concentrations, with a maximum size of 5.483 m found in 20% ethanol and a minimum size of3.907 m found in 50% ethanol. The findings of this study agree with those of previous studies.

Entrapment Efficiency

After establishing that bilayer vesicles were present in the gel system, ultra- centrifugation was used to examine the vesicles' potential for drug entrapment. Ultra- centrifugation was

employed to separate the drug-containing gel vesicles from thefree drug and un-entrapped drug.

Table 5: Drug entrapment efficiency of Ketoconazole Gel

Formulation code	Entrapment efficiency(%)			MEAN
F1	72.19	71.75	71.82	71.92
F2	66.91	67.12	68.53	67.52
F3	60.05	60.00	60.01	60.02
F4	**79.91**	**79.62**	**79.33**	**79.62**
F5	58.01	55.96	54.96	56.31
F6	39.39	42.32	42.76	41.49

Ultracentrifugation results showed that the gel formulation containing 30% ethanol (EF4) achieved the highest entrapment efficiency (79.62%), nearly twice that of the 50% ethanol formulation (EF6). Entrapment efficiency increased with ethanol concentration up to 30% w/w but declined at higher levels due to increased vesicle membrane permeability. A phospholipid concentration of 3% was found to be optimal, as deviations reduced entrapment efficiency, supporting previous findings by Jain NK et al.

Particle size, PDI, and zeta potential were measured using a Malvern Zetasizer Nano ZS. Increasing the concentrations of phospholipon 90G and ethanolic neem extract led to a sudden increase in particle size and PDI, indicating that vesicle physicochemical properties can be modulated by the lipid-to-ethanol ratio. Smaller particle size and ethanol content enhance skin penetration. Formulation EF5 showed an optimal particle size (169.5 ± 4.6 nm) and PDI (0.407 ± 0.01), making it suitable for topical use. Its high negative zeta potential (−37.9 ± 1.0 mV) indicates good stability and resistance to aggregation.

Table-6: Organoleptic characters of Ketoconazole gel

Organoleptic Characteristics	Color: golden yellow Greasiness:Nongreasy Grittiness:Free fromgrittiness Ease of application: Easily/smoothly applied Skin irritation: No skin irritation
Washability	Easily washable without leaving any residue on the surface of the skin.
Spreadability	6.25cm/sec

In-Vitro Drug Permeation Studies

In-vitro diffusion studies were conducted to evaluate drug release from Ketoconazole gel formulations using a Franz diffusion cell. Drug penetration was measured spectroscopically, accounting for sampling losses, and the cumulative amount released was calculated per unit membrane area. Steady-state transdermal flux was determined from the slope of the linear portion of the cumulative release vs. time graph values with time .

Anti fungal studies

Antifungal activity was measured in vitro, and the zone of inhibition decreased from G6 to G0 to LG to HE gels (i.e., 18.53, 7.83, 12.4, and 10.73 mm, respectively). Because the G2 gel demonstrated the most inhibitory effect relative to the other formulations, it is possible that increased diffusion of vesicles carrying Ketoconazole through fungal cell walls is responsible for the G6 gel's increased in vitro antifungal activity. Students' t-test and analysis of variance were used to identify differences that met the criteria for statistical significance at the $P< 0.05$ level.

4. SUMMARY & CONCLUSION

Transdermal drug delivery systems (TDDS) offer advantages over traditional methods, such as steady blood levels, fewer side effects, and improved patient compliance, but skin's barrier limits drug penetration. Vesicular systems, like ethosomes, can enhance transdermal delivery by improving solubility and skin absorption.

This study developed ketoconazole (a BCS Class-II drug) ethosomes using statistical optimization (Box-Behnken design) and compared different preparation methods, selecting the TFH approach. The optimized ethosomes were incorporated into a carbopol gel. Characterization (FTIR, DSC, SEM) confirmed drug integration, vesicle size (<200 nm), homogeneity, and entrapment efficiency (~79%).

In vitro studies showed ethanol and sonication enhanced vesicle size reduction, drug release, and skin deposition, following non-Fickian kinetics. Ethosomal gels showed superior penetration and prolonged drug retention compared to standard gels, suggesting ketoconazole gel is an effective transdermal strategy.

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