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Design, Formulation and Evaluation of Clotrimazole Loaded Transethosomal Gel for the Treatment of Candidiasis

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ABSTRACT: The purpose of the present study was to design develop and characterize the clotrimazole loaded transethosomal gel formulation for antifungal activity. The transethosomes were prepared by cold method. A 2^3 factorial design was implemented in this research. Three independent variables: Type of surfactant (X1), Soya lecithin concentration (X2), Ethanol concentration (X3) in the design while two responses: Entrapment efficiency (Y1), In vitro drug release (Y2). Total 8 formulations were prepared using different concentrations of phospholipid (Soya lecithin) and edge activator (Span 80 and tween 80). The developed transethosomes were characterized for FTIR, drug content, EE, particle size, zeta potential, SEM, in vitro drug release and release kinetics. The zeta potential of Optimized formulation was -27.1 mV, the particle size was at the range of 28.18 nm and Polydispersity index was 0.236 nm. The transethosomal formulation was incorporated into the Carbopol gel base to get transethosomal gel. The formulated gel was estimated for viscosity, drug content, EE, pH, Spreadability and stability study for 30 days. Span 80 formulations exhibit a faster drug release and entrapment efficiency than tween 80 formulations. Optimized gel of In vitro release exhibits an 89.85 % at the end of 8th hr. Optimised formulation follows zero order kinetics and Higuchi kinetics its R^2 value is 0.8922. The viscosity of the gel formulation was 3528 cps. The drug content and EE was found to be 98.45 % and 87.65 % respectively. Stability studies prove that there is very less change in the EE, hence the formulation was found to be stable.

KEYWORDS: Transethosomes, clotrimazole, Higuchi kinetics,Polydispersity index

I. INTRODUCTION

The oral route is frequently chosen for drug delivery because of its ease of administration. Still, these formulations pose significant drawbacks like gastrointestinal discomfort, unpleasant taste, and decreased bioavailability due to first-pass metabolism. An alternate strategy, continuous intravenous injection, is regarded as a high-dose drug management approach for avoiding hepatic 'first pass' metabolism and maintaining a long-lasting, consistent drug level. However, this necessitates hospitalization of patients and careful monitoring under medical help. Hence, the health sector currently reaps significant benefits from transdermal drug delivery technologies that reduce plasma drug level fluctuations for repeated treatment, avoid organ toxicity, and early metabolism, limit dose-based side effects, and prevent gastrointestinal discomfort and poor bioavailability. It also provides benefits such as controlled drug delivery, lower doses, and improved patient compliance. Topical drug delivery of active pharmaceutical carriers, which a drug is delivered in the targeted area under controlled system can have a significant effect on efficacy. The main advantage of the topical drug delivery is to bypass first pass metabolism. Topical drug carriers are used for the localized effect of application and deliver the drug into underlying percutaneous layers of the skin. The polar drug molecules are encapsulated into the hydrophobic channel carrier which is prevented from hydrolysis and enzymatic degradation. The ethosomal carriers are vesicular systems that comprise phospholipids, water and a moderately increased concentration of ethanol. Ethosomal systems are of three types namely classical ethosomes, binary ethosomes and transethosomes (TEs). These are of novel generation and developed in 2012. They usually comprises of ethosomes and an edge activator or a penetration enhancer. The transethosomes improves the physical and chemical qualities of the therapeutic drug enclosed in dermal and transdermal delivery. Topical treatment of fungal infections has various advantages, including the ability to target the infection site, reduced risk of systemic adverse effects, improved treatment efficacy, and high patient compliance. A range of topical antifungal agents have been employed in treatment of various dermatological infections.

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These antifungal medicines are currently accessible in creams, gels, lotions, and sprays in traditional dose forms. The effectiveness of topical antifungal treatment is determined on drug penetration through the target tissue.

When antifungals are applied topically, the drug components must penetrate through the stratum corneum; the skin's outermost layer, to reach the lower layers, notably the viable epidermis. New carrier systems for licensed and investigational medications are being developed as alternate techniques for topical treatment of fungal infections of the skin. Antifungal chemicals can be delivered to the skin more effectively using carriers such as colloidal systems and vesicular carriers. The conventional liposomes show the drawback of less permeation into the deeper region of skin and they accumulate at the outer layer of stratum corneum. Transfersomes and the liposomes having the addition of edge activator like span 60, Span 80, Span 25, tween 80 and sodium cholate. Transfersomes improve the skin deposition of many drugs. But they can't reach the stratum corneum deep enough. The Transethosome show the presence of high amount of ethanol with edge activator. The novel lipid vesicles is also known as the deformable or elastic liposomes. They have the great ability to intact the skin and deliver the drug into ultradeformable vesicles (UDV) were developed in the beginning of the 1990's. The UDV are more deformable than the conventional the epidermis and dermis layers or even to the systemic circulation. Transethosomes are lipid vesicles which are made up of transfersomes and ethosomes. It was first introduced by Song et al in 2012 where he characterized the high content of ethanol (up to 30%). Transethosomes have the irregular spherical shape and higher values in both vesicles elasticity and skin permeation studies. This cause due to the rearrangement of lipid bilayer in combination of ethanol and edge activator. **(10 Bold)**

II. MATERIALS AND METHODS

MATERIALS:

Clotrimazole was obtained as a gift sample from Sai Mirra Innopharm pvt.Ltd., India. Soya Lecithin was obtained as a gift sample from S.J. Pharma Limited, Chennai. Ethanol was purchased from Changshu Hongshen Fine chemicals, India. Tween 80 was purchased from Oxford fine chem Lab, India, Span 80 was purchased from NR chem Lab, India.

METHODS

I. PREFORMULATION STUDIES

Preformulation testing is an investigation of physical and chemical properties of a drug substance and combined with excipients. It is the first step in the rational development of dosage forms.

1. DRUG-EXCIPIENT INTERACTION STUDIES

Physical Compatability Study:

100 mg of each Clotrimazole, Tween 80, Span 80, Soya Lecithin were weighed. Individual Clotrimazole, Tween 80, Span 80, Soya Lecithin along with admixture of drug and excipients in airtight screw cap amber coloured vials were kept at room temperature as well as 40 ºC±2 ºC / 75% ± 5% RH for 30 days. Change in colour was observed after 10, 20, 30 days.

2. Chemical Compatability Study:

The possibility of drug-excipient (soya lecithin, surfactant) interactions were investigated by FT-IR spectrum study. The FT-IR spectrum of pure drug and combination of drug with excipient were recorded using Shimadzu FT-IR Spectrophotometer. The spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. The IR Spectra of the test sample were obtained by Pressed Pellet Technique using potassium bromide.

3. DETERMINATION OF MELTING POINT

For determination of melting point USP method was followed. Small quantity of drug was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which drug started to melt and the temperature when the entire drug gets melted was noted.

4. DETERMINATION OF λ_{max}

10 mg of drug was weighed accurately and transferred to 10 ml of volumetric flask. Then Methanol was added to dissolve the drug completely. The volume was made up to 10 ml with solvent. The prepared sample was 1000 µ g/ml. 1 ml of above solution was then transferred to another 10 ml volumetric flask and diluted it upto the mark with solvent. This sample was 100 µ g/ml. 1 ml of above solution was then transferred to another 10 ml volumetric flask and diluted it up to the mark with solvent. This sample was 10 µ g/ml.

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clotrimazole solution (10 µ g/ml) was scanned in the uv range of 200-400 nm using UV-Visible Spectrophotometer.

5. STANDARD CURVE FOR CLOTRIMAZOLE

Preparation of pH 7.4 Phosphate Buffer Solution

11.45 g Potassium dihydrogen phosphate and 28.80 g of disodium hydrogen phosphate was weighed and dissolved in 1000ml of distilled water.

Preparation of Standard Solution of clotrimazole

100 mg of clotrimazole was accurately weighed and transferred in to 100 ml volumetric flask dissolved in pH 7.4 phosphate buffer solution.

Preparation of Working Standard Solution of Clotrimazole

From the stock solution, different aliquots are taken and made up to 100 ml with buffer to give concentration of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml of clotrimazole respectively. The absorbance of each solution was measured by UV Visible Spectrophotometer at 262 nm using phosphate buffer solution pH 7.4 as a blank. The graph of concentration versus absorbance was plotted.

6. SOLUBILITY STUDIES

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking certain amount of drug sample in 1 ml of solvent e.g. water, methanol, ethanol, pH buffer 6.8, 7.2 in small test tubes and well solubilized by shaking upto saturation.

II. Formulation of Clotrimazole Loaded Transethosomal Suspension

The cold method was used for the preparation of clotrimazole transethosomes. A solution of phosphotidylcholine and edge activator in ethanol at 30 ^{0}C act as organic phase. The aqueous phase was heated at 30 ^{0}C and then delivered to the organic phase drop wise with constant stirring at 700 rpm with the help of magnetic stirrer. The suspension will be cooled down at room temperature of 25 ^{0}C. Finally, the suspension will be homogenized at 2000 rpm and stored in a refrigerator for further study. This method was found to give more stable transethosomes.

PREPARATION OF GEL BASE

Carbopol 934 (1% w/v) was accurately weighed and dispersed into double distilled water (80 ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10 ml of propylene glycol was added to this solution. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.8. Transethosomal preparation corresponding to 1% w/w of clotrimazole was incorporated into the gel base to get the desired concentration of drug in gel base.

OPTIMIZATION OF CLOTRIMAZOLE TRANSETHOSOMES USING 2^3 FACTORIAL DESIGN

A 2^3 factorial design was developed to statistically optimize the formulation factors and evaluate the main effects, interaction effects on the independent factors. A 2 factor 2 level factorial design was used to explore independent factor response on dependent variables and constructing actual and coded factors with Design Expert Software (version 13.0) and a matrix comprising 2 factors, 2 levels, 8 runs is selected for the optimization study followed by ANOVA to determine the significance of each factor. The replicates are 3 Independent and 2 dependent variables are listed in table.

Table 1: Summary of Experimental design

Independent variables	Units	Level	
		Low (-1)	High (+1)
X1 = Type of Surfactant	ml	Tween – 80	Span – 80
X2 = Soya Lecithin conc.	mg	300	400
X3 = Ethanol Conc.	Ml	20	30
Dependent variables	**Units**	**Constraints**	

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Y1= Entrapment Efficiency	%	Maximize
Y2= % Drug Release	%	Maximize

Table 2: Formulation of Clotrimazole loaded transethosomes (2^3 factorial design)

S.NO	Formulation code	Drug (g)	Type of Surfactant	Surfactant (ml)	Lecithin (mg)	Ethanol (ml)
1	F1	1	Tween-80	0.3	300	20
2	F2	1	Span-80	0.3	300	20
3	F3	1	Tween-80	0.4	400	20
4	F4	1	Span-80	0.4	400	20
5	F5	1	Tween-80	0.3	300	30
6	F6	1	Span-80	0.3	300	30
7	F7	1	Tween-80	0.4	400	30
8	F8	1	Span-80	0.4	400	30

III.CHARACTERIZATION OF CLOTRIMAZOLE TRANSETHOSOMAL SUSPENSION

1. Scanning electron microscopy (SEM)

Shape and morphology of the Transethosomes are investigated using scanning electron microscopy. Transethosomes are negatively stained with 2% w/v aqueous solution of phosphotungstic acid on a carbon-coated copper grid. The grid are examined under scanning electron microscope with resolution of 2.4 A° at accelerating voltage of 200 kV.

2. Determination of entrapment efficiency

Entrapment efficiency of Transethosomes is determined by ultracentrifugation method. Transethosomes are separated by ultracentrifugation at 15,000 rpm for 60 minutes at a temperature of 4 ºC. The sediment and supernatant liquid is separated, the amount of drug in the sediment was determined by rupturing the vesicles using methanol and the amount of drug was quantified spectrophotometrically. Entrapment efficiency is determined by the following equation;

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount entrapped API}}{\text{Total amount of API}} \times 100$$

3. Vesicle size and Zeta potential

Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS). The size of transethosomes ranges between tens of nanometers to microns and is influenced by the composition of the formulation. Zeta potential is an important and useful indicator of particle surface charge, which can be used to predict and control the stability. In general, particles could be dispersed with proper stability when the absolute value of zeta potential was above 30 mV due to the electric repulsion between particles.

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4. **In vitro drug release study**

The in vitro drug release was conducted in PBS of pH 7.4 using Franz diffusion cell. Dialysis membrane was used as the diffusion barrier between the donar and receptor compartments. The receptor compartment was filled with 25 ml of PBS of pH 7.4 and maintained at a temperature of 37 ^{0}C with a stirring speed of 100 rpm. The membrane was fixed between the donor and receptor compartments and 1 ml of the suspension was placed in the donor compartment over the dialysis membrane. Aliquots were withdrawn at 1, 2, 3, 4, 5, 6, 7, 8 h followed by replacing the fresh buffer medium in the receptor compartment to maintain sink conditions. The sample were analysed at 262 nm by UV spectroscopy after dilution with PBS of pH 7.4 to quantify the cumulative drug release through Clotrimazole Transethosomes.

IV.CHARACTERIZATION OF CLOTRIMAZOLE INCORPORATED TRANSETHOSOMAL GEL

1. Physical appearance and homogeneity

The physical appearance and homogeneity of the prepared gels were tested by visual observations after the gel had been set in the container. They were analysed for their appearance and the existence of any aggregates.

2. **Measurement of pH**

The pH of each formulation was determined by dispersing 0.5 gm of gel in 50 ml of water. Checked using Digital pH meter with constant temperature. This was previously calibrated by pH 4.0 and 7.0 and then electrode was washed with demineralized water. The electrode was then directly dipped in to gel formulation and constant reading was noted.

3. Viscosity

The viscosity measurements of prepared topical transethosomes based gel were measured by Brookfield viscometer. 10 gm of gel formulation was measured by rotating the spindle no.63 at 10 rpm .

4. Spreadability

The 2 gm of gel was placed on one slide that was fixed and another slide was placed on it. A weight of 100 gm was allowed to rest for 5 min on the upper glass slide. The 20 gm of weight was used to lift the upper glass slide. The time taken to travel 6 cm by movable glass slide to separate it away from the lower glass slide was noted. Then, spreadability was determined using the formula.

S = M.L/ T

Where,

M = Weight attached to upper slide

L = Length moved by slide

T = Time taken (sec)

5. Drug Content

100 mg of transethosomal gel was weighed in a beaker, and 20 ml of methanol was added. This solution was mixed before being filtered via Whatman filter paper no.1. Then, 1 ml of the filtered solution was placed in a volumetric flask with a capacity of 10 ml, and the volume was raised to 10 ml using methanol. A UV-Spectroscope with a maximum wavelength of 262 nm was used to examine the solution.

The % drug content of transethosomal preparation was determined by using following formula, % drug content = Sample absorbance/ Standard absorbance

6. In vitro drug release study

In vitro drug release was carried out by franz diffusion cell. An egg membrane (soaked in phosphate buffer 24 hours before use) was fixed between donar and receptor compartment with the aid of an adhesive. 1 gm medicated gel was introduced in the cell (donor compartment) and cell was immersed in a beaker containing 25 ml of 7.4 pH phosphate buffer as receptor compartment. The entire surface of the cell was in contact with receptor compartment which was agitated using magnetic stirrer and a temperature 37 ^{0}C was maintained. Samples 1 ml of the receptor compartment was taken at 1 hour interval of time over a period 8 hours with same amount replaced. The sample was analyzed for clotrimazole at 262 nm against blank using UV spectroscopy. Amount of clotrimazole released at various time intervals was calculated with the help of calibration curve and plotted against time.

7.Stability study

The accelerated stability studies were performed. According to ICH guidelines accelerated stability study has to done for new drug product. For the study, sample was placed at specified storage conditions of 5 ^{0}C and accelerated stability condition at 25 ^{0}C for 30 days. The stability study formulation was stored in a borosilicate container to avoid any contact between

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the formulation and the container glass. The formulations were tested for physical changes.

III. RESULTS AND DISCUSSION

Preformulation studies organoleptic properties

The clotrimazole drug colour is White powder and the odour is odourless.

1. Solubility studies

Solubility of clotrimazole pure drug in Ethanol, Methanol, Distilled water, phosphate buffer pH 7.4 was studied and the values are given in Table

Table No.1: Solubility of clotrimazole pure drug in various media

S.NO	Solvent	Inference
1	Methanol	Freely soluble
2	Ethanol	Freely soluble
3	Distilled water	Insoluble

2.Determination of Melting Point

The melting point of the drug was determined by capillary method and was found to be 148 ^{0}C.

3.Determination of λ_{max} for Clotrimazole

The maximum absorbance of the clotrimazole was studied. The maximum absorbance of the drug clotrimazole was found to be 262 nm. Hence the wavelength of 262 nm was selected for analysis of drug in dissolution media.

4.Standard Calibration Curve of Clotrimazole

The Ultraviolet Spectrophotometric method was used to analyze the calibration curve of clotrimazole. The absorbance of the drug in pH 7.4 phosphate buffer of concentration ranging from 2-10 µg/ml was measured at a wavelength of 262 nm against blank.

Table No.2: Data for calibration curve of clotrimazole in phosphate buffer pH 7.4

S.NO	Concentration (µ g/ml)	Absorbance
1	0	0
2	2	0.098
3	4	0.216
4	6	0.343
5	8	0.478
6	10	0.578

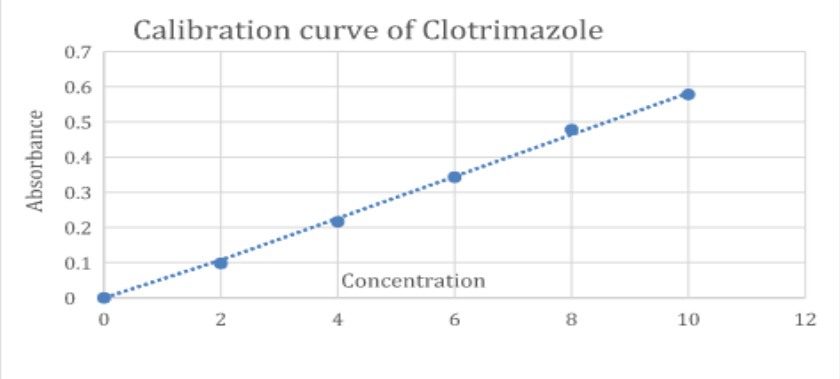

Fig 1: Calibration curve of clotrimazole

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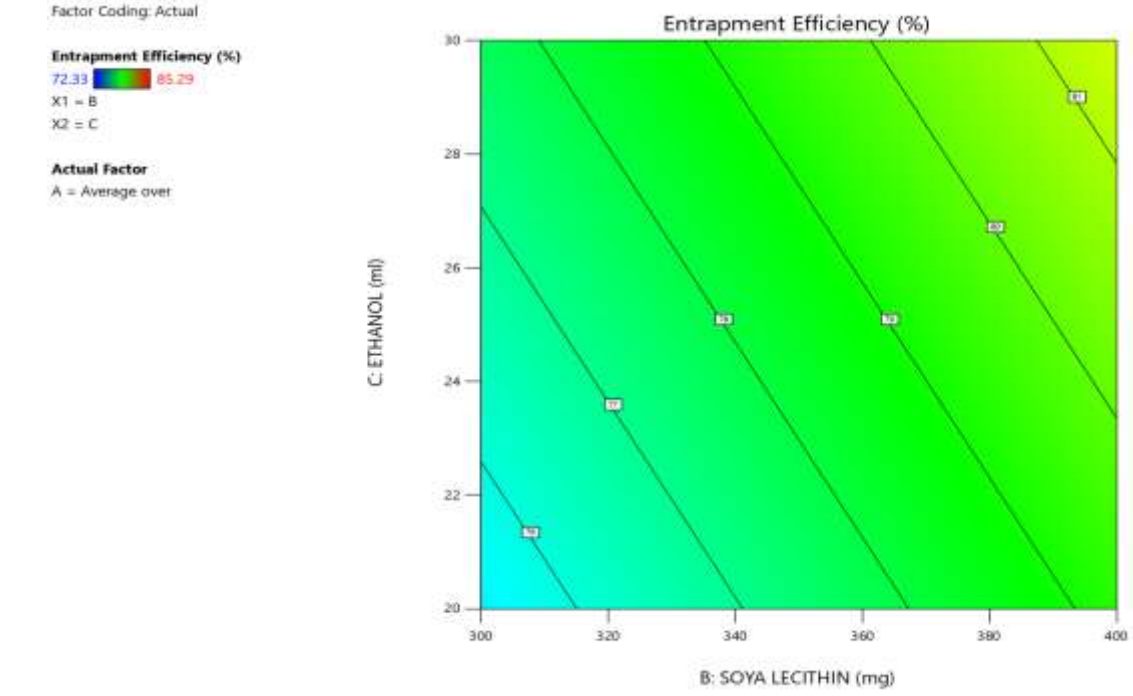
Table No.3: Actual summary of 2^3 Factorial Design for Clotrimazole Transethosomes

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	**Run**	**A: TYPE OF SURFACTANT (TWEEN-80 & SPAN- 80)**	**B: CONC. OF SOYA LECITHIN**	**C: CONC. OF ETHANOL**	**ENTRAPMENT EFFICIENCY**	**CUMULATIVE % DRUG RELEASE**
		ml	mg	ml	%	%
6	1	SPAN 80 OF 0.3	300	30	81.92	84.09
5	2	TWEEN 80 OF 0.3	300	30	73.68	75.08
4	3	SPAN 80 OF 0.4	400	20	83.78	86.15
7	4	TWEEN 80 OF 0.4	400	30	77.38	79.88
1	5	TWEEN 80 OF 0.3	300	20	72.33	73.90
3	6	TWEEN 80 OF 0.4	400	20	75.02	77.90
8	7	SPAN 80 OF 0.4	400	30	85.29	88.21
2	8	SPAN 80 OF 0.3	300	20	78.22	82.02

Table 4: Fit Summary for Entrapment efficiency

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**0.0005**		**0.9723**	**0.9366**	**Suggested**
2FI	0.8206		0.9441	0.4893	
Quadratic					**Aliased**

CONTOUR PLOTS



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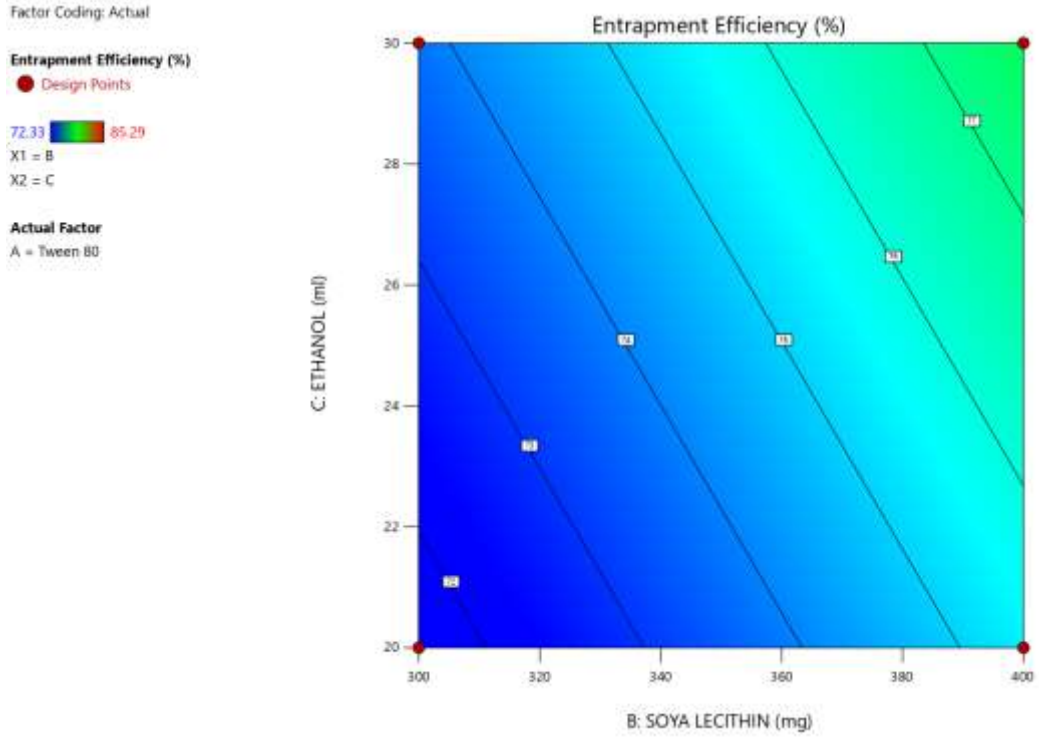

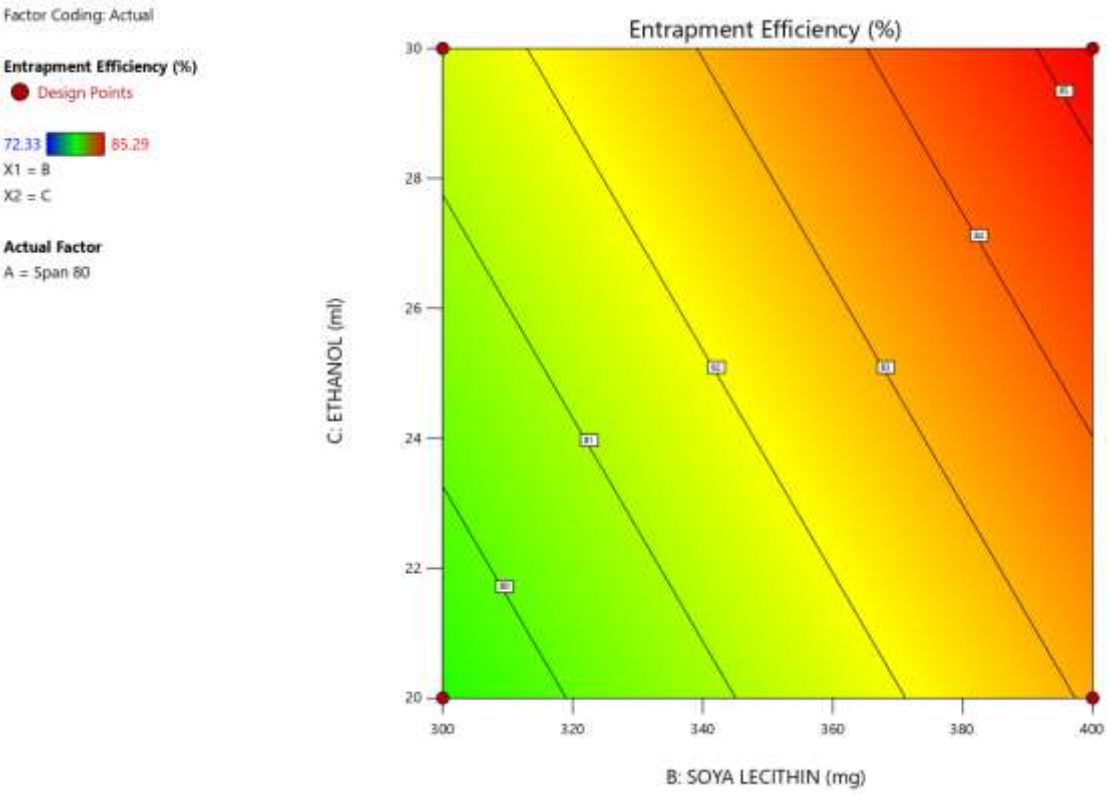

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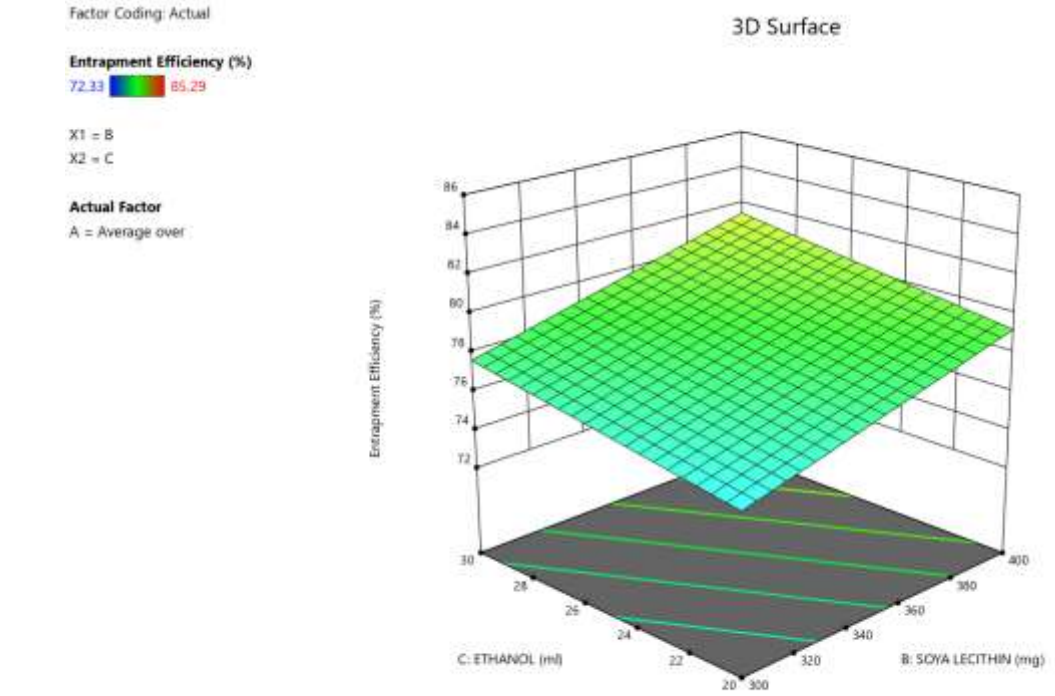
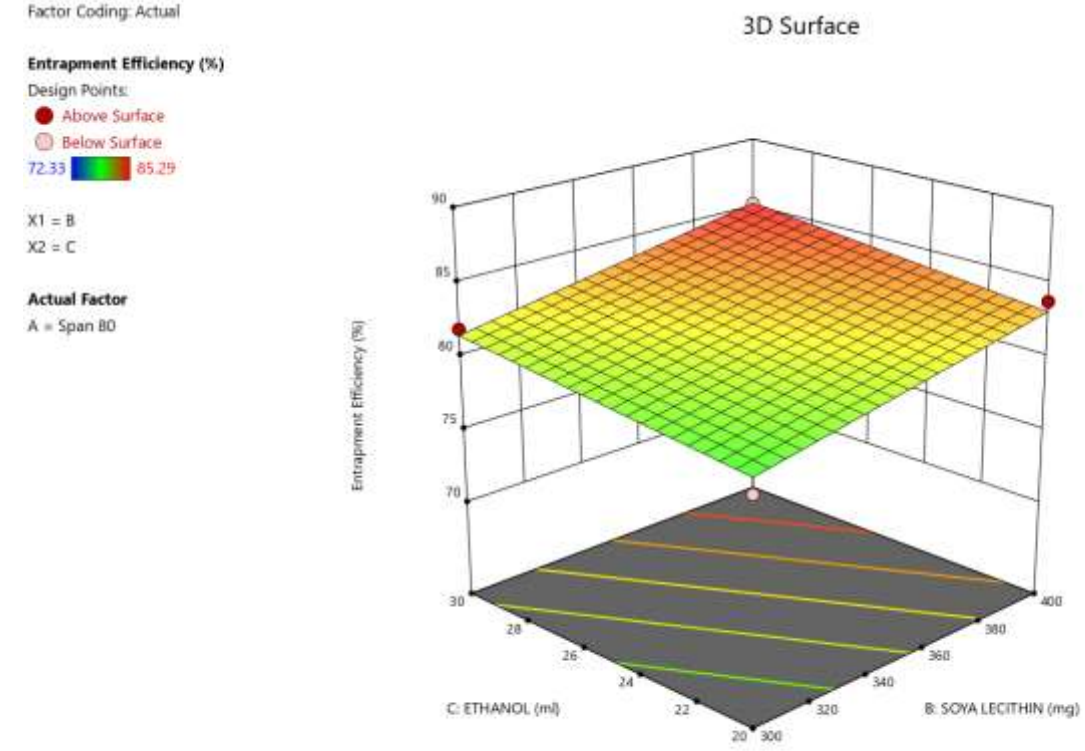
3D SURFACE





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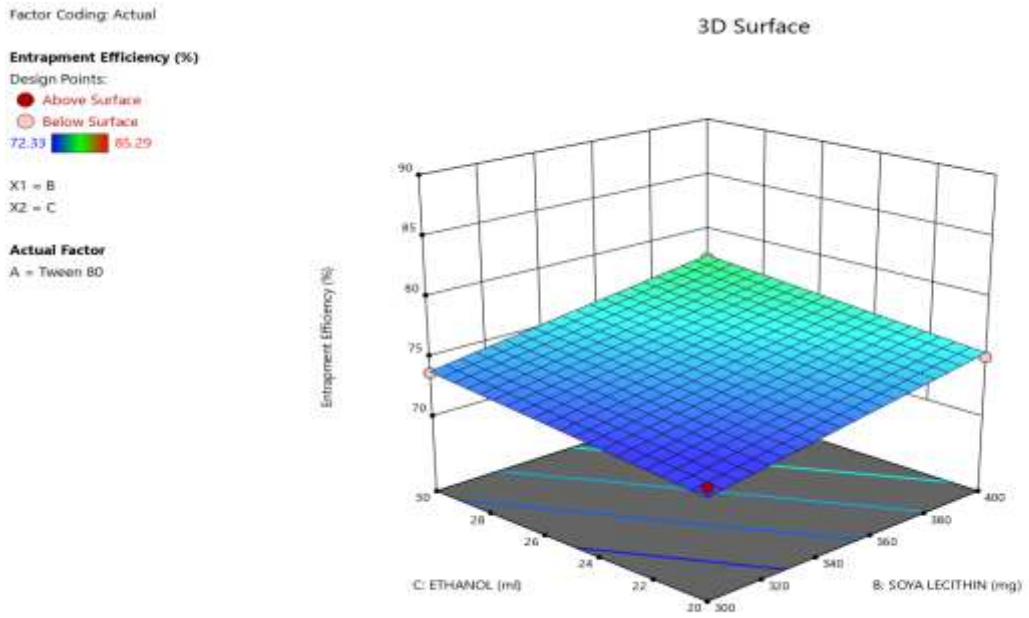
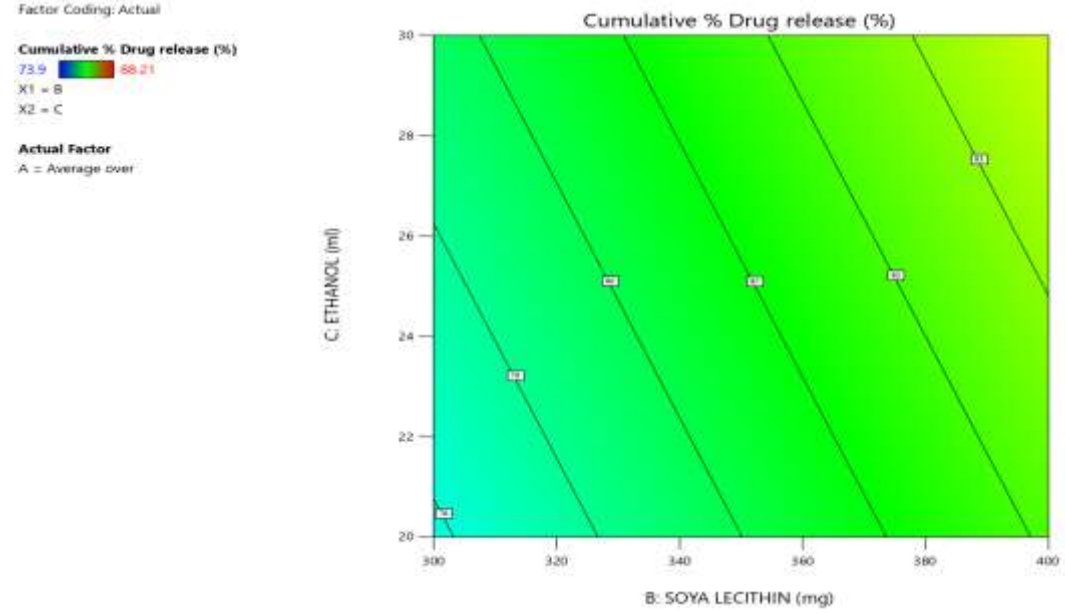


Table No.5: Fit Summary for % Drug Release

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**		**0.9970**	**0.9932**	**Suggested**
2FI	0.6199		0.9969	0.9717	
Quadratic					**Aliased**

CONTOUR PLOTS



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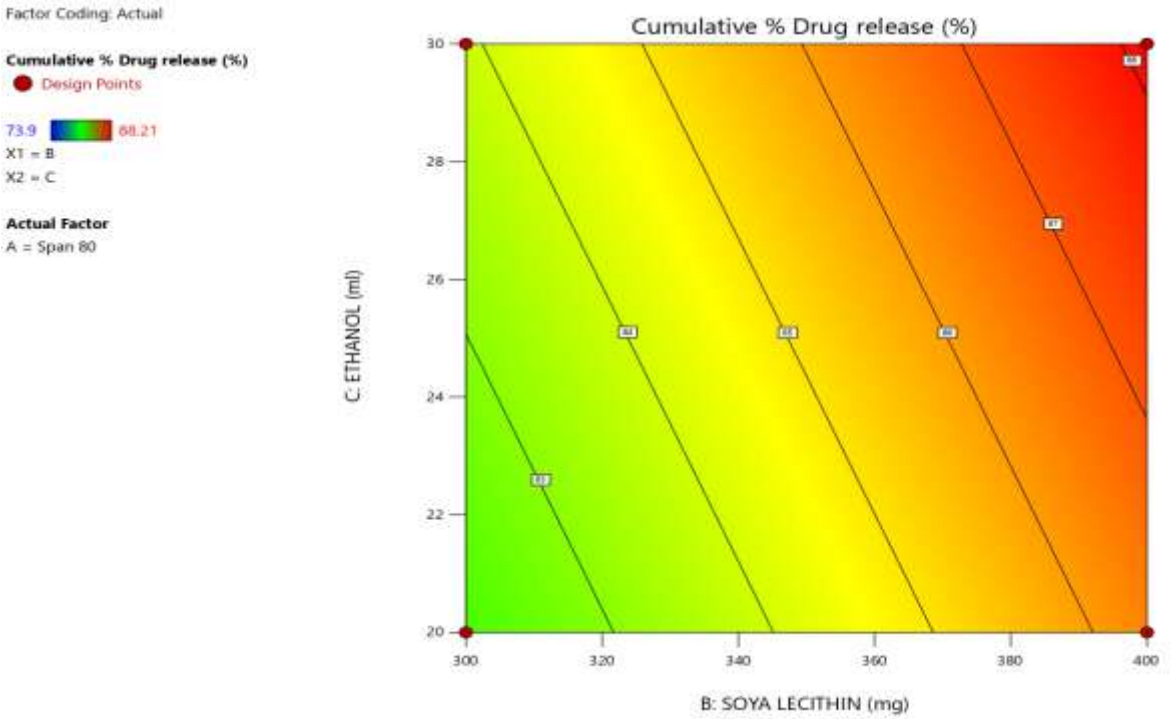

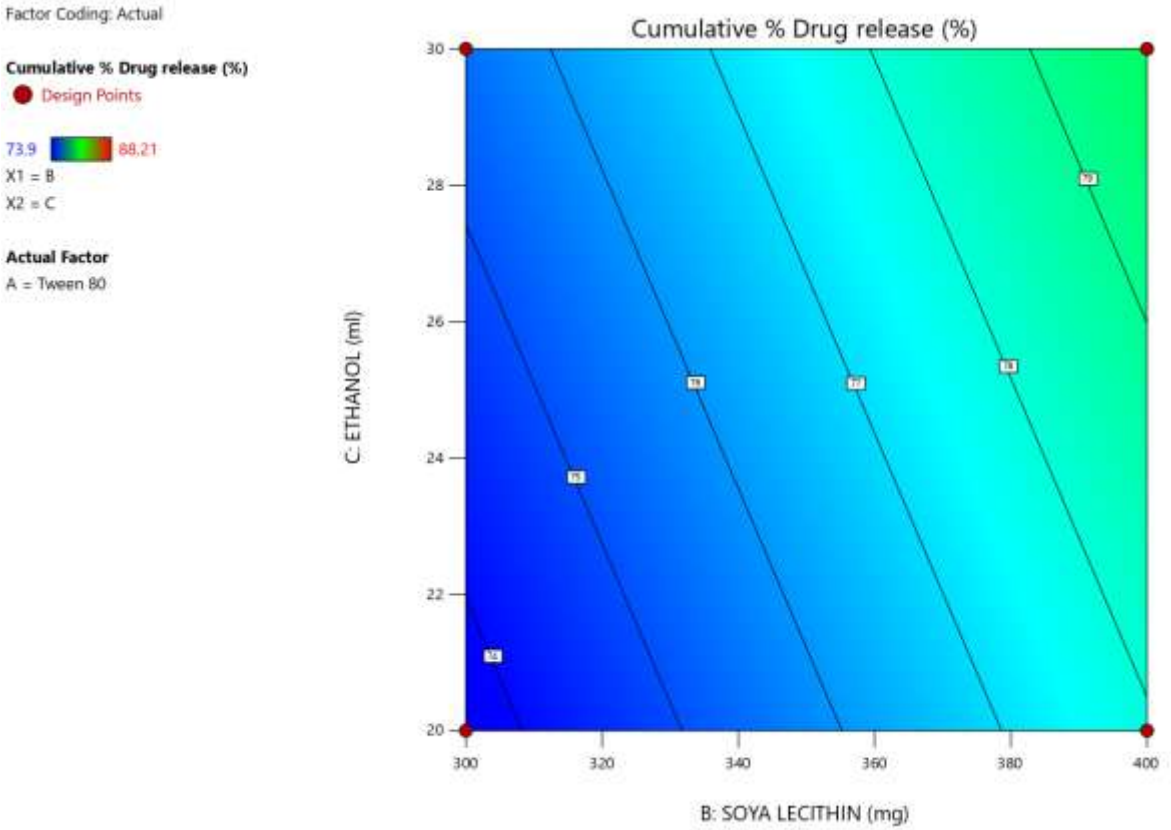

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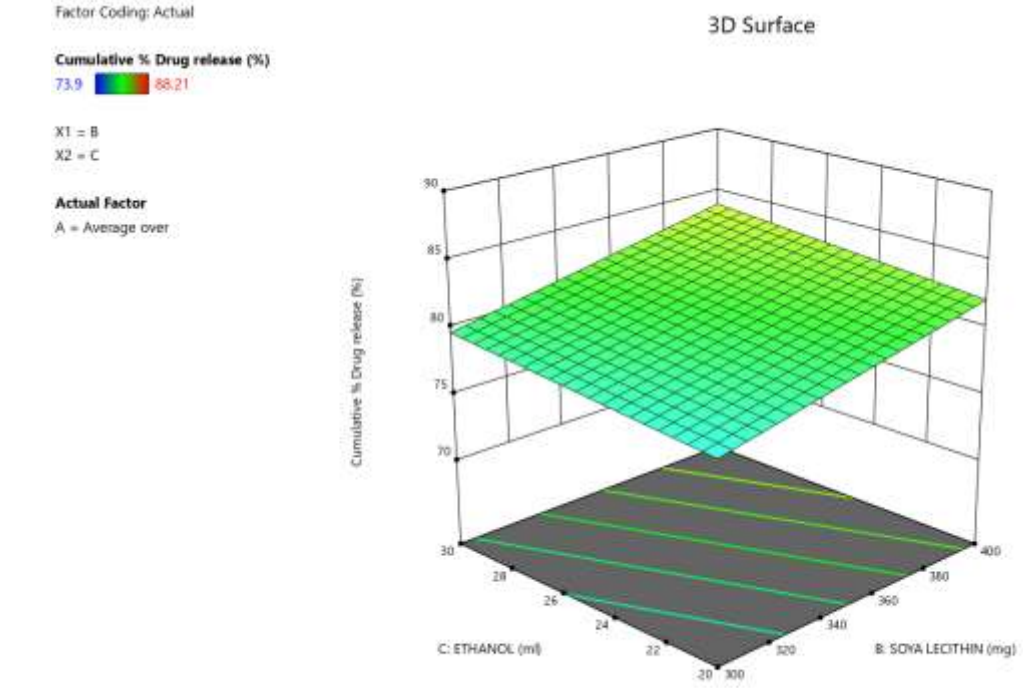

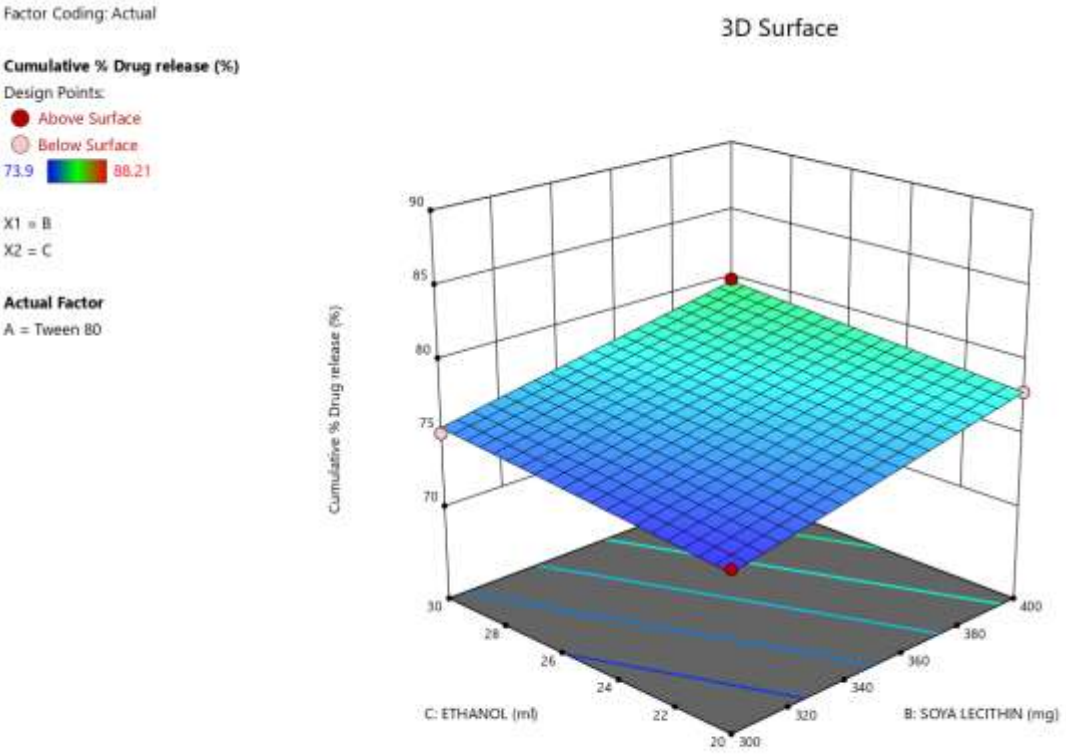

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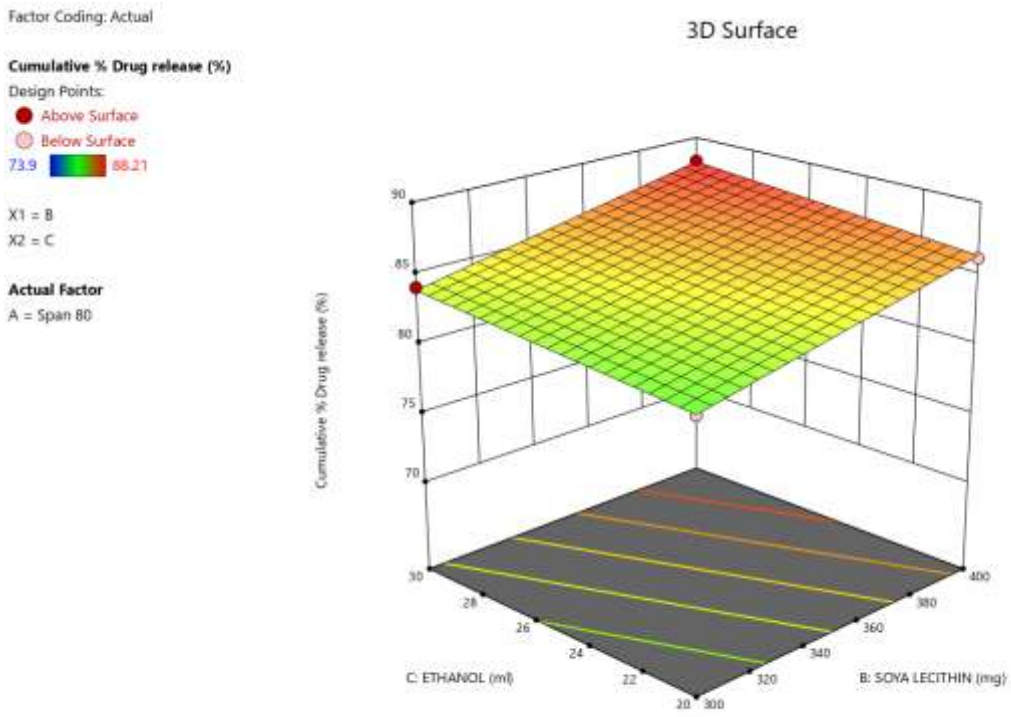

CHARACTERIZATION OF TRANSETHOSOMAL FORMULATION

1. DETERMINATION OF ENTRAPMENT EFFICIENCY

Table No.6: Entrapment Efficiency of Transethosomal Suspension

S.NO	Formulation Code	Entrapment Efficiency (%)
1	F1	72.33
2	F2	78.22
3	F3	75.02
4	F4	83.78
5	F5	73.68
6	F6	81.92
7	F7	77.38
8	F8	85.29

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2. DETERMINATION OF IN VITRO DRUG RELEASE

Table No.7: In vitro Drug Release of Suspension F1-F4

Time (Hours)	Cumulative Percentage drug release			
	F1	F2	F3	F4
0	0	0	0	0
1	7.65	8.92	8.20	9.51
2	15.78	18.13	16.91	19.31
3	24.15	27.73	26.01	29.62
4	33.08	37.79	37.43	40.48
5	42.63	48.19	49.45	51.72
6	52.65	59.04	56.80	62.71
7	63.04	70.28	64.62	74.20
8	73.90	82.02	77.90	86.15

Table No.8: In vitro drug release for Suspension F5-F8

Time (Hours)	Cumulative Percentage drug release			
	F5	F6	F7	F8
0	0	0	0	0
1	7.95	9.21	8.62	9.76
2	16.07	18.77	17.50	19.90
3	24.70	28.66	26.85	30.38
4	31.62	39.01	36.74	41.16
5	44.26	49.66	47.18	52.14
6	56.49	60.64	54.91	63.67
7	64.05	72.13	68.81	75.63
8	75.08	84.06	79.88	88.21

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3. Morphology of Transethosomes by Scanning Electron Microscopy (SEM) Analysis

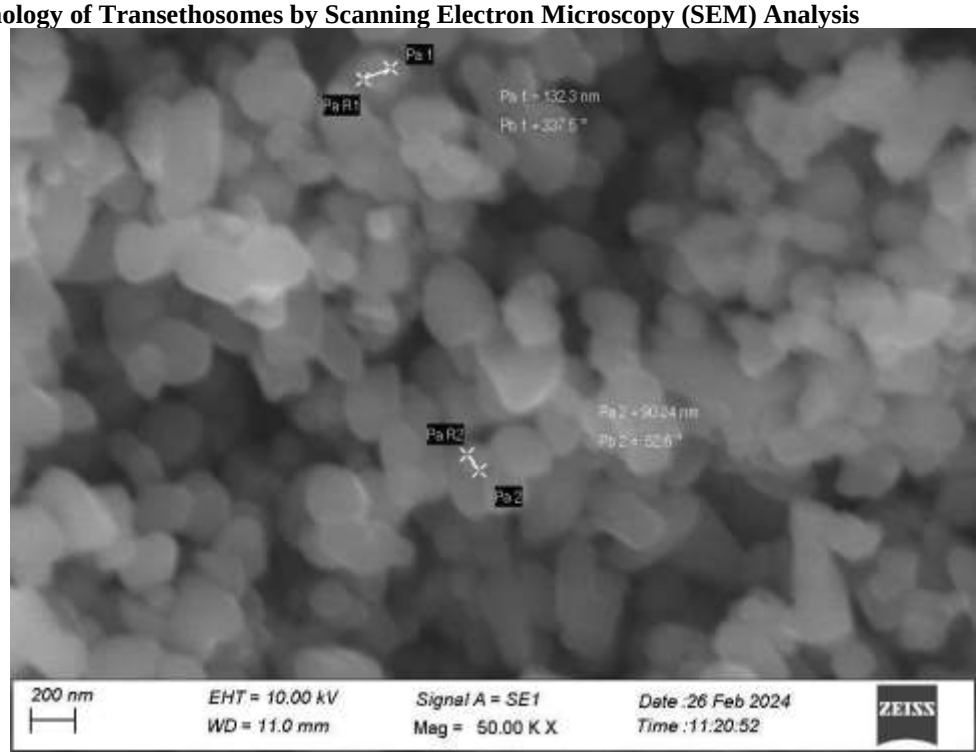

Fig 2: SEM image (1 µm)

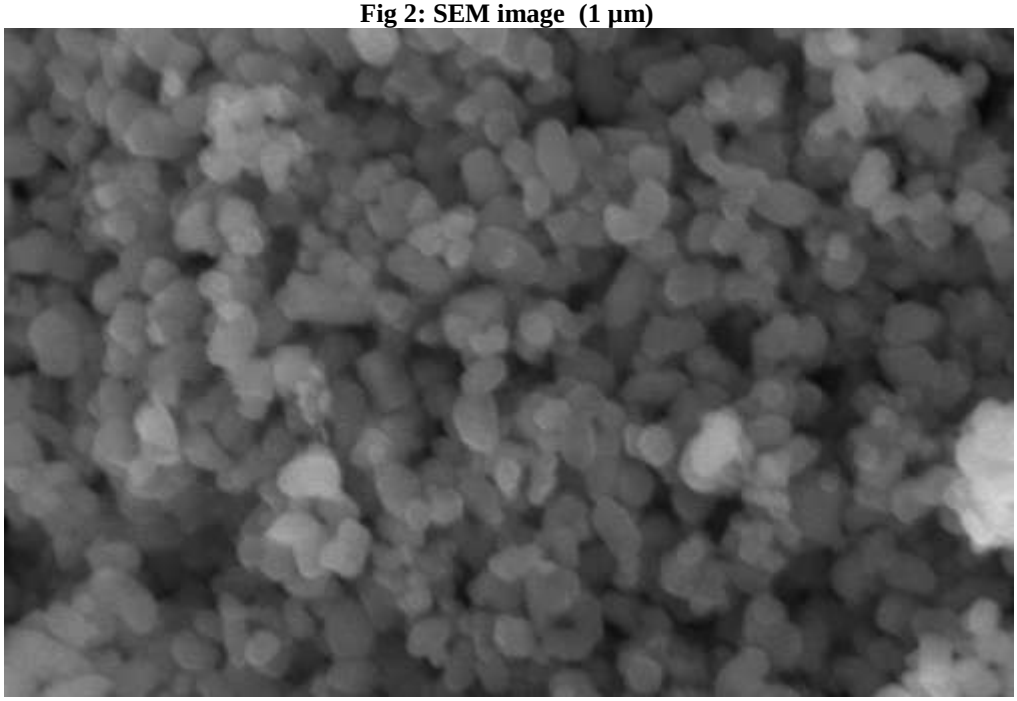

Fig 3 : SEM image of optimized Suspension (200 nm)

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4. Determination of Zeta Potential of Optimized Transethosomal Suspension

Zeta Potential Report

v2.2

Malvern

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Sample Details

Sample Name: Gel
SOP Name: mansettings.nano
General Notes:

File Name: Particle size and Zeta-8.dts
Record Number: 520
Date and Time: March 06, 2024 11:44:41 ...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

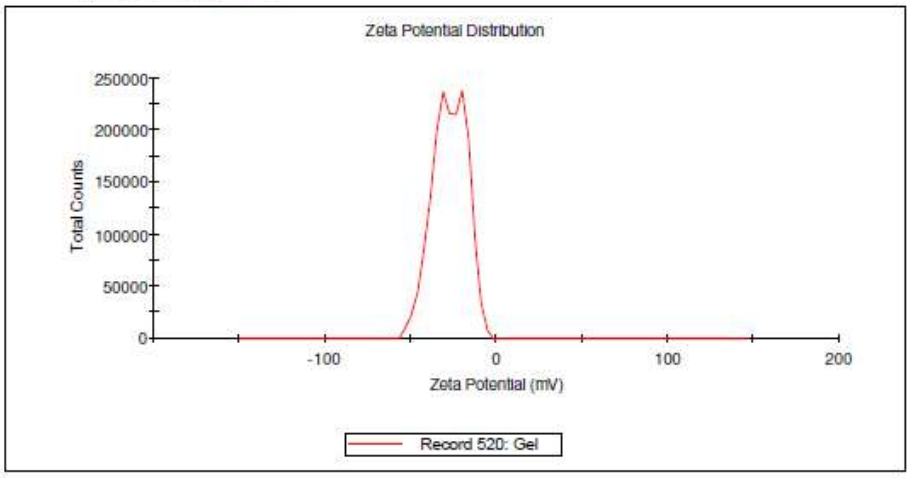
System

Temperature (°C): 25.0
Count Rate (kcps): 360.5
Cell Description: Clear disposable zeta cell
Zeta Runs: 12
Measurement Position (mm): 2.00
Attenuator: 6

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -27.1	**Peak 1:**	-32.3	59.8	6.92
Zeta Deviation (mV): 9.59	**Peak 2:**	-18.4	40.2	4.41
Conductivity (mS/cm): 2.39	**Peak 3:**	0.00	0.0	0.00

Result quality : Good



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Zetasizer Ver. 6.32
Serial Number : MAL1037088

File name: Particle size and Zeta-8
Record Number: 520
6 Mar 2024 4:05:44 PM

Fig 4: Zeta potential of optimized Suspension

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5. Determination of Particle Size and Polydispersity of Optimized Transethosomal suspension

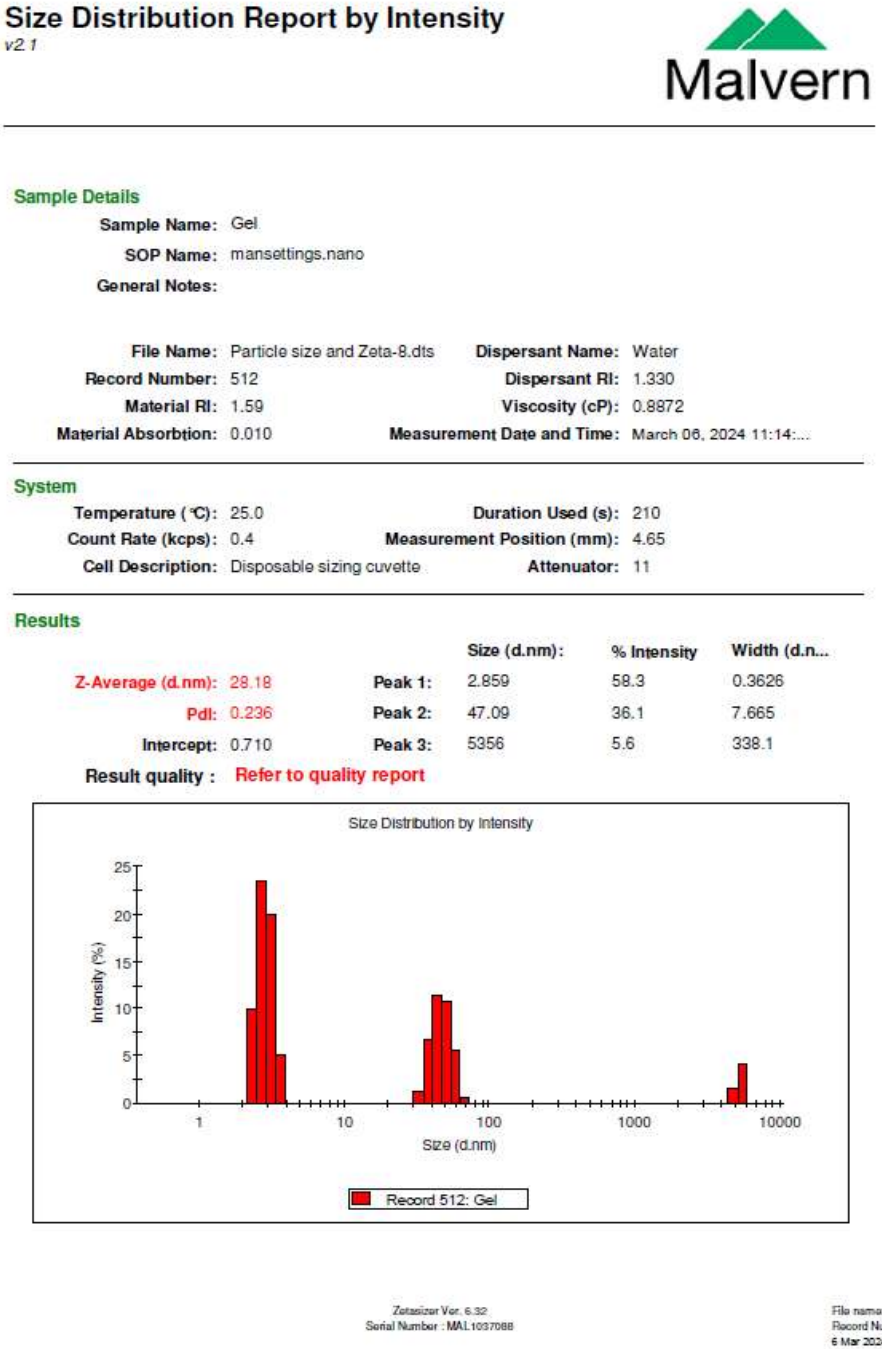

Fig 5: Particle size and polydispersity index of optimized suspension

Characterization Of Clotrimazole Transethosomal Gel

1. Homogeneity

All the gel formulations (F1-F8) shows good homogeneity with absence of lumps. Gels were found to be transparent and were free from

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presence of particles, uniformity of gel, aggregates, foreigh matter and phase separation.

2.Determination of pH

The pH of different formulations from F1 to F8 were showed. The pH varies from one formulation to another.

3.Spreadability

Spreadability diameter for different formulations F1-F8 showed good spreadability i.e. gel is easily spreadable. The results are shown in table

4.Drug content

The drug content of all the formulations from F1 to F8 are shown in **Table No.9** There is no much difference in the drug content of each formulations. So, the effect of polymers ratios is less considerable here.

5.Viscosity

All the formulations of transethosomal were subjected to Brookfield viscometer used to measure the viscosity (in cps) by dropping a cone attached to a holding rod from distance of 10 cm in such a way that, it should fall on centre of the glass cup filled with gel. The results are shown in **Table No. 9.**

Table No. 9: Evaluation of formulation for transethosomal gel

Formulation code	Clarity	Homogeneity	pH	Spreadability (cm)	Drug content	Viscosity (cps)
F1	Clear	Homogenous	5.93	2.5	87.67%	3268
F2	Clear	Homogenous	6.42	3.2	94.41%	3459
F3	Clear	Homogenous	6.14	2.8	90.37%	3351
F4	Clear	Homogenous	6.67	3.4	97.44%	3501
F5	Clear	Homogenous	6.04	2.6	88.35%	3340
F6	Clear	Homogenous	6.54	3.3	95.42%	3498
F7	Clear	Homogenous	6.26	2.9	92.72%	3356
F8	Clear	Homogenous	6.72	3.6	98.45%	3528

6.In vitro drug diffusion study

The in vitro drug diffusion study of optimized clotrimazole transethosomal gel was done in franz diffusion cell assembly using phosphate buffer pH 7.4 as the diffusion medium.

Table No.10: In vitro drug diffusion of optimized clotrimazole transethosomal gel

Time (Hours)	Cumulative Percentage drug release (%)
	Optimized transethosomal gel
0	0
1	9.63
2	19.86
3	30.47
4	41.66
5	53.32
6	65.27
7	77.44
8	89.85

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RELEASE KINETICS OF OPTIMIZED CLOTRIMAZOLE LOADED TRANSETHOSOMAL GEL

Table No.11: Release kinetics study of optimized transethosomal gel

Time (in hours)	Log time	Square root of time	Cumulative % drug release	Cumulative % drug remaining	Log cumulative % drug release	Log cumulative % drug remaining	Cube root of % drug release
0	∞	0	0	0	0	0	0
1	0	1	9.63	90.37	0.9836	1.9560	4.1957
2	0.3010	1.4142	19.68	80.32	1.2940	1.9048	4.1404
3	0.4771	1.7320	30.47	69.53	1.4838	1.8421	4.0717
4	0.6020	2	41.66	58.34	1.6194	1.7659	3.9866
5	0.6989	2.2360	53.32	46.68	1.7260	1.6691	3.8758
6	0.7781	2.4494	65.22	34.78	1.8143	1.5413	3.7244
7	0.8450	2.6457	77.44	22.56	1.8889	1.3533	3.4899
8	0.9030	2.8284	89.95	10.05	1.9540	1.0021	3.003

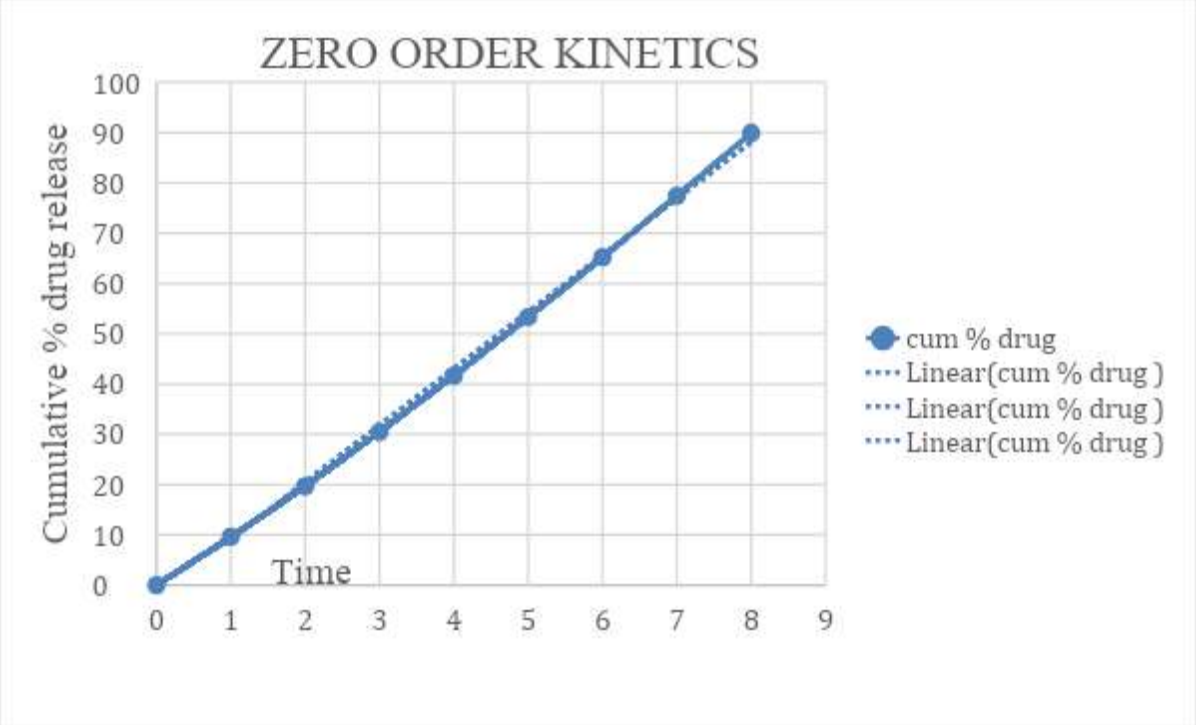

Fig 6: Zero order kinetics of optimized transethosomal gel

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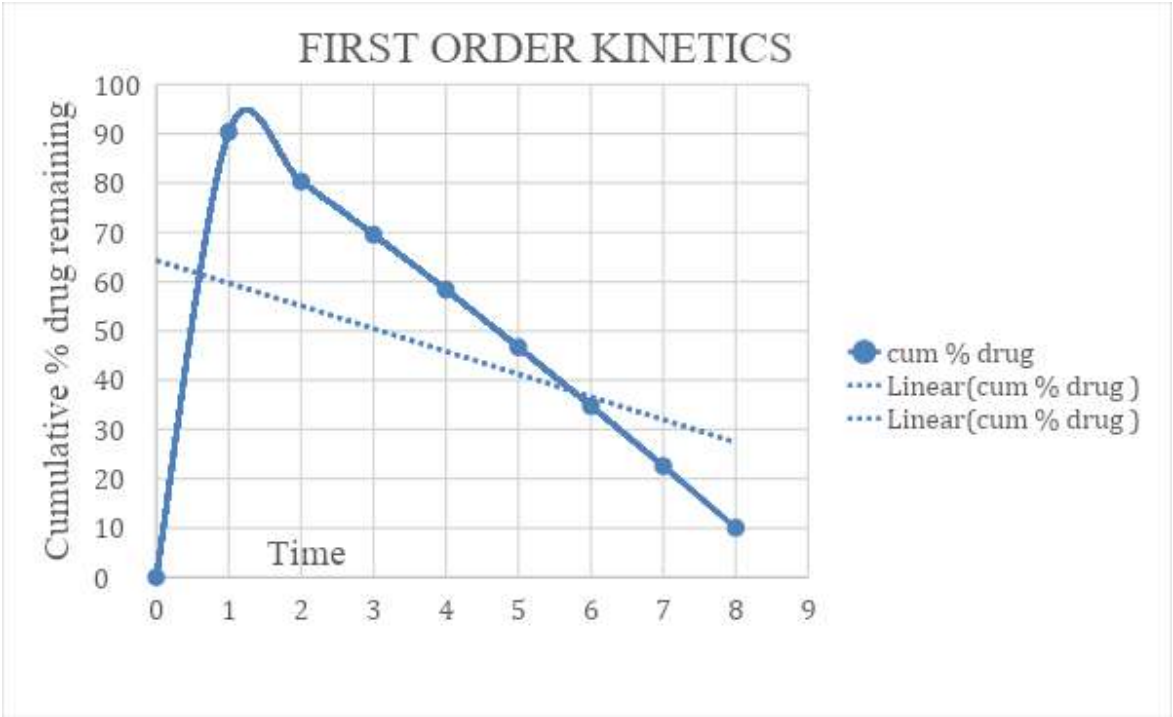

Fig 7: First order kinetics of optimized transethosomal gel

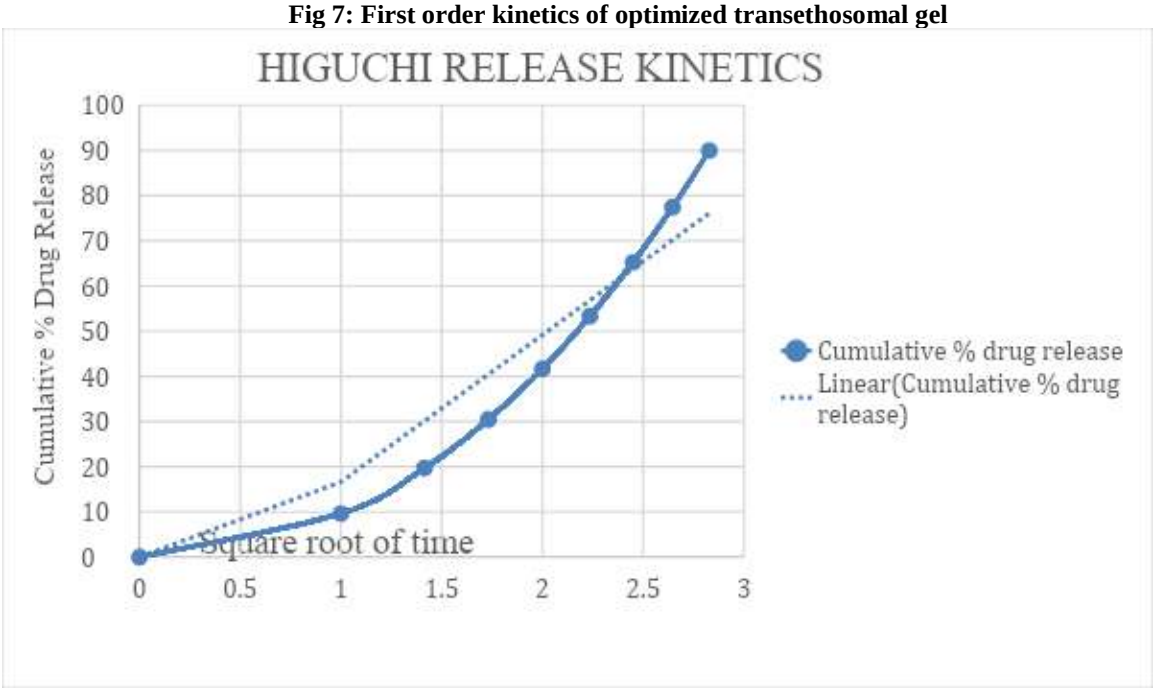

Fig 8: Higuchi release kinetics of optimized transethosomal gel

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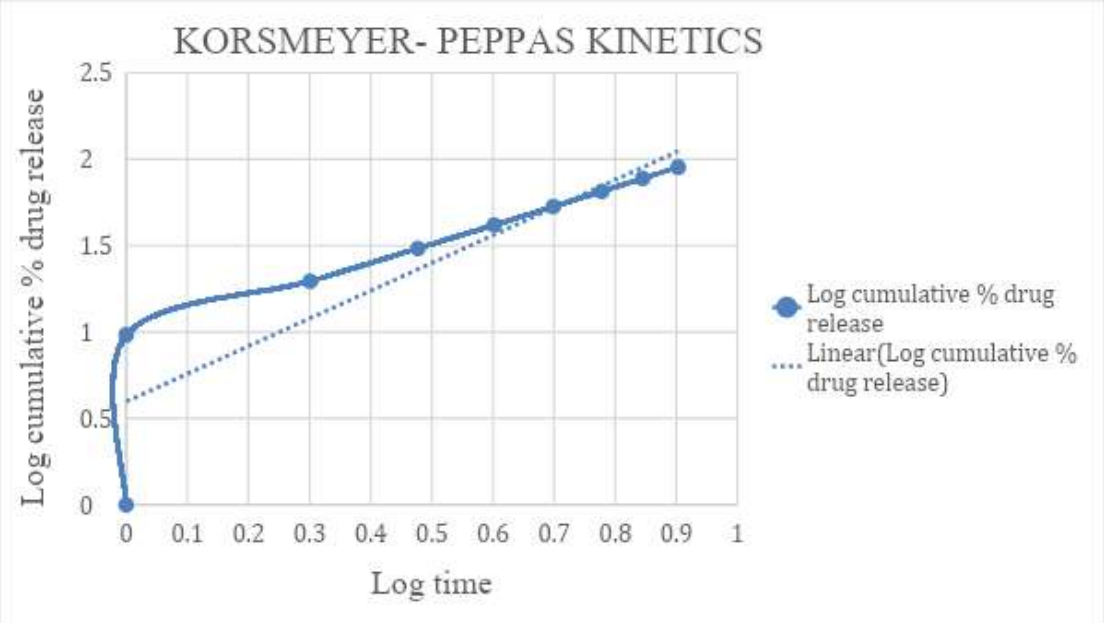

Fig 9: Korsmeyer-peppas kinetics of optimized transethosomal gel

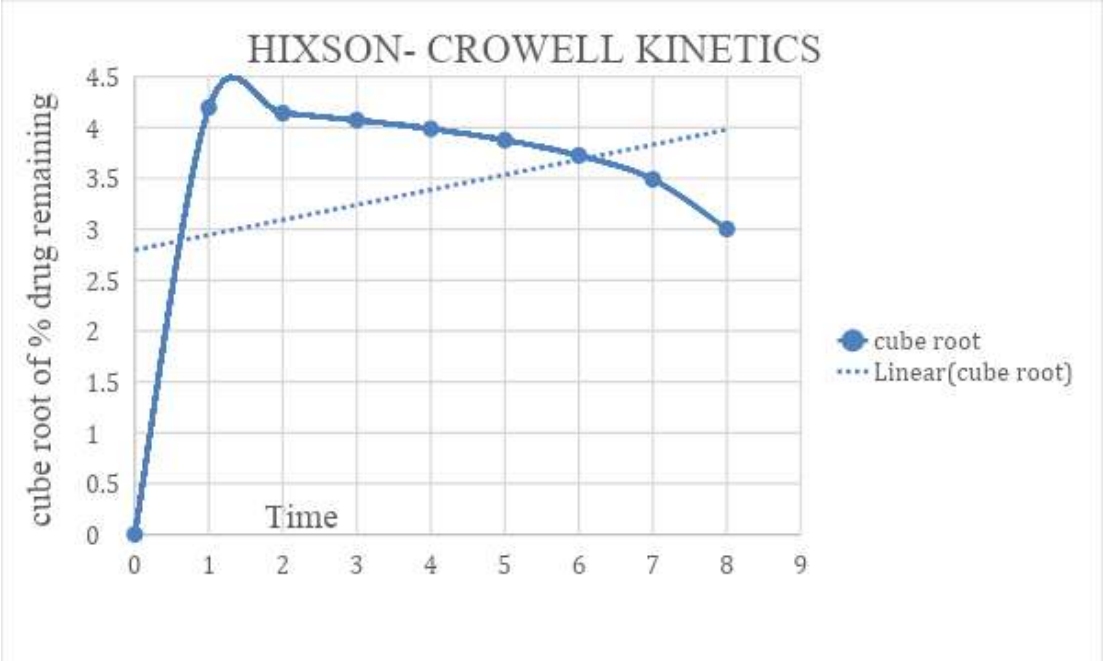

Fig 10: Hixon- Crowell kinetics of optimized transethosomal gel

Thus, the release kinetics of the optimized formulation was best fitted into Higuchi model and showed zero-order drug release with anomalous diffusion (Non-Fickian diffusion) mechanism.

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7. STABILITY STUDIES OF OPTIMIZED TRANSETHOSOMAL GEL

Table No.12: Stability study of optimized transethosomal gel

Stability condition	Entrapment efficiency (%)			Drug content (% w/w)		
	Initial	After 15 days	After 30 days	Initial	After 15 Days	After 30 days
Room Temperature	87.65	87.42	86.94	98.45	98.27	97.92
4 ºC±2 ºC	87.65	87.55	87.09	98.45	98.31	98.11

IV. CONCLUSION

In the present study, the transethosomes vesicle of clotrimazole were prepared and incorporated into the gel for the treatment of candidiasis. The purpose was to achieve high % entrapment efficiency, and sustained release on application. The method of preparation used was cold method followed by probe sonication. The vesicle were incorporated into the gel. The formulation gives homogeneous distribution of the vesicle size as the PDI value was found to be 0.236. The vesicles were found to have small size of 28.18 nm thus they can penetrate deep into the dermis layer. The entrapment efficiency was also found to be higher 87.65% more amount of drug can in entrapped. The drug release was found to be 89.85% at 8 hours and showed sustain release. It is concluded that cold method technique is a best method for successful incorportation of poorly water soluble drug clotrimazole into transethosomes preparation made with span 80 as the surfactant had higher entrapment efficiency compared to tween 80. The administration of the drug as gel type formulations enhances its penetration across stratum corneum and reduce the side effects by site specific targeting.

REFERENCES

[1]. Abdallah MH, Elghamry HA, Khalifa NE, Khojali WMA, Khafagy ES, Shawky S, El-Horany HE, El-Housiny S. Development and Optimization of Erythromycin Loaded Transethosomes Cinnamon Oil Based Emulgel for Antimicrobial Efficiency. Gels.**2023** Feb 6;9(2):137. doi:10.3390/gels9020137.

[2]. Abdellatif AAH, Aldosari BN, Al-Subaiyel A, Alhaddad A, Samman WA, et.al. Transethosomal Gel for the Topical Delivery of Celecoxib: Formulation and Estimation of Skin Cancer Progression. Pharmaceutics. **2022** Dec21;15(1):22. http://dx.doi.org/10.3390/pharmaceutics15010022.

[3]. Abdulbaqi IM, Darwis Y, Assi RA, Khan NAK. Transethosomal gels as carriers for the transdermal delivery of colchicine: statistical optimization, characterization, and ex vivo evaluation. Drug Des Devel Ther. **2018** Apr 9;12:795-813. doi:10.2147/DDDT.S158018.

[4]. Jessy Shaji, Shamika S. Parab. Formulation Development of Tranexamic Acid loaded transethosomal patch for Melasma, Research Journal of Pharmaceutical Dosage Forms and Technology.**2022**;14(1):7-6. Doi:10.52711/0975-4377.2022.00002

[5]. Sundar VD, Divya P. Design, Development and characterization of tramadol hydrochloride loaded transethosomal gel formulation for effective pain management. Indian Journal of Pharmaceutical Education and Research. 2020;54(2s): S88-97.

[6]. Nousheen K, Din FU, Jamshaid H, Afza R, Khan SU, Malik M, Ali Z, Batool S, Zeb A, Yousaf AM, Almari AH, Alqahtani S, Khan S, Khan GM. Metformin HCl-loaded transethosomal gel; development, characterization, and antidiabetic potential evaluation in the diabetes induced rat model. Drug Deliv. **2023** Dec;30(1):2251720. doi:10.1080/10717544.2023.2251720.

[7]. Zaki RM, Seshadri VD, Mutayran AS, Elsawaf LA, Hamad AM, Almurshedi AS, Yusif RM, Said M. Wound Healing Efficacy of Rosuvastatin Transethosomal Gel, I Optimal Optimization, Histological and In Vivo Evaluation. Pharmaceutics. **2022** Nov 19;14(11):2521. doi:10.3390/pharmaceutics14112521.