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Original Article

Enhanced Antifungal Efficacy of Transferosomal Gel Containing Clotrimazole and Miconazole Nitrate: A Novel Approach for Topical Treatment by QbD

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ABSTRACT

Aim: This study focuses on developing and evaluating a transferosomal gel containing clotrimazole and miconazole nitrate, antifungal medications used to treat fungal infections, including superficial tinea and nail infections. **Materials and Methods:** The MI-CTM transferosomes were created using rotary film evaporation and optimized using the Box-Behnken statistical design. They were evaluated for drug concentration, viscosity, Spreadability, pH, and *in vitro* release kinetics after being embedded in a Carbopol 934 gel. **Results:** Particle sizes ranging from 145±0.604 nm to 760±0.684 nm and a zeta potential ranging from -2.8 to -41.8, the produced MIC transferosomes exhibited a high EE% ranging from 15.6±0.66%) to (80.25±1.85%). Transferosomes' surface morphology was assessed using a scanning electron microscope, and it was discovered that the vesicles had a spherical form. A 24 hr *in vitro* release study was conducted for the optimized formulation, showing improved drug release of 86.94% and 89.87% CDR for clotrimazole and miconazole nitrate, respectively. After conducting a kinetic release research, the formulation for miconazole nitrate and the clotrimazole medication followed the non-Fickian transport mechanism described by Peppas and first order kinetics, respectively. There was no noticeable deterioration of the medication based on the stability data, which included no appreciable changes in pH, drug content, or cumulative percentage drug release. **Conclusion:** Miconazole nitrate and clotrimazole in transferosomal gel, thus, increase medication application frequency while simultaneously enhancing patient compliance.

Keywords: Transferosomes, Transdermal drug delivery system, Antifungal, Drug release.

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INTRODUCTION

The most common form of skin disease worldwide is fungus-induced infections, with superficial infections ranking among the ten most common types. Fungus infections are currently one of the main causes of morbidity and death in immunocompromised individuals. Medical professionals face a great deal of difficulty in treating these infections since they are often found on the outer layers of the skin, hair, nails, and mucous membranes.[1] The skin's outermost layer of protection, the stratum corneum, keeps xenobiotics out, limits the permeability of transdermal drug delivery, which makes it less desirable than other routes of administration despite its potential advantages over other routes.[2] IDEA AG, a German firm, has registered the word "transferosome," which is derived from the Greek word "soma," which means "body," and the Latin word "transferre," which means "to transport across," as a trademark. The non-ionic surfactant monolayer vesicles (niosomes) or stiff lipid bilayers (liposomes) are the flexible, highly deformable, and stress-responsive lipid-based transferosomes.[3] The medicine Miconazole Nitrate (MI) and Clotrimazole (CTM) an imidazole-grouped wide-spectrum anti-fungal, is used to treat candidiasis. Because to MI and CTM has limited water solubility and extensive hepatic transformation, its systemic efficacy is minimal. MI and CTM work by suppressing ergosterol production, which causes the fungal cell membrane to burst,

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and by inhibiting peroxidase, which causes peroxide to build up inside the cell and cause cell death.[4]

The vesicular drug-carrier technology, transferosome, has recently been found to improve transdermal drug administration when applied to skin without occlusive contact. Being far more deformable than typical liposomes, transferosomes are artificial vesicles.[5] Formulation development has been remarkably successful when a systematic methodology and DOE are applied. While having complete control over the number of tests, DOE enables the formulation scientist to evaluate numerous components and their relationships.[6] A diverging kind of RSM design for statistical formulation optimization is the Box-Behnken Design (BBD). Because it requires fewer trials and takes less time, this design is significantly more efficient than the traditional methods of dosage form optimisation.[7]

The study's objective was to create a transferosomal gel that included miconazole nitrate and clotrimazole, the advantage of transferosomal gel containing clotrimazole and miconazole nitrate over conventional dosage forms lies in its enhanced permeability and prolonged retention at the site of application. This innovative formulation utilizes transferosomes, which are lipid-based vesicles that may efficiently transport the antifungal medicines into the skin's deeper layers by piercing the stratum corneum. By encapsulating clotrimazole and miconazole nitrate within transferosomes, the gel ensures targeted delivery, improved bioavailability, and prolonged therapeutic effects. Moreover, compared to traditional dosing forms like creams or ointments, the gel formulation provides superior patient compliance and simplicity of application.

MATERIALS AND METHODS

Materials

Miconazole nitrate and Clotrimazole were procured from Yarrow Chem. Products, Mumbai, India. soya lecithin was procured from techno scientific products, Bangalore. Carbopol-934, Span-80 and tween-80 were procured from S.D Fine Chem. Ltd, Mumbai, India. All Other reagents and chemicals employed were of analytical purity grade and procured from standard manufacturers.

Formulation of Transferosomes

The transferosomes were made using the conventional rotary flash evaporation procedure. Specifically, the drug, phospholipid, and surfactant were added to a dry, clean, round-bottom flask. The mixture of lipids was then dissolved in a 2:1 volume ratio solution of methanol to chloroform. Rotary evaporation was used to evaporate the organic solvent at 60°C under reduced pressure. Overnight, final solvent remnants were vacuumed out. After one hour of room temperature rotation at 60 rotations per minute, 6.8 pH buffer was used to hydrate the lipid film that had been formed (above the lipid transition effects). After the resultant vesicles were enlarged for 2 hr at room temperature, Large Multilamellar Vesicles (LMLV) was formed. To produce smaller vesicles, LMLVs were ultrasonically treated for 20 min at 40°C.[8]

Formulation of transferosomal gel

By employing the rotary flask evaporation method and 1% weight/weight of Carbopol as the gel base, miconazole nitrate and clotrimazole-loaded transferosomal gel was created. The transferosomal solution mentioned above was then mixed continuously with Carbopol, methyl paraben, and propyl paraben, which were dissolved in propylene glycol and utilized as preservatives in a 10:1 ratio, until a homogenous gel was produced.[9]

Formulation Optimization

The 3-factor, 3-level Box-Behnken design of Response Surface Methodology (RSM) was employed to determine the optimum concentration of the selected factors and their interaction in the ensuing desired particle size, Zeta potential and entrapment efficacy as mentioned in (Table 1).

Evaluation of transferosomes

Determination of Entrapment Efficiency (%EE)

The whole transferosomal suspensions were ultracentrifuged for 30 min at 10°C and 20,000 rpm.

Following centrifugation, 50 mL of 6.8 pH buffer were added to dilute 1 mL of supernatant, and the absorbance of clotrimazole and miconazole nitrate was then determined at 232 nm and 263 nm, respectively, using a UV-vis spectrophotometer.[10]

The drug entrapment efficiency was calculated using the following formula:

$$EE\% = [\text{Amount of entrapped MI / Total amount of MI}]\ 100.$$

Determination of Particle Size and Zeta Potential

For each produced TFS Transferosome, the Dynamic Light Scattering (DLS) technique was used at 25°C with a Malvern Zetasizer (Malvern, UK) to quantify the particle size, zeta potential, and polydispersity index. The TFS-loaded transferosomal colloidal dispersion was diluted with phosphate buffer (purified water) prior to measurements.[11]

Table 1: The complete work plan for optimization for the preparation of transferosomes containing miconazole nitrate and clotrimazole drugs by using Box Behnken design.

Independent Variables	Dependent Variables
Soya Lecithin	Entrapment Efficiency (%)
Span-80	Particle Size (nm)
Tween-80	Zeta Potential (mV)

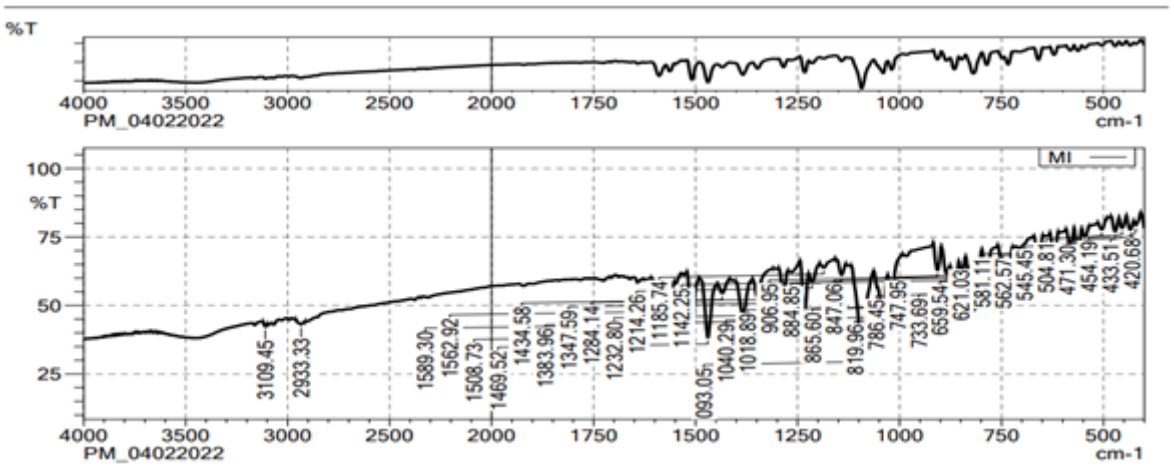

Figure 1: Infrared Spectrum of Miconazole Nitrate.

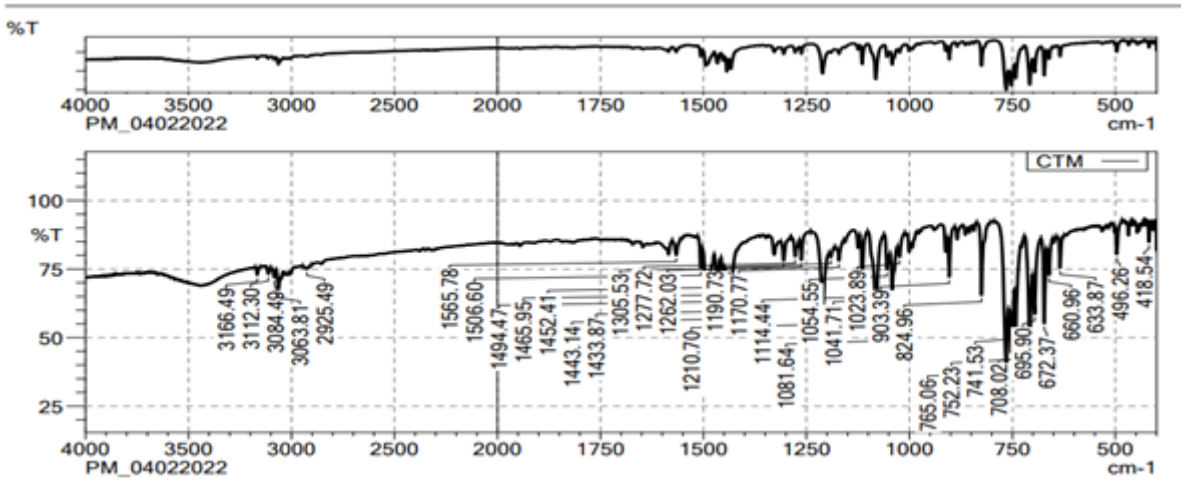

Figure 2: Infrared Spectrum of Clotrimazole.

Photo-Microscopic Analysis and Scanning Electron Microscope

The vesicles were examined using an optical microscope, and images of them at a 10x magnification were taken with a camera attachment. To ascertain the morphology of the optimized recipe, SEM examination was employed. To enable the transferosomes to stick to the collodion and dry, one drop of an optimized transferosomal solution was placed on a copper grid covered with collodion and left there for around 2 min. Then, a drop of uranyl acetate solution was added, and a grid was allowed to sit for a minute. A SEM analysis was carried out after the material had dried.[12]

Characterization of miconazole nitrate and clotrimazole loaded transferosomal gel

Homogeneity

For patients to comply, it is essential to ascertain the homogeneity of semisolid dosage forms given topically to the skin. To do this, a little quantity of transferosomal gel filled with MI-CTM was squeezed between the thumb and index finger. The consistency's homogeneity was evaluated.[13]

Determination of pH

A calibrated pH meter was used to measure the pH of the transferosomal gel formulation. Three average samples were used to obtain the readings.[13]

Spreadability

By pressing 1 g of gel between two clear, spherical glass slides, the Spreadability of each batch of transferosomal gel was evaluated. They were allowed to spread out for a maximum of 5 min. To determine the Spreadability, the diameter of the produced circle was measured.[14]

Drug Content Estimation

To a beaker of precisely weighed topical transferosomal gel equivalent to 100 mg, 20 mL of phosphate buffer was added. After

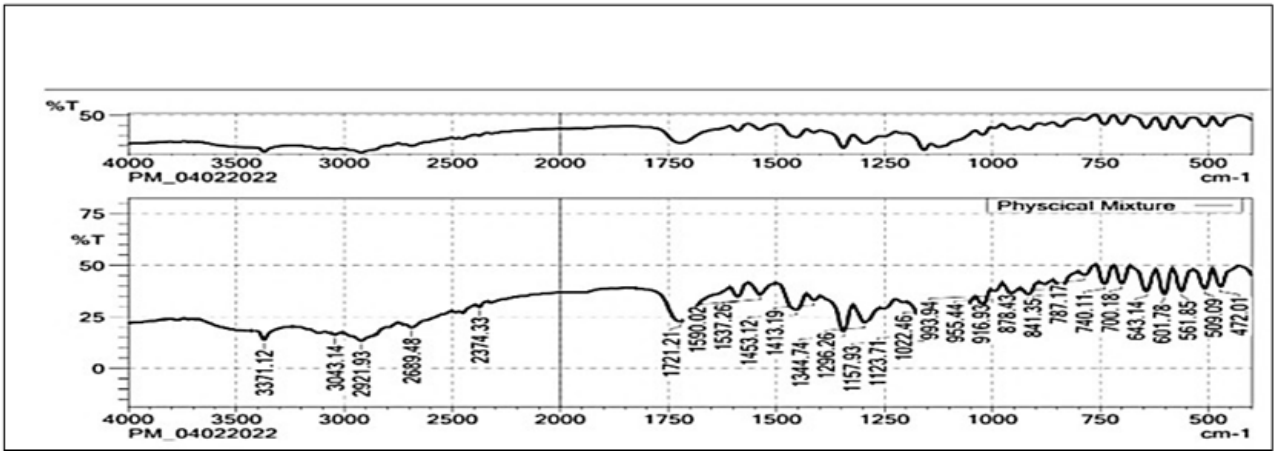

Figure 3: Infrared Spectrum of Physical Mixture

thoroughly mixing the material, Whatman filter paper no. 1 was employed to filter it. Then, using phosphate buffer, 1.0 mL of the filtered solution was added to a volumetric flask with a 10 mL capacity. This solution was examined using a UV-Spectroscope at maximum wavelengths of 232 nm and 263 nm for miconazole nitrate and clotrimazole, respectively, and the proportion of drug content may be calculated using the formula below.[14]

[% drug content= practical drug content/theoretical drug content ×100]

Viscosity and rheological studies

Using a Brookfield viscometer with spindle number 4 spinning at a speed of 5 to 25 rpm at 25°C, the viscosity of MI-CTM loaded transferosomal gels was determined.[15]

In vitro drug release studies

Diffusion study was conducted in vitro using Franz diffusion cells and a dialysis membrane. 20 mL of 6.8 pH PBS and 1 g of transferosomal gel loaded with MI-CTM were placed in the donor and recipient compartments, respectively. Two millilitre aliquots of samples were extracted from the sampling port at predetermined intervals. The removed samples were replaced with an equivalent amount of fresh dissolving media. The study was conducted in triplicate, and a UV spectrophotometer was used to quantify the means and standard deviations. Miconazole nitrate and clotrimazole were also checked in the samples, respectively, at 232 nm and 263 nm. A 24 hr release study was conducted. Time and the total amount of medication released were plotted.[16]

Drug Release Kinetic Studies

Drug release kinetics may be examined using a variety of mathematical models that account for drug release levels over a range of 0 to 24 hr. The best mathematical models to describe the dissolution profiles were found using these approximations. The following plots were made: First-order kinetic model: cumulative% drug remaining vs time; Higuchi model: cumulative% drug release versus square root of time; Korsmeyer-Peppas model: cumulative% drug release versus log time; Zero-order kinetic model: cumulative% drug release versus time.[17-19]

Short term Stability Studies

A pharmaceutical product's stability may be summed up as its capacity to maintain its physical, chemical, therapeutic, and toxicological properties over the course of its shelf life in a given formulation and container. The duration of research and storage requirements are specified by ICH.

Accelerated Testing: 40°C±2°C/75% RH±5% for 6 months

The best formulations of MI-CTM loaded transferosomal gel were kept in screw-capped, little glass bottles with an amber tint and submitted to accelerated stability testing for a brief period of time in accordance with ICH requirements. For duration of three months, drug content, pH, and *in vitro* drug release analyses were conducted on the samples.

RESULTS

Drug excipients compatibility studies

Using IR spectroscopy, the drug-polymer interactions were investigated. From this study, it was found that characteristic peaks of miconazole nitrate and clotrimazole drug showed below. For pure drugs, drug polymer mixes, and drug excipient mixtures, FTIR analyses were conducted. Spectrum (Figures 1-3) of the drugs and excipients exhibited the notable peaks with respect to functional groups. Both the drug with polymer and the drug with excipients exhibit a physical mixture spectrum that indicates no significant interaction between the drug, polymer, and excipients. In the drug polymer combination's spectra, the distinctive drug peak remained unchanged.[20]

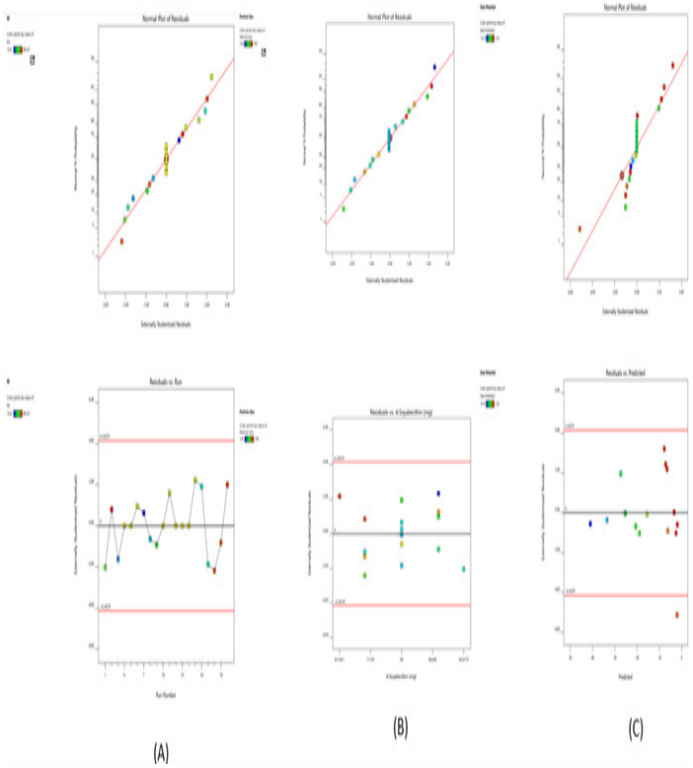

Figure 4: Normal probability and model residuals versus test orders for (A) EE, (B) PS and (C) ZP.

EVALUATION OF TRANSFEROSOMES

Preparation of transferosomes containing miconazole nitrate and clotrimazole

Box-Behnken design was possible to examine how several factors interacted to determine the zeta potential, minimum particle size, and maximum Entrapment Efficiency (EE%). (Table 2) lists the observed responses from a total of 20 predicted experimental trials. The percentage of drug entrapped in the experimental formulations ranged from 15.6% to 80.25%, while the particle size was found to be between 145 nm and 724.3 nm, and the zeta potential was found to be between -2.8 Mv and -41.8 Mv. To examine all of the experiment findings for the individual responses, the Fx model and ANOVA were employed.

For EE, there is less than a 0.2 difference between the Adjusted R2 of 0.9518 and the Predicted R2 of 0.8062. Appropriate Precision measures the signal-to-noise ratio. More over four is the ideal ratio. A strong signal may be seen in the ratio of 22.927. This method makes navigating the design area easier. The PS and ZP showed similar findings (0.8216 predicted and 0.9561 adjusted) and (0.8192 predicted and 0.9550 adjusted), respectively; nevertheless, the ZP ratio of 21.821 indicates an adequate signal (Table 3).

The selected responses were indicated by a strong correlation between the experimental and anticipated values. Remaining values are guaranteed to be under regular dispersion, or straight linearity by the points, thanks to the probability distribution (Figure 4). The visual plot analysis is suitable, but the conventional statistical procedures are not relevant. Moreover, a general residual plot was utilized to evaluate and ensure the accuracy of the adjusted model (external studentized residuals vs. typical probability percent).

To investigate the intervention of quantitative impacts of the fact factors, an ANOVA was used. Multiple regression analysis was performed on the data to produce polynomial equations. The following are the equations that were derived from the potential optimal model's output:

Equations generated for coded factors,

EE: +64.60-2.97*A+7.43*B+3.26*C -2.17*AB -8.11*AC-19.56*BC-10.07*A^2 +0.9154*B^2-3.79*C^2

PS: 270.044+-91.3177*A+-30.0142*B+-96.1982*C+10.675*AB+-41.55*AC+-124.45*BC+103.463*A^2 + 31.4086*B^2 + 70.9535*C^2

ZP: -25.2414+-3.99563*A+-10.2106*B+1.56698*C+-2.6*AB+0.675*AC+0.825*BC+4.01845*A^2+0.535954*B^2 + 7.11205*C^2

It is possible to anticipate the reaction for certain amounts of each element by using the equation expressed in terms of coded factors. The factors are coded with a +1 for high levels and a -1 for low levels. By comparing the factor coefficients, the coded equation may be used to determine the relative effect of the factors.

(Table 4) displayed the ANOVA coefficients and corresponding p-values for three responses. The obtained outcomes were used to calculate the model coefficients' significance. Moreover, RSM examined and assessed how each individual parameter affected the replies in Figure 5.

It is possible to optimize a number of series of models developed from experimental research by using the desire function [D]. A formulation consisting of 85.2059 mg of soy lecithin, 30 mg of Span-80, and 23.3146 mg of Tween-80 may satisfy the criteria of the ideal formulation when utilizing this technique to desirability. Consequently, using these ideal concentrations might lead to an EE of 78.5125 percent, a particle size of 145 nm, and a zeta potential of -33.5546 mV. An enhanced formulation of transferosomes including Miconazole Nitrate and Clotrimazole was created and evaluated using these predicted ideal concentrations as mentioned in (Figure 6).

The relative error of the prepared formulation values Entrapment efficiency, Particle size, Zeta potential to predicted values of the same by DOE software were mentioned in (Table 5) and the practical results were mentioned in the (Figure 7).

Table 2: Optimized Design Formulation.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A:Soyalecithin	B:Span-80	C:Tween-80	EE	Particle Size	Zeta Potential
		mg	Mg	mg	%	nm	mV
6	1	90	0	30	54	370.8	-3.3
4	2	90	30	0	80.25	654	-34
2	3	90	0	0	25.2	438.5	-7.5
19	4	80	15	15	64.6	268	-25.3
20	5	80	15	15	64.6	268	-25.3
14	6	80	15	40.2269	61.8	325	-4.1
1	7	70	0	0	15.6	495	-6
8	8	90	30	30	28	145	-24.8
13	9	80	15	-10.2269	46	618	-4.1
15	10	80	15	15	64.6	280	-25.3
7	11	70	30	30	59.5	325	-15.6
17	12	80	15	15	64.6	268	-25.3
16	13	80	15	15	64.6	268	-25.3
18	14	80	15	15	64.6	268	-25.3
11	15	80	-10.2269	15	59.47	450	-3.6
10	16	96.8179	15	15	35.44	366.9	-21.7
9	17	63.1821	15	15	36.85	760	-4
12	18	80	40.2269	15	74.97	269.3	-41.8
5	19	70	0	30	74	650	-2.8
3	20	70	30	0	76.5	724.3	-20.4

Table 3: Model statistical summary.

Response	Models	R^2	Adju.R^2	Pred.R^2	Adequate Precision	Sequential *p*-value	Remarks
EE%	Quadratic	0.9746	0.9518	0.8062	22.927	<0.0001	Suggested
PS	Quadratic	0.9769	0.9561	0.8216	22.972	<0.0001	Suggested
ZP	Quadratic	0.9763	0.9550	0.8192	21.821	<0.0001	Suggested

Microscopic Observation of Prepared Transferosomes

Using an optical microscope equipped with a camera attachment, the created transferosome formulation's shape was examined (Figure 8).

Surface Morphology of Optimized Transferosomes

To support the vesicular properties, optical and scanning electron microscopy were used to evaluate the shape of the transferosome vesicles. The transferosomes loaded with MI and CTM, which were made from soy lecithin, span-80, and tween-80, were easily recognized as spherical vesicles with a consistent size distribution as (Figure 9).

Evaluation of Optimized Transferosomal gel

The prepared optimized transferosomal gel were subjected to various analysis like appearance as (Figure 10), Homogeneity, pH and Spreadability results were mentioned in (Table 6).

Viscosity and rheological studies

The viscosity and rheological characteristics of the MI-CTM loaded topical gel were measured using a Brookfield digital viscometer with spindle no. 4. Consistency in a gel system is determined by the ratio of the solid to liquid fraction, which creates the structure. The topical gel loaded with MI-CTM was found to have a viscosity within the range of 16640 cps at 5 rpm. (Table 7) tabulates the obtained viscosities. The formulations' rheological investigations were examined by graphing the shear rate V/s viscosity, as shown in (Figure 11).

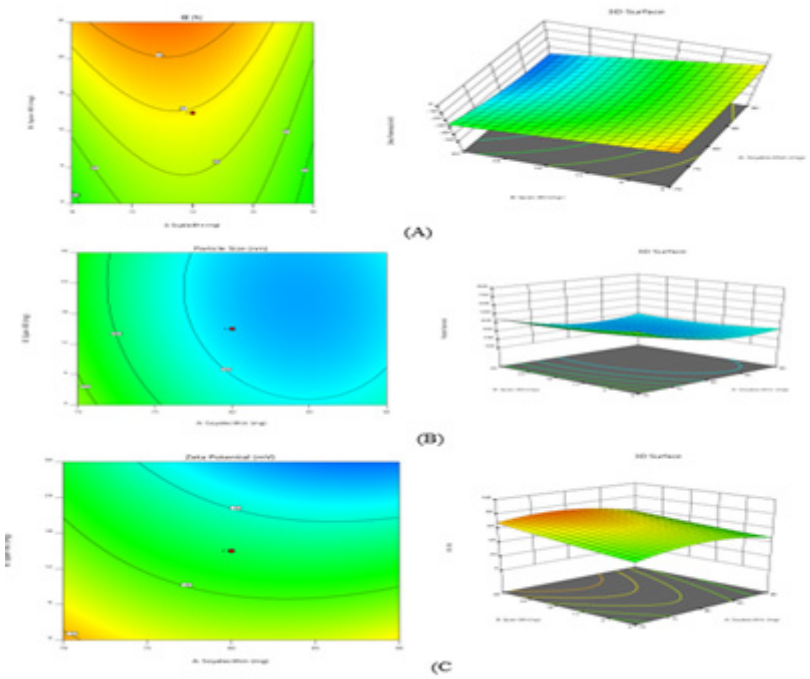

Figure 5: Contour plots and 3-D Response surface plots for (A) EE, (B) PS and (C) ZP.

Table 4: ANOVA coefficients table for both the responses.

	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
EE	64.60	-2.97	7.43	3.26	-2.17	-8.11	-19.56	-10.07	0.9154	3.79
p-value	< 0.0001	0.0219	< 0.0001	0.0139	0.1605	0.0002	< 0.0001	< 0.0001	0.4105	0.0052
PS	270.044	-91.3177	-30.0142	-96.1982	10.675	-41.55	-124.45	103.463	31.4086	70.9535
p-value	< 0.0001	< 0.0001	0.0148	0.0148	0.4424	0.0110	< 0.0001	< 0.0001	0.0102	< 0.0001
ZP	-25.2414	-3.99563	-10.2106	1.56698	-2.6	0.675	0.825	4.01845	0.535954	7.11205
p-value	< 0.0001	0.0002	< 0.0001	0.0449	0.0156	0.4674	0.3776	0.0001	0.4395	< 0.0001

Drug content estimation

The UV Spectrophotometer was used to estimate the drug content. The optimized formulation's drug content was determined to be 96.45% w/w for miconazole nitrate and 94.85% w/w for clotrimazole, respectively. This indicates a satisfactory level of content uniformity.

In vitro drug release studies

For duration of 30 min to 24 hr, an *in vitro* drug release research was conducted to examine the drug releases from the MI-CTM loaded transferosomal gel carrying topical gel. Table 8 displays the in vitro release characteristics of the topical gel including transferosomal gel loaded with MI-CTM. It was discovered that the improved formulation had the greatest order of *in vitro* drug release data. The total percentage of drug release during a 24 hr period was recorded. The drug release data for the topical gel medication loaded with MI-CTM that was manufactured and delivered at various time intervals is displayed in Table 8. Miconazole nitrate and clotrimazole were reported to release their drugs 86.94% and 89.87%, respectively, from MI-CTM loaded transferosomes containing topical gel in a 24-hr period. The in vitro drug release study's outcome is displayed in (Figure 12).

Drug release kinetics

Drug release kinetics may be examined using a variety of mathematical models that account for drug release levels over a range of 0 to 24 hr and the results were mentioned in (Table 9) and drug release kinetic studies of different models of Miconazole nitrate and Clotrimazole based optimized formulation were displayed in Figures 13 and 14 respectively.

Stability Studies

For three months, the stability investigations were conducted at 4°C±2°C and 25°C±2°C/60% RH. Transferosomal gels loaded with MI-CTM were analysed for pH, drug content, and in vitro

Table 5: Percentage (%) Error of Response Parameter for Selected Optimized Formulation.

Parameter	DOE Software Results	Obtained Practical Results	Error Percentage (±) %
EE (%)	70.1681	68.8	1.3
Particle Size (nm)	247.42	233.5	13.92
Zeta Potential (mV)	-36.2022	-29.2	7

Table 6: Evaluation of Optimized Formulation of MI-CTM loaded topical gel.

Formulation	Appearance	Homogeneity	pH	Spreadability g.cm/sec
Optimized formulation of MI-CTM loaded topical gel.	Transparent	Smooth	6.99±0.40	5.43-7.3±0.460

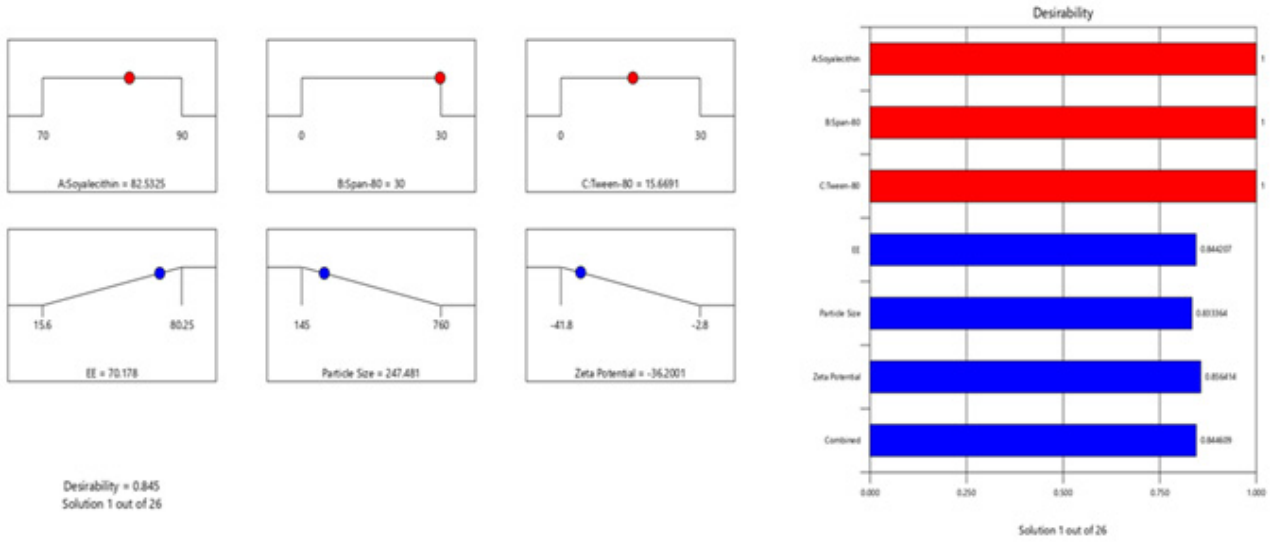

Figure 6: Desirability graphs- Ramp and Bar Graphs.

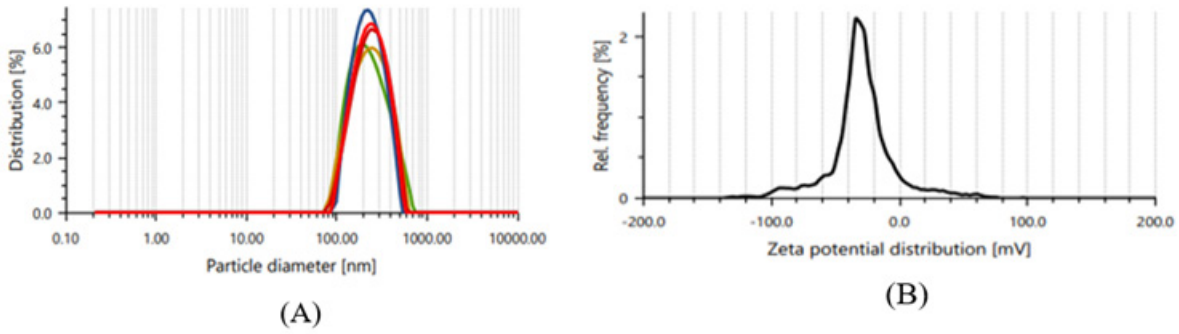

Figure 7: Obtained practical result of optimized formulation.(A) Particle Size and(B) Zeta Potential.

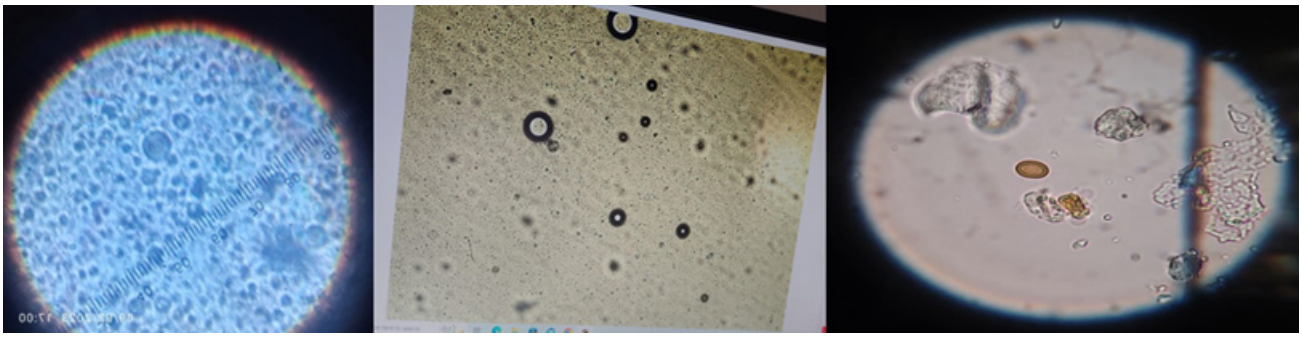

Figure 8: Microscopic Observation of Prepared Transferosomes.

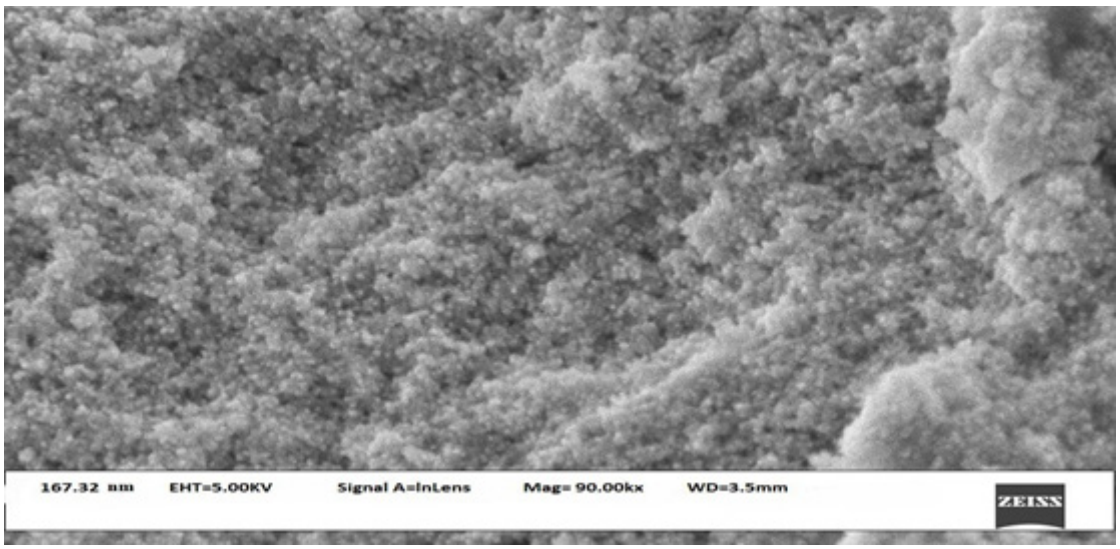

Figure 9: SEM of Optimized Transferosomal Gel.

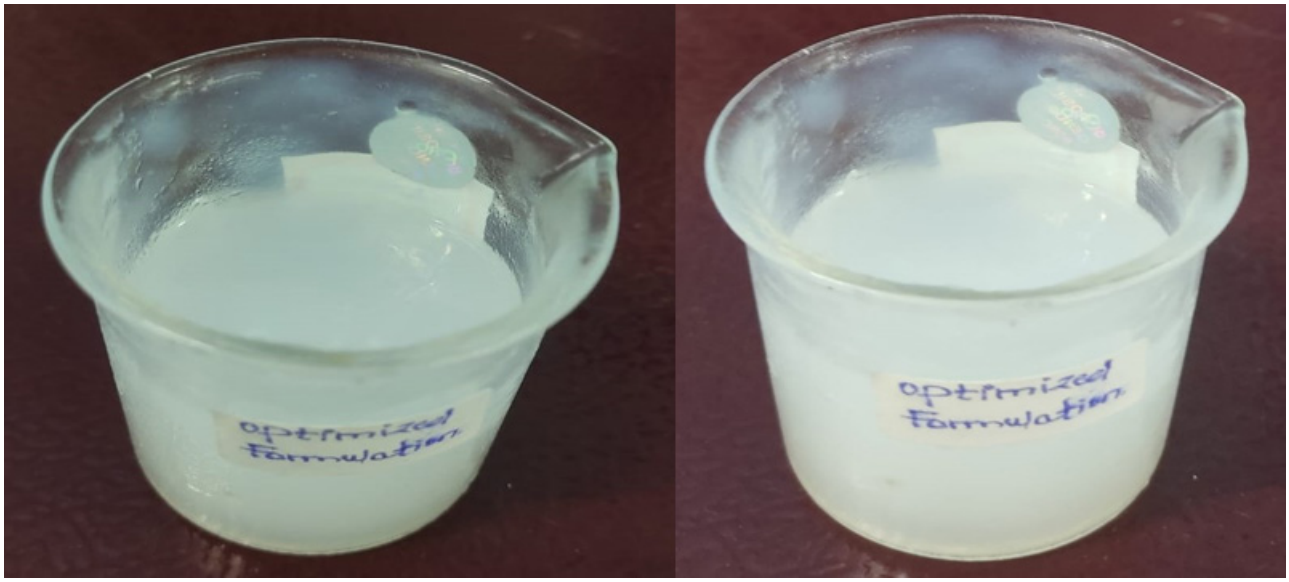

Figure10: Optimized Gel.

release investigations. The stability study findings are listed in (Table 10).

The pH, drug content, and *in vitro* release characteristics of the transferosomal gels did not significantly alter, according to the stability study results. For the duration of the investigation, the generated transferosomal gel formulation remained physiochemically stable.

DISCUSSION

The study used FTIR and IR spectroscopy to examine drug-polymer interactions. The distinctive peaks of the drugs clotrimazole and miconazole nitrate were discovered. The maximum Entrapment Efficiency (EE%), minimum particle size, and zeta potential were ascertained using the Box-Behnken design. The experimental formulations had a range of drug entrapment percentages (15.6% to 80.25%) and particle sizes (145 nm to 724.3 nm). To investigate the form of the transferosome formulation, optical and scanning electron microscopy were employed. It was determined that

Table 7: Viscosity of optimized formulation MI-CTM Loaded topical gel.

Shear Rate (RPM)	Viscosity in cps
	optimized formulation MI-CTM loaded transferosomal gel
5	16640
10	9320
15	7546
20	5260
25	3589

Table 8: *In vitro* drug release data of prepared transferosomal gel.

Time interval (min)	%Cumulative % drug release of MI-CTM loaded optimized transferosomal gel	
	MI	CTM
0	0	0
30	6.140	6.45
60	11.20	14.55
120	20.55	22.40
240	35.97	33.16
360	40.33	43.29
480	50.47	48.60
600	60.38	56.64
1080	78.51	70.50
1440	86.94	89.87

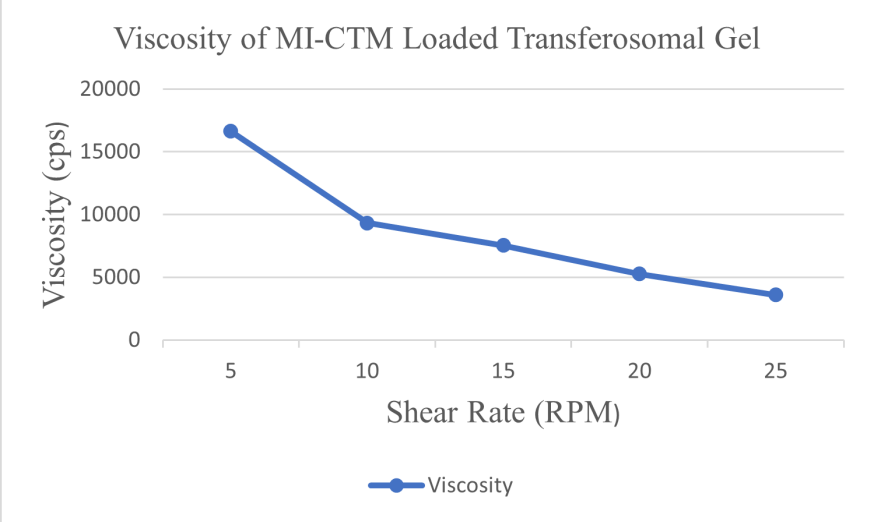

Figure 11: Rheological Profile of MI-CTM Loaded Transferosomal Gel.

Table 9: Regression Values of Kinetic Release Studies of Miconazole Nitrate and Clotrimazole.

Formulation	Zero- order kinetics	First order kinetics	Higuchi Plot	Peppas Model		Kinetic Model	Release Mechanism
	R^2	R^2	R^2	R^2	n		
Optimized formulation of Miconazole Nitrate	0.9097	0.9973	0.99	0.9915	0.6429	First order kinetics	Non-Fickian Transport
Optimized formulation of Clotrimazole	0.9221	0.9595	0.9892	0.9925	0.6288	Peppas	Non-Fickian Transport

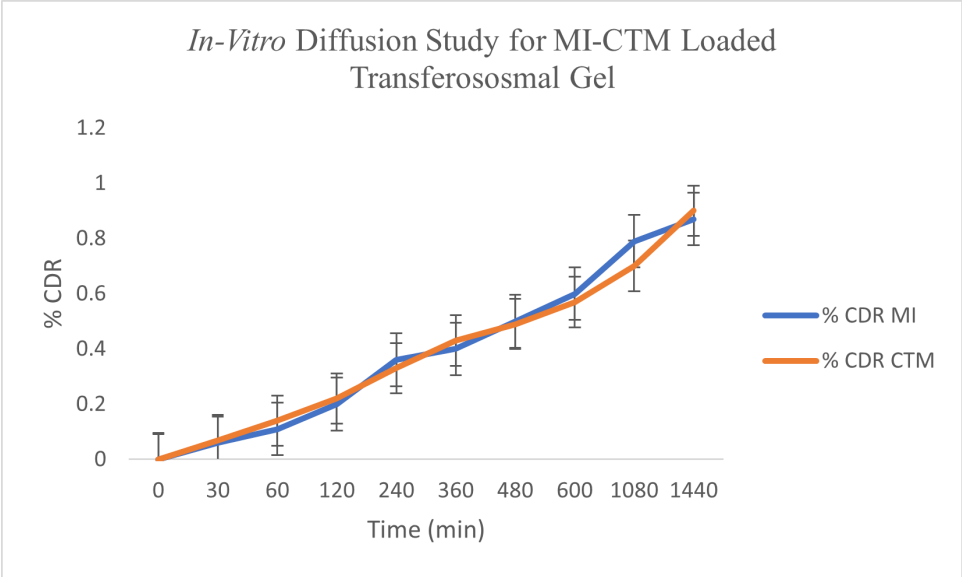

Figure 12: *In vitro* drug release profile of optimized formulation.

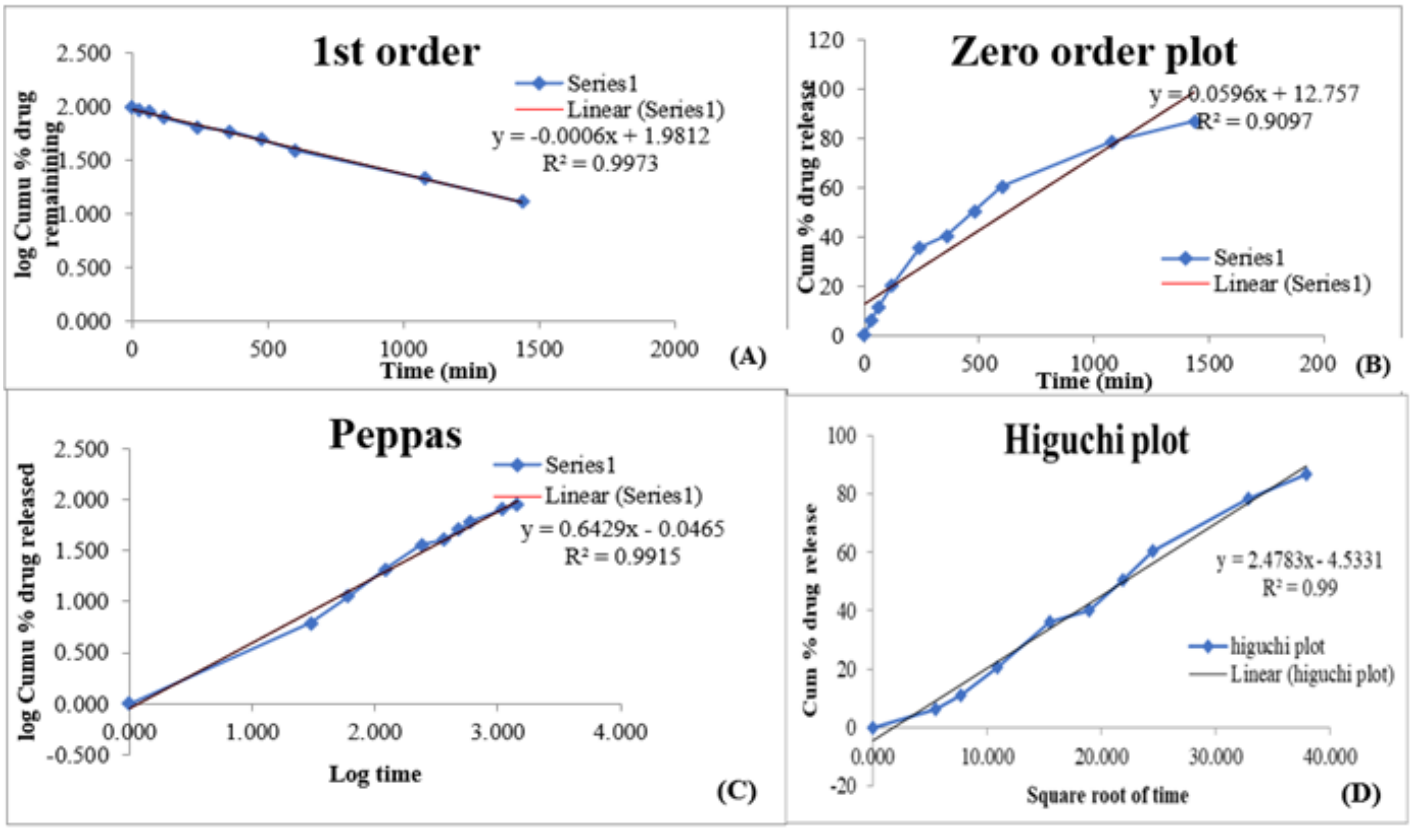

Figure 13: Optimized formulation(A)First order release, (B)Zero order release, (C)Peppas model, (D)Higuchi model of Miconazole Nitrate.

the transferosomes containing CTM and MI were spherical vesicles with a regular size distribution. A number of analyses were performed on the improved transferosomal gel, including appearance, homogeneity, pH, and Spreadability measurements. A Brookfield digital viscometer was used to measure the topical gel loaded with MI-CTM's viscosity and rheological properties. The drug content for miconazole nitrate and clotrimazole in the optimized formulation was found to be 96.45% w/w and 94.85% w/w, respectively, showing good content uniformity. An analysis of 30 min to 24 hr of *in vitro* drug release study showed that the enhanced formulation had the highest order of *in vitro* drug release data. Mathematical models were used to analyze the drug release kinetics, and three months of stability studies were carried out at 4°C±2°C and 25°C±2°C/60% RH. Over the

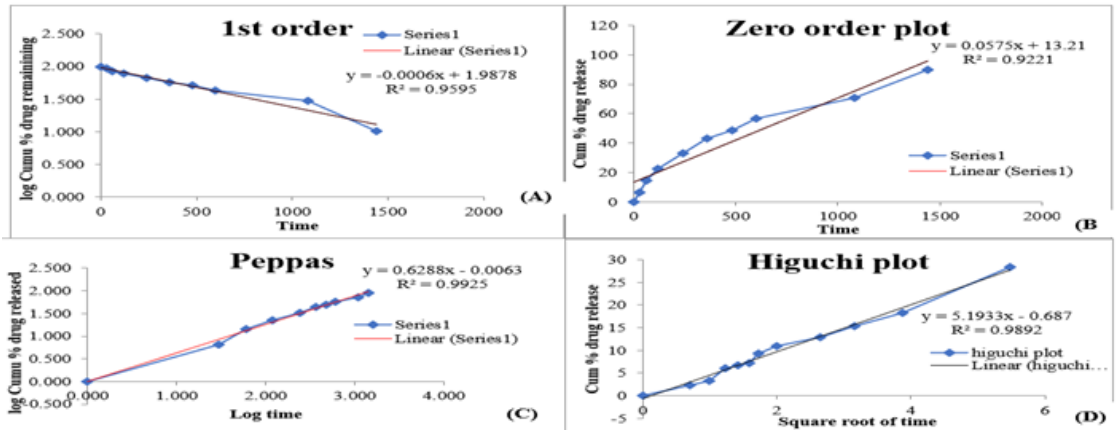

Figure 14: Optimized formulation(A)First order release, (B)Zero order release, (C)Peppas model, (D)Higuchi model of Clotrimazole.

Table 10: Stability studies of Optimized Transferosomal Gel.

Months	4°C±2°C					25°C/60% RH				
	pH	Drug content (%)		% CDR		pH	Drug content (%)		% CDR	
		MI	CTM	MI	CTM		MI	CTM	MI	CTM
Initial	6.99±0.4	96.45±0.5	94.85±0.2	86.9±0.1	89.8±0.1	6.99±0.4	96.45±0.5	94.85±0.2	86.9±0.2	89.9±0.1
1	6.96±0.2	96.43±0.5	94.83±0.6	86.6±0.1	89.8±0.1	6.96±0.2	96.43±0.5	94.83±0.6	86.1±0.1	89.6±0.2
2	6.95±0.4	96.30±0.6	94.85±0.5	86.5±0.1	89.8±0.1	6.95±0.4	96.30±0.6	94.85±0.5	86.1±0.3	89.3±0.3
3	6.92±0.2	96.28±0.3	94.82±0.4	86.2±0.1	89.8±0.1	6.92±0.2	96.28±0.3	94.82±0.4	86.2±0.2	89.1±0.2

course of the study, the created transferosomal gel formulation remained physiochemically stable, as evidenced by the pH, drug content, and *in vitro* release properties of the gels not changing considerably.

CONCLUSION

The research aims to produce topical transferosomal gel of miconazole nitrate and clotrimazole using a rotary film evaporation technique with a variable phospholipid: span 80: tween 80 ratio. The results show satisfactory drug solubility, melting point, and compatibility with excipients. Entrapment efficiency depends on lipid concentration and particle sizes are below 724 nm. The rheological study shows non-Newtonian flow, and the formulations sustained drug release for extended periods. The drug release kinetics showed first order release kinetics and peppas model for miconazole nitrate and clotrimazole respectively and follows non-Fickian transport mechanism for the both drugs. The formulations were stable for 3-month stability studies at specific temperature and relative humidity, indicating no drug degradation during the study period. This transferosomal gel formulation can be used in future for treatment of skin fungal infections with improved patients' compliance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MI-CTM: Miconazole nitrate and Clotrimazole; **PS:** Particle size; **CDR:** Cumulative drug release; **MI:** Miconazole nitrate; **DOE:** Design of experiment; **CTM:** Clotrimazole; **LMLV: %:** Percentage; **nm:** Nano meter; **mv:** Millivolts; **EE:** Entrapment efficiency; **SEM:** Scanning electron; **MI:** Microscopy; **PBS:** Phosphate buffer solution; **ICH:** International council of harmonization;

RH: Relative humidity; **FTIR:** Fourier transferomer infrared spectroscopy; **ZP:** Zeta potential.

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