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Formulation and Evaluation of Ferulic acid Loaded Transethosomal Gel

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ABSTRACT

The current study used the Box–Behnken design (BBD) to develop and optimize a transethosomal gel loaded with ferulic acid for topical administration. Phospholipids, ethanol, and Span 60 were combined to create transethosomes, which were then cold-prepared and evaluated for entrapment efficiency, zeta potential, and vesicle size. With a vesicle size of 181.28 ± 0.41 nm, an entrapment efficiency of 78.98 ± 0.85%, and a zeta potential of –22.91 ± 0.6 mV, the improved formulation (F4) demonstrated good stability.

In vitro drug release studies using a Strat-M® membrane in Franz diffusion cells demonstrated **96.7% cumulative release** over 24 hours, following **Higuchi kinetics** ($R^2 = 0.9905$), suggesting diffusion-controlled sustained release. The gel displayed favorable physicochemical properties (pH = 6.5, viscosity = 4990 ± 33.5 cP, spreadability = 11.56 ± 0.4 g·cm/s). Permeation studies confirmed enhanced drug permeation from the transethosomal gel compared to plain gel (**84.3% vs. 39.0%** over 24 h).

With its enhanced penetration, regulated release, and possible anti-inflammatory properties, transethosomal gel is a viable cutaneous delivery method for ferulic acid.

HIGHLIGHTED POINTS

1. **Novel Topical Delivery System:** Developed and evaluated a Ferulic acid-loaded transethosomal gel for enhanced topical delivery, aiming to harness its anti-inflammatory effects.

2. **Optimized Formulation:** Utilized Box-Behnken design (BBD) to optimize Transethosomes formulation, identifying soya lecithin and Span 60 as key drivers for entrapment efficiency and vesicle size.

3. **Stable Nano vesicular System:** The optimized transethosomal formulation (F4) evidence a favorable particle size (181.28 nm), high entrapment efficiency (78.98%), also moderate stability (zeta potential −22.91 ± 0.6 mV).

4. **Sustained Drug Release:** Ferulic acid was released from the Transethosomes over a 24-hour period in vitro, with release kinetics that matched the Higuchi model and suggested diffusion-controlled release.

5. **Enhanced Permeation:** The transethosomal gel significantly enhanced drug release and permeation compared to a plain gel, suggesting its potential for improved dermal delivery of Ferulic acid.

1. INTRODUCTION

One of the most significant and acceptable target areas for medication distribution is the skin. Its intrinsic limitations, however, restrict its applicability in this field. With a surface area of 1.8 square meters, it is the biggest organ in the body and accounts for 16% of the total body weight. Apocrine glands, sweat glands, hair, nails, and oil glands are among the many derivatives found in the skin. Because it controls bodily fluids, it serves to shield the body from harmful substances and microorganisms. Water, phospholipid, ethanol, and edge activator are all present in transethosomes, which are lipid-based vesicular drug carriers. Phospholipids' main function is to act as a carrier by delivering drug particles straight to the skin. There is a hydrophilic head and a hydrophobic tail in the lipid vesicular system. The edge activator is what softens the bilayer of transethosomes. Additionally, it can render the vesicle permeable. The most crucial characteristics of ethanol for the development of nano-vesicular systems are its adaptability and flexibility, which enable them to readily pass through

extremely tiny holes in the stratum corneum as a result of the fluidization process. The lipid bilayer is transposed as a result of the reaction between the edge activator and ethanol, which may also provide a more flexible structure that more readily penetrates the skin's deeper layer.

Transethosomes Content high amount of ethanol content, about 30%. It consists of merits of Transferosomes as well as Ethosomes. Transethosomes indicate presence of phospholipids, including phosphatidylcholine, presence of a significant quantity of ethanol, and an edge activator causing enhancement of permeation. Shape of the vesicles: irregular spherical. Absorption: Absorption of drugs from transethosomes probably occurs through both ethanol effect and Transethosomes effect. Sophisticated medication delivery system Advanced medication delivery methods have been developing daily since the advent of modern technology, solving issues with traditional and contemporary drug delivery while also mitigating negative effects. At initial time, drugs were used orally by humans and subsequently slight advancement resulted in human to take those drugs from a transdermal route by avoiding the first pass metabolism and irritation in stomach. It has been demonstrated that transethosomes (TEs) are efficient transdermal carriers for the administration of several medication classes, including antifungal, anti-inflammatory as well as anticancer medications. (1)

According to numerous research, amide derivatives of Ferulic acid increase insulin production, stop low density lipoprotein from oxidizing, and stop inflammatory illnesses caused by the cyclooxygenase COX-2. Furthermore, a number of other studies have shown that Ferulic acid derivatives have superior physicochemical and antioxidant properties. According to these research, Ferulic acid derivatives have a variety of potential uses, including anti-inflammatory action, anticancer, antiatherogenic, anticarcinogenic, and antibacterial agents Additionally, it has been demonstrated that a number of Ferulic acid derivatives are more lipophilic, which improves their capacity to pass through lipid-rich cell membranes and enhances their antioxidant properties.(2) Examining ferulic acid's anti-inflammatory properties and exploring its potential for the creation of transethosomes were the main goals of this study. By varying the proportions of lipid, ethanol, and Span 60 utilized in the formulation process, the Ferulic acid-Transethosomal gel was adjusted. Box–Behnken design (BBD), one of the subsets of the design of experiments (DOE) approach, helps create higher-order models that need fewer runs than other factorial designs. Thus, BBD application saves money and time by minimizing the number of trials. Combining the statistical and mathematical methods needed for optimization modeling and analysis of trials where multiple factors affect the output, BBD aims to maximize a system's output while minimizing the number of runs. One of the most effective optimization strategies for response surface methods is the three-factor, three-level Box-Behnken design. For investigating second-order polynomial models of quadratic response surfaces, this approach is perfect. The relationships between a number of variables and variations in vesicle size and % entrapment efficiency were examined using Box–Behnken design (BBD) analysis. Additionally evaluated were the improved Ferulic acid-Transethosomal gel formulation's vesicle morphology, penetration characteristics, dermatokinetics, and Ferulic acid release in vitro. The research clearly predicts that the optimal Ferulic acid-Transethosomal gel formulation will provide the appropriate amount of Ferulic acid for efficient anti-inflammatory action. (3)

2. MATERIALS AND METHODS

2.1. Materials

FA was purchased from Arti Drugs Limited Pvt. Ltd, Mumbai. PDEA's Seth Govind Ragunath Sable Pharmacy College, Saswad provided Span60, cholesterol, soya lecithin, methanol , ethanol, chloroform, carbapol 940, triethanolamine, potassium dihydrogen orthophosphate, propylene glycol were obtained from loba chemicals pvt. Mumbai, India. Every chemical utilized was of analytical quality and didn't require any additional purification.

2.2. Preparation of Ferulic acid loaded Transethosomes

The cold approach was utilized to create transethosomes loaded with ferulic acid (FA). This method involves mixing two phases at a cool temperature of 30 °C in a water bath. To form the vesicles, an aqueous phase is added to the organic phase while continuously stirring. Ferulic acid, Span 60, and soy lecithin are dissolved in ethanol at 30 °C to create the organic phase. Meanwhile, the aqueous phase, which consists of Millipore water, is also heated to 30 °C. Gradually, the aqueous phase is mixed into the organic phase while stirring at a steady rate of 1200 revolutions per minute using a magnetic stirrer (REMI Lab 1 MLH, India). After that, the mixture is stored in a refrigerator at 4 °C until it's ready for further characterization. The stirring continues for 45 minutes to achieve the transethosomal dispersions. Finally, the transethosomal dispersion is agitated ultrasonically with a probe sonicator (SPECTRALAB instruments Pvt Ltd) and then kept at 4 °C in the refrigerator until it's time for further analysis (4)

2.3. Ferulic acid-loaded transethosomes optimization via Box-Behnken design (BBD)

A number of factors that could affect the optimal properties of transethosomes for topical distribution have been found based on the literature research, and it was discovered that three variables (factors), including the concentration of ethanol, phospholipid, and Span 60, may have necessary for topical administration to be successful. Using Design Expert® (Version 7.1.6, Stat Ease Inc., Minneapolis, MN), we applied response surface methodology to create a Box–Behnken design featuring three components, each with three levels. This software-driven experimental design included 17 runs, complete with five

repeated center points, allowing us to measure the outcomes effectively. The goal of using the Box–Behnken design was to explore how varying concentrations of phospholipid, surfactant, and ethanol influenced our dependent variables, specifically entrapment efficiency and vesicle size. You can find the independent and dependent variables used in the experimental design listed in Table 1. We also assessed the size and entrapment efficiency of the Ferulic acid-loaded Transethosomes vesicles. **As shown in Table no.1 and 2.**

Table no.1. Independent and Dependent variables for formulation of transethosomes

Variables	Levels		
Independent Variable	-1	0	+1
Ethanol	10	15	20
Soya lecithin	100	120	140
Span 60	10	20	30
Dependent Variable			
Particle size (nm)			
Entrapment efficiency %			

Table 2. Optimizing Transethosomal Preparations with Independent Factors and the Outcomes of Dependent Variables

Formulation code	Ethanol (ml) (X1)	Soya lecithin (mg) (X2)	Span 60 (mg) (X3)	EE (%) (Y1)	Vesicle size (nm) (Y2)
1	20	140	20	82.49 ± 1.4	203.88± 2.3
2	15	140	30	84.19 ± 1.2	210.56± 2.7
3	15	100	30	72.44± 0.3	204.34± 2.8
4	20	120	30	78.98± 0.5	181.28± 1.4
5	15	120	20	80.99± 1.3	178.98± 0.7
6	15	120	20	81.01± 0.8	177.56± 1.3
7	15	120	20	81.47± 1.5	178.14± 1.5
8	20	100	20	73.11± 0.4	198.18± 2.3
9	15	140	10	88.99± 0.4	211.98± 2.4
10	10	100	20	75.11± 0.2	196.55± 0.8
11	10	120	10	83.98± 0.6	185.1± 1.2
12	15	120	20	82.99± 0.7	178.99± 1.9
13	15	120	20	81.37± 0.1	178.98± 0.8

14	20	120	10	82.41± 0.8	181.77± 1.9
15	15	100	10	75.89± 0.2	202.14± 1.01
16	10	140	20	86.04± 1.2	204.44± 2.3
17	10	120	30	79.49± 1.2	184.24± 2.04

2.4. Particle size and Zeta Potential

The average vesicle size (PS) and electrostatic potential of charge of suspended Transethosomes (TEs) were evaluated as crucial characteristics for stability studies. The zeta potential (ZP) of the charged particles was used to determine whether they were repelling or attracting one another. The prepared FA-loaded Transethosomes' mean PS and ZP were determined using the Zetasizer HAS 3000 (Malvern, UK). (5)

2.5. Entrapment Efficiency (%EE)

Transethosomal suspensions' entrapment efficiency (% EE) was ascertained indirectly. Clear supernatants were produced when transethosomal suspensions were ultracentrifuged (C-24, Remi instruments, India) for 30 minutes at 12,000 rpm. The gathered supernatants were put through spectrophotometric (Shimadzu, Japan) testing to measure the amount of FA in transethosomal formulations. At ¥ max = 320 nm. Measurements of the quantities of both free and total drugs were made. A calibration curve was used to determine the drug's concentration. The standard curve calibration equation (R2 = 0.9952) was used to calculate the amount of free drug in the supernatant. The proportion of EE was calculated using the following formula after three different readings were obtained: %EE = Total drug – drug supernant/ Total drug *100.

2.6. Selection of optimized Transethosomal formulation.

All 17 transethosomal formulations were prepared according to 3 factors and 3 levels of Box-Behnken design using design experts. The values corresponding to the F1-F17 batches represented particle size and entrapment efficiency, which clearly demonstrated that the formulations differed significantly from one another.

2.7. Zeta Potential

To ascertain whether the optimized formulation of Transethosomes (F4) are colloidal, zeta potential analysis is performed. Using a zeta potential analyzer, the suitably diluted Transethosomes from the pronoisome dispersion were determined using the laser Doppler velocimetry method and electrophoretic light scattering. A temperature adjustment of 25°C was made. The measurements were used to determine the measurement standard deviation, mean zeta potential values, and vesicle charge.

2.8. Scanning electron microscopy.

The size of transethosome particles is a crucial feature. Surface morphology (smoothness, roundness, and aggregation formation) of Transethosomes (F4) was investigated using scanning electron microscopy (SEM) tailored formulation. Transethosomes were used to cover the double-sided tape that was affixed to aluminum stubs. The aluminum stub was injected into the vacuum chamber of a scanning electron microscope. A gaseous secondary electron detector was used to evaluate the morphological features of the samples.

2.9 Invitro Drug release

The optimized Ferulic acid loaded transethosomal formulation in vitro release profile for topical administration was assessed using a synthetic Strat-M® membrane in a Franz diffusion cell setup. This membrane was selected for topical permeation investigations because of its multilayered structure and permeability properties that resemble those of human skin. 2 mL of the transethosomal gel formulation, which contained 10 mg of medicine, was carefully added to the donor compartment. Phosphate-buffered saline (PBS, pH 7.4) was used as the dissolving medium in the receptor compartment, kept at a steady temperature of 37 ± 0.5°C. A magnetic stirrer continuously mixed the fluid to ensure proper sinking conditions and uniform distribution. To keep the volume consistent, 1 mL samples were taken from the receptor compartment at various time points—0, 1, 2, 4, 6, 8, 12, and 24 hours—and immediately replaced with an equal volume of fresh PBS. The extracted samples were analyzed using UV spectroscopy at the drug's λ max to determine the total amount of drug released.

2.10 Preparation Ferulic acid loaded Transethosomal gel

The refined formulation's transethosomal suspension (F4) was mixed with a Carbopol 934 gel foundation to create a transethosomal gel filled with ferulic acid. In distilled water, carbopol 934 (0.5% w/v, 1% w/v, and 1.5% w/v) was dissolved while being continuously swirled, and the mixture was left to hydrate overnight to guarantee full swelling of the polymer. Triethanolamine (TEA) was subsequently added slowly to the dispersion while stirring gently until a different gel was

formed, on the basis viscosity adjusting the pH close to the physiological range (pH 6.8–7.4).The carbapol 934 (1%w/v) was found to be consistent and clear to incorporate into gel.

To ensure that the Transethosomes were uniformly dispersed within the gel matrix, the prepared Ferulic acid-loaded TE suspension was added gradually to the Carbopol gel under stirring conditions. Stirring was continued until a uniform transethosomal gel was obtained until it was required again.

To ensure that the Transethosomes were uniformly dispersed within the gel matrix, under stirring conditions, the produced Ferulic acid-loaded TE suspension was progressively added to the Carbopol gel. Stirring was continued until a uniform transethosomal gel was obtained until it was required again. (6)

2.11 Evaluation of Transethosomal gel

2.11.1 Physical assessment

The adjusted gel formulation's color, transparency, and homogeneity were evaluated.

2.11.2 Viscosity:

The Brookfield viscometer (Model DV-II+ Viscometer, India) was used to measure the viscosity of the Transethosomal gel formulation. After the gel sample was put in a beaker, the dial reading was taken at 60 rpm and 25 °C.

2.11.3 PH Measurement:

The pH of Ferulic acid (F4) was determined using a pH meter that had been calibrated using standard buffer solutions with pH values of 4 and 7. After dissolving the necessary quantity of gel in 100 milliliters of distilled water, it was left for 2 hours. Three individual measurements of the pH of Transethosomal gel were taken, and average results were calculated.

2.11.4 Spreadability (7)

The ability of gel to spread over the skin's surface is one of its most crucial properties. A gel composition must have the ideal spreadability. Two glass slides were used to measure the prepared Ferulic acid -containing Transethosomal gel's spreadability. One glass slide was fixed, and using a conventional weight, the second slide was pushed over the chosen slide. A gel sample was positioned at the designated halfway of the glasses. Equation was used to calculate spreadability.

2.12. In vitro Permeation study

Using a Franz diffusion cell method, an in vitro permeation research was conducted to evaluate the topical distribution capacity of the transethosomal gel formulation loaded with ferulic acid. Because of its multilayered structure and permeability profile that closely resemble human skin, the Strat-M® synthetic membrane was used as the diffusion barrier, making it appropriate for simulating in vivo circumstances.

The donor compartment of the Franz diffusion cell was evenly coated with 1 gram of a transethosomal gel that was loaded with ferulic acid and included 10 milligrams of medication. To mimic the conditions of the skin's surface, we filled the receptor compartment with phosphate-buffered saline (PBS, pH 7.4) and kept it at a temperature of 37 ± 0.5°C. During the experiment, a magnetic stirrer was employed to keep the receptor fluid agitated, ensuring both sink conditions and uniformity. To keep the volume and concentration gradient steady, we took 1-milliliter samples from the receptor compartment at specific intervals, including 0, 1, 2, 4, 6, 8, 12, and 24 hours.) and immediately replaced with new PBS. A validated UV-Visible spectrophotometric test was used to assess the amount of medication that infiltrated the membrane from the collected samples µg/cm², the total amount of drug penetrated per unit area, was shown against time. Permeation characteristics such as enhancement ratio (ER), permeability coefficient (Kp), and steady-state flow (J) were calculated. To ascertain the release mechanism and diffusional properties of the drug from the transethosomal gel matrix, drug release kinetics were investigated by comparing the permeation data to several kinetic models (zero-order, first-order, Higuchi, and Korsmeyer–Peppas). (8)

3. RESULT AND DISCUSSION

3.1. Transethosomes Optimization via Box-Behnken Design:

This study illustrates the influence of various parameters on formulation performance, utilizing insights derived from recent testing data and specialized design software. The empirical workplace values for drug entrapment efficacy (Y1) and vesicle size (Y2) were calculated using Design-Expert13 software (Table 2). Three-dimensional surface graphs, contour plots, and mathematical models were employed to illustrate each reaction. Tables display the findings of the analysis of variance (ANOVA) along with the related statistical variables and after the removal of irrelevant components.

3.1.1. Independent factors' effects on drug entrapment efficiency (9, 10)

As shown in fig no. 1, 2, 3 and 4 respectively.

Table no. 3. Shows that ANOVA for entrapment efficiency

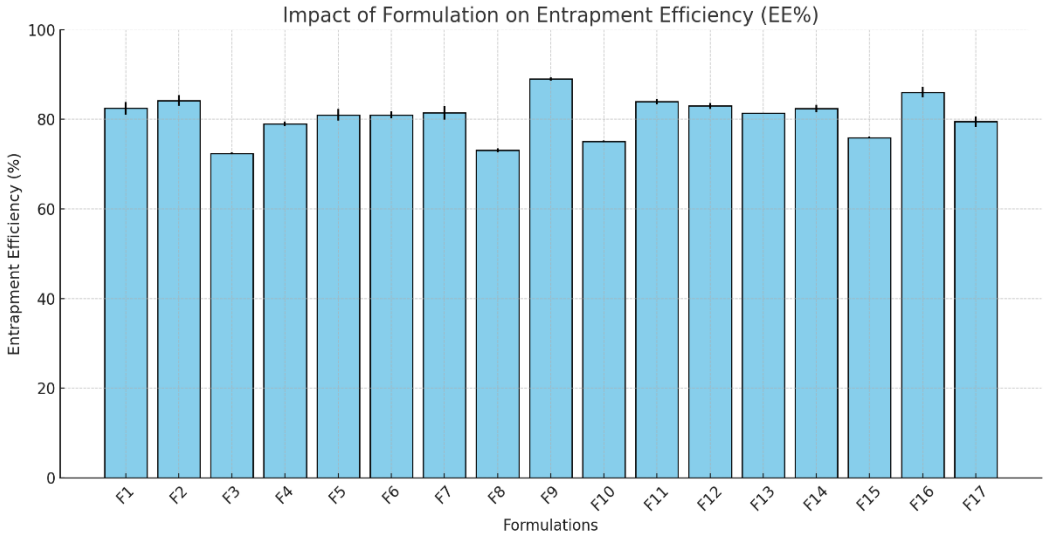
Table no. 3 ANOVA for Quadratic model

Response 1: EE%

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	310.55	9	34.51	35.46	< 0.0001
A-ethanol	7.28	1	7.28	7.48	0.0291
B-soya lecithin	254.93	1	254.93	261.98	< 0.0001
C-span 60	32.68	1	32.68	33.59	0.0007
AB	0.6006	1	0.6006	0.6172	0.4578
AC	0.2809	1	0.2809	0.2887	0.6077
BC	0.4556	1	0.4556	0.4682	0.5158
A^2	2.50	1	2.50	2.57	0.1530
B^2	10.89	1	10.89	11.19	0.0123
C^2	0.7410	1	0.7410	0.7615	0.4118
Residual	6.81	7	0.9731		
Lack of Fit	4.10	3	1.37	2.01	0.2550
Pure Error	2.72	4	0.6791		
Cor Total	317.36	16			

Table no.4 Fit Statistics

Std. Dev.	**0.9864**		**R^2**	**0.9785**
Mean	**80.64**		**Adjusted R^2**	**0.9509**
C.V. %	**1.22**		**Predicted R^2**	**0.7802**
			Adequate Precision	**20.3617**



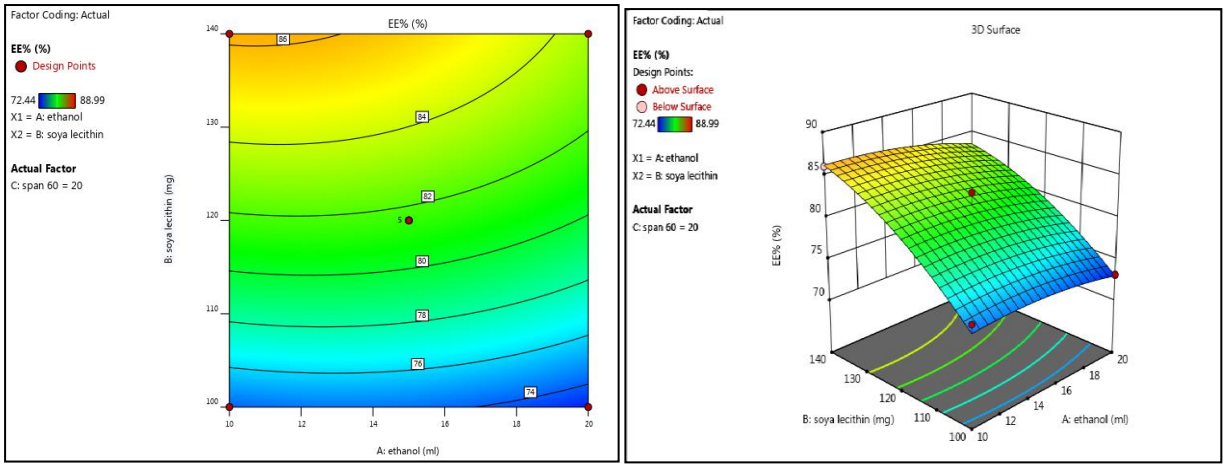


Fig no.2. Effect of ethanol and soya lecithin on EE

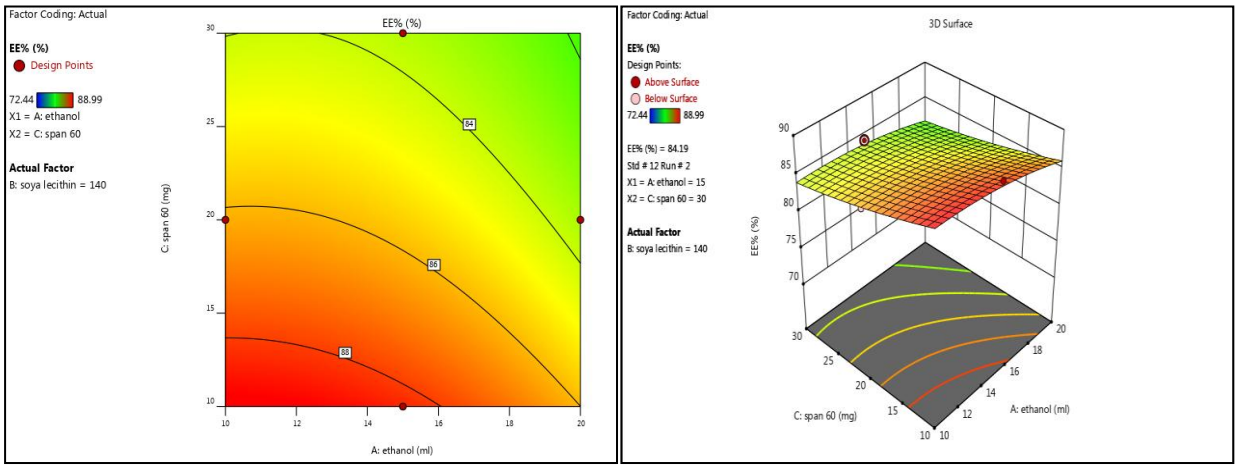


Fig no. 3. Effect of span 60 and ethanol on EE

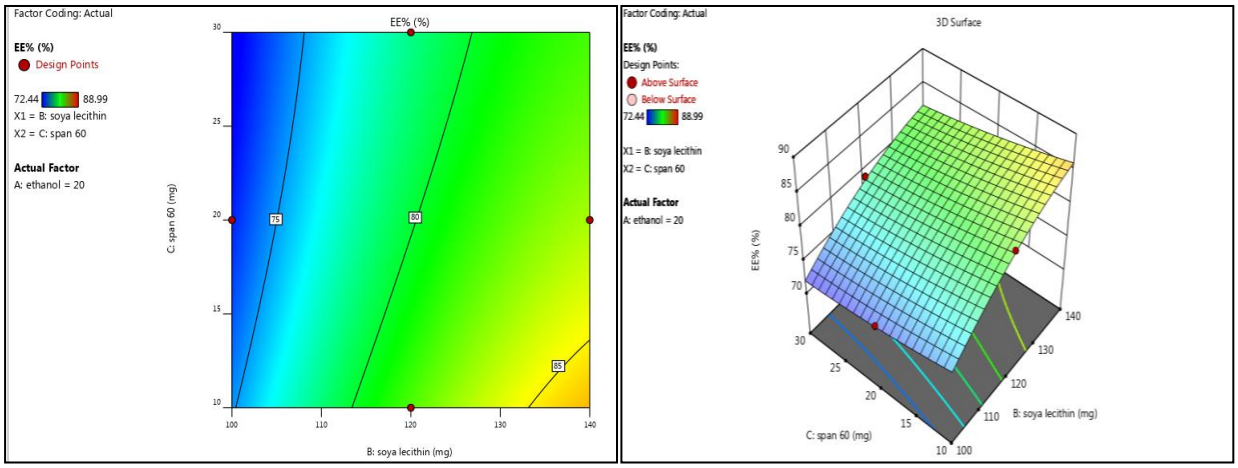


Fig no. 4. Effect of span 60 and soya lecithin on EE

A-ethanol:

No significant linear change in EE.

B-soya lecithin:

As soya lecithin initially ↑, EE ↓ (up to an optimum), then as soya lecithin continues to ↑, EE ↓. (Based on typical response surface plots like the one seen previously).

C-span 60:

This suggests that as Span 60 initially ↑, EE ↑ (up to an optimum), then as Span 60 continues to ↑, EE ↑. (Similar to soya lecithin, implying an optimal mid-range for Span 60).

Interactions (AB, AC, BC):

Combinations of changes in factors (e.g., A & B together) ↔ No statistically significant *interactive* effect on EE. This means the effect of one factor doesn't significantly change depending on the level of another in a synergistic/antagonistic way for the linear interactions.

Response 1: EE%

EE% = +81.37- 0.9537 A +5.65 B – 2.02 C -0.3875 AB + 0.2650 AC – 0.3375 BC – 0.7705 A^2 - 1.61 B^2 + 0.4195 C^2

There is factor coding.

Sum of squares is **Type III - Partial**

The importance of the model is highlighted by its F-value of 35.46. There's only a 0.01% chance that such a high F-value could be due to random noise.

In this case, the model terms A, B, C, and B2 are considered significant since their P-values are below 0.0500. If the P-values exceed 0.1000, then those model terms aren't significant. If your model has a lot of unnecessary terms—aside from those needed to keep the hierarchy—consider reducing it for better performance.

When we look at the Lack of Fit, it's not a major concern, as indicated by the Lack of Fit F-value of 2.01, which has a 25.50% chance of being attributed to noise. Overall, the lack of fit isn't significant

Table no.5 Shows Fit Statistics

Table no.5 Response 2: Vesicle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2577.26	9	286.36	204.33	< 0.0001	significant
A-ethanol	3.41	1	3.41	2.43	0.1630	
B-soya lecithin	109.89	1	109.89	78.41	< 0.0001	
C-span 60	0.0406	1	0.0406	0.0290	0.8696	
AB	1.20	1	1.20	0.8555	0.3858	
AC	0.0342	1	0.0342	0.0244	0.8802	
BC	3.28	1	3.28	2.34	0.1701	
A^2	3.90	1	3.90	2.78	0.1392	
B^2	2265.30	1	2265.30	1616.33	< 0.0001	
C^2	128.76	1	128.76	91.87	< 0.0001	
Residual	9.81	7	1.40			
Lack of Fit	8.10	3	2.70	6.32	0.0535	not significant
Pure Error	1.71	4	0.4274			
Cor Total	2587.07	16				

The Predicted R2 of 0.7802 and the Adjusted R2 of 0.9509 are quite close, with a difference of less than 0.2, which is a good sign.

We measure the signal-to-noise ratio using Adequate Precision, and ideally, we want that ratio to be over four. With a signal

strength of 20.362, you're in a good spot. This model allows you to effectively navigate the design space.

Final Formula with Coded Elements

EE% = +81.37-0.9537 A +5.65 B – 2.02 C -0.3875 AB + 0.2650 AC – 0.3375 BC – 0.7705 A^2 - 1.61 B^2 +0.4195 C

When you have specific amounts of each element, you can make predictions about the reaction using an equation that's based on coded factors. Typically, the high and low levels of the components are represented by +1 and -1, respectively. By looking at the coefficients of these factors, you can figure out how important each element is in the equation.

Conclusion:

Soya lecithin and Span 60 are the key drivers for EE, both showing optimal concentrations. Ethanol, within the tested range, appears to have less direct impact. The model is statistically robust and adequately explains the observed variability

Fit Statistics Table No. 6

Std. Dev.	**1.18**		**R^2**	**0.9962**
Mean	**191.59**		**Adjusted R^2**	**0.9913**
C.V. %	**0.6179**		**Predicted R^2**	**0.9489**
			Adequate Precision	**36.7935**

3.1.2 Independent factors' effects on Vesicle size (11)

As shown in fig.no.5, 6, 7 and 8 respectively

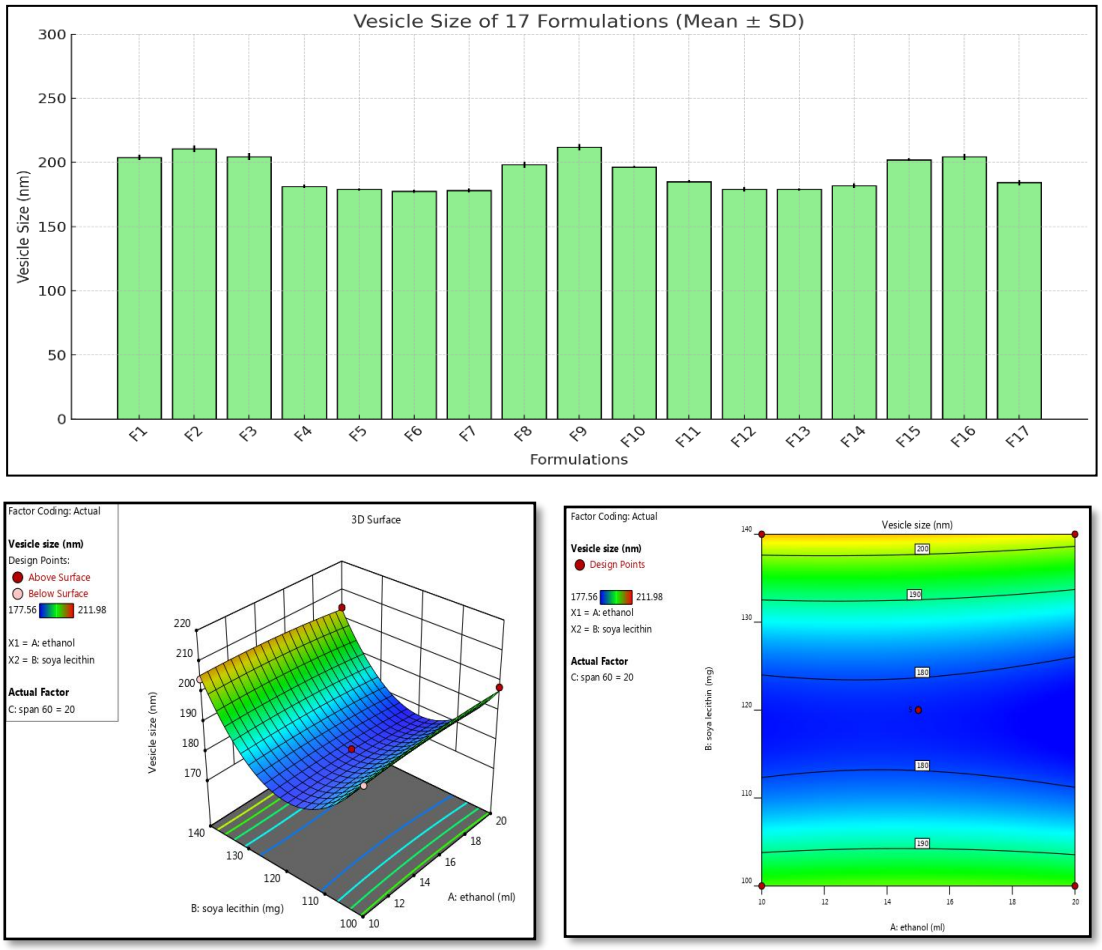


Fig No. 6.. Efect of ethanol and soya lecithin on vesicle size

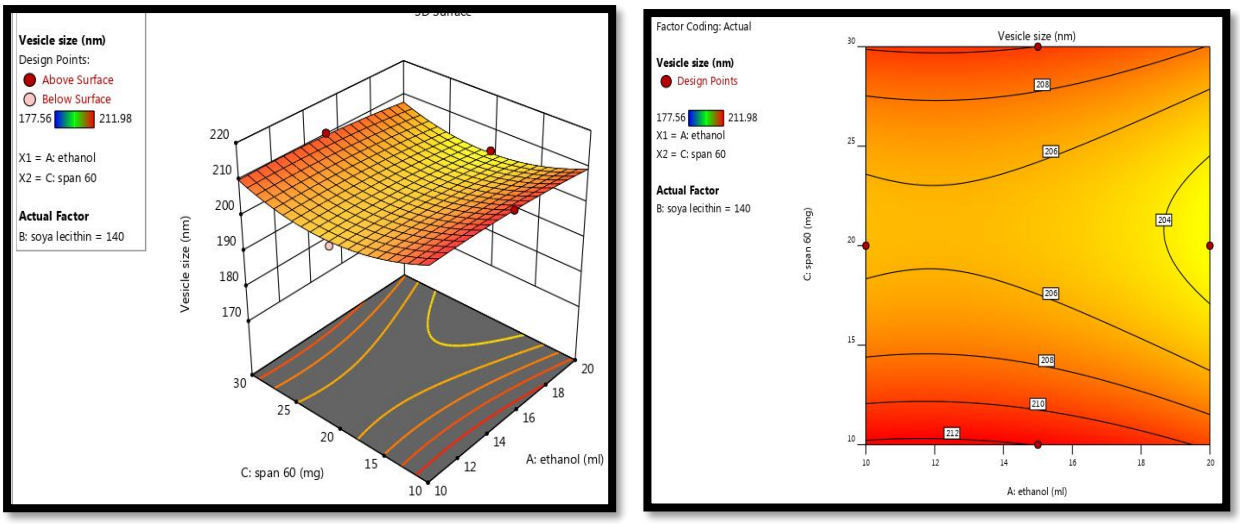


Fig No. 7. Efect of ethanol and span 60 on vesicle size

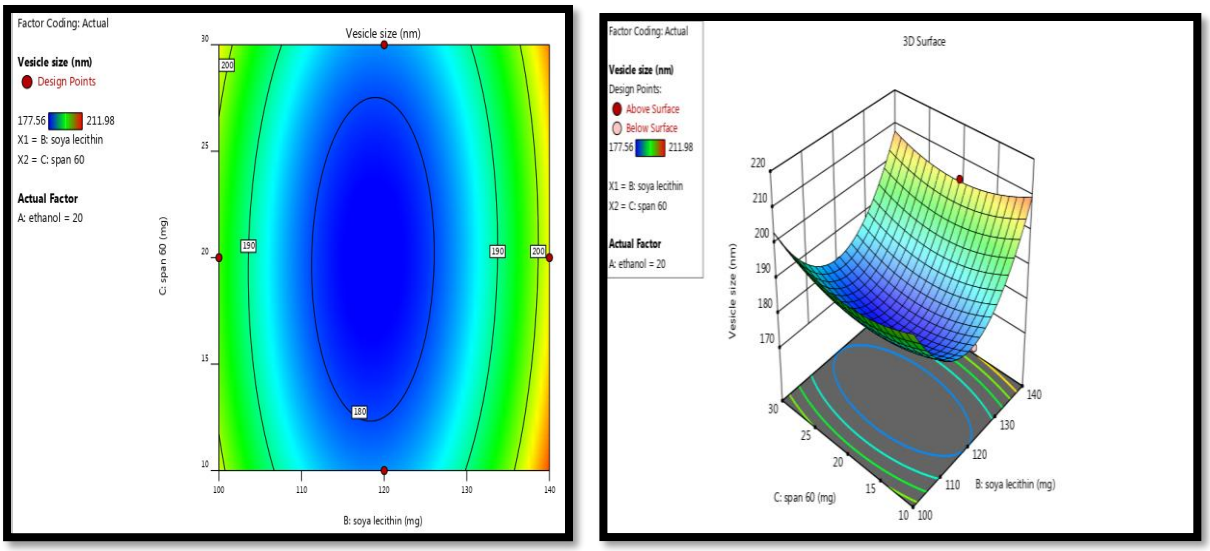


Fig No.8. Efect of soya lecithin and span 60 on vesicle size

A-ethanol:

Vesicle size did not significantly alter linearly..

B-soya lecithin:

As soya lecithin initially ↑, vesicle size ↓ (up to an optimum), then as soya lecithin continues to ↑, vesicle size ↑. (Based on typical response surface plots like the one seen previously).

C-span 60:

This suggests that as Span 60 initially ↑, vesicle size ↓ (up to an optimum), then as Span 60 continues to ↑, vesicle size ↑. (Similar to soya lecithin, implying an optimal mid-range for Span 60).

Interactions (AB, AC, BC):

Combinations of changes in factors (e.g., A & B together) ↔ No statistically significant *interactive* effect on vesicle size. This means the effect of one factor doesn't significantly change depending on the level of another in a synergistic/antagonistic way for the linear interactions.

There is factor coding.

Partial Type III sum of squares

The importance of the model is highlighted by its impressive F-value of 204.33. There's only a 0.01% chance that such a high F-value could be due to random noise.

In this case, model terms are deemed significant when the P-value falls below 0.0500, and here, B, B2, and C2 stand out as key terms. If the P-values exceed 0.1000, those model terms aren't considered significant. If your model is cluttered with unnecessary terms—aside from those needed to keep the hierarchy intact—consider reducing it to enhance its effectiveness.

With a Lack of Fit F-value of 6.32, noise has a 5.35% probability of producing such a high number. A model that does not fit is not what we want. The chance is extremely low (less than 10%), which is worrying.

Table No. 7 Shows Fit statistics

Table no.7.Optimize Values of Ferulic acid loaded Transethosomes

Responses	Predicted Value	Observed Value
Entrapment Efficiency (%)	**78.8713 %**	**78.98±0.85**
Vesicle size (nm)	**186.463 nm**	**181.28±0.41**

With a difference of less than 0.2, the Predicted R2 of 0.9489 and the Adjusted R2 of 0.9913 show a solid level of agreement. The Adequate Precision measures the signal-to-noise ratio, and ideally, this ratio should be over four. Your ratio of 36.794 indicates a strong signal. This model allows for effective navigation through the design space.

Final Formula with Coded Elements

Vesicle size = +178.53-0.6525 A +3.71 B – 0.0712 C -0.5475 AB + 0.0925 AC – 0.9050 BC – 0.9625 A^2 +23.19 B^2 + 5.53C^2

When you have specific amounts of each element, you can make predictions about the reaction using an equation that incorporates coded factors. Typically, the high and low levels of these components are represented by +1 and -1, respectively. By looking at the coefficients of the factors, you can figure out how important each element is in the equation.

.Conclusion:

Soya lecithin and Span 60 are the key drivers for vesicle size, both showing optimal concentrations. Ethanol, within the tested range, appears to have less direct impact. The model is statistically robust and adequately explains the observed variability.

3.2. Optimize formulation of FA loaded Transethosomes:

The final optimized formulation was found to have an overall desirability function of 1, with ethanol = 20 ml, soya lecithin = 120 mg, and span 60 = 30 mg. **Table no. 5.Optimize Values of Ferulic acid loaded Transethosomes** The predicted percentage error was determined by comparison the experimental observed values with the expected values, as indicated in Table No. 7. The optimized Transethosomes formulation (F4) yields a good EE of 78.98% and a vesicle size of 181.28 nm. When compared to the optimized formula produced by Box-Behnken Design (BBD) in Design of Experiments (DOE), this formulation is thought to be the best.with 3 factors at 3 levels As shown in fig no. 9. (12)

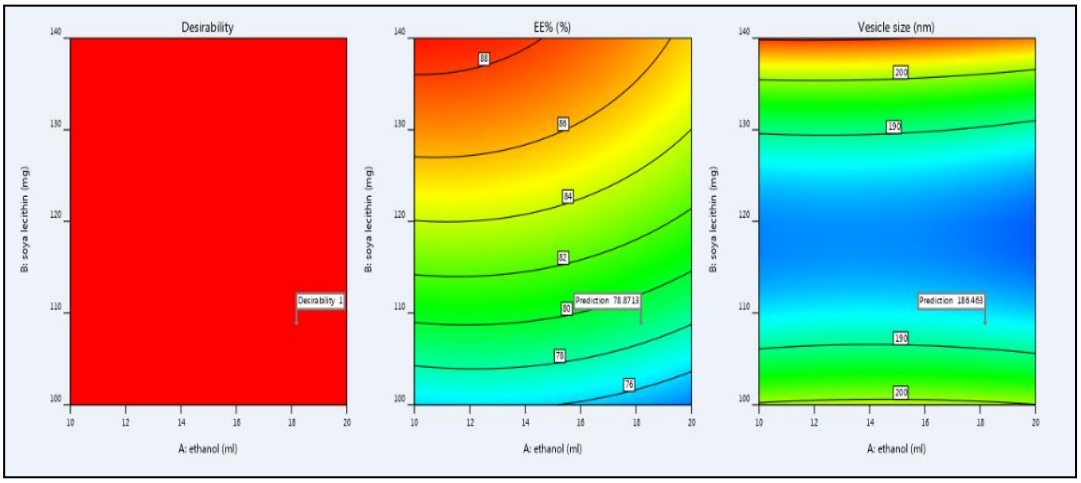


Fig no. 9 Contour Plot of Optimized Formulation as A Function of Y1 and Y2 (a) For Overall Desirability (D); (A) For EE%; (C) For Vesicle size

3.3. Zeta potential:

A value of -22.91 mv suggests moderate to good stability for your transethosomal formulation**.** There's enough electrostatic repulsion between the Transethosomes to prevent rapid aggregation or Fusion, which is crucial for maintaining their integrity and thus their drug delivery function.

The optimized Ferulic acid-loaded transethosomal formulation had a zeta potential of −22.91 ± 0.6 mV, which represents moderate negative surface charge. Although values above ±30 mV are commonly viewed as highly stable, a zeta potential of −20 to −30 mV is usually sufficient for stabilizing colloidal systems, especially in non-ionic surfactant-based systems. The low standard deviation indicates stable and reproducible measurements. (13) As in Fig no.10 Zeta potential of optimized batch (F4) Transethosomes

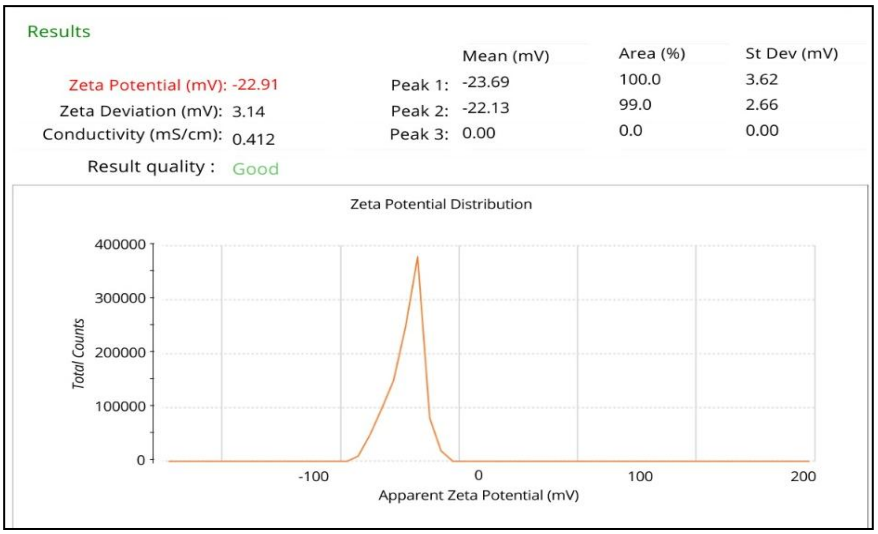


Fig no.10 Zeta potential of optimized batch (F4) Transethosomes

3.4. Scanning Emission Microscope:

As Fig no. 11 SEM images depicting surface morphology of optimized batch (F4) transethosomes. The SEM photograph shows an assembly of round or nearly round particles, which are typical morphologies for Transethosomes. The particles are of varying sizes and seem to be aggregated or clustered in groups in some regions. The Transethosomes surfaces have some roughness or texture. Some of the particles seem to be intact and clearly defined, while some others may display minute deformations or abnormalities. (14)

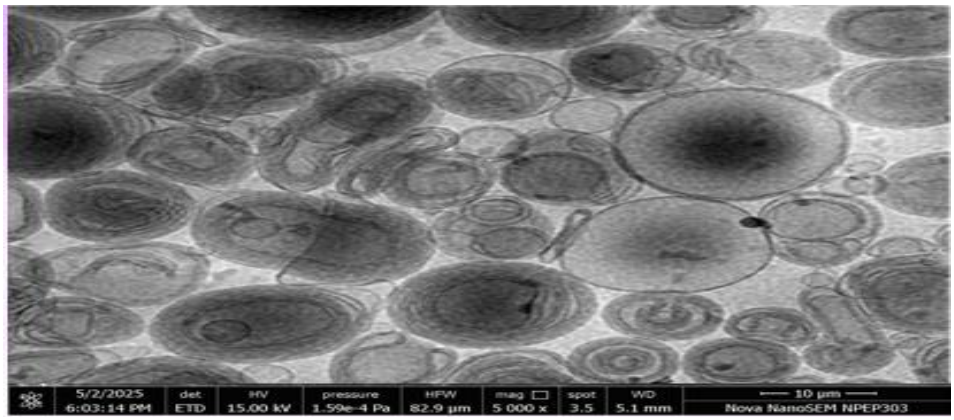


Fig no. 11 SEM images depicting surface morphology of optimized batch (F4) Transethosomes

3.5. Invitro Drug release study (15)

As shown in **Table no.8: % Drug release from Ferulic acid loaded Transethosomes F4 Formulation and Fig No.12: Cumulative % Drug release from Ferulic acid loaded Transethosomes F4 Formulation.** The cumulative percentage release of Ferulic acid from Transethosomes over 24 hours is shown in fig12. Within the first hour, a first burst release of

about 22% was seen, most likely as a outcome of the API's surface-associated release. The ferulic acid-loaded Transethosomes showed a prolonged and regulated release pattern in vitro, indicating their promise as a dermal delivery method. This was followed by a sustained release phase that lasted up to 24 hours. To assess skin penetration and treatment effectiveness, more in-vivo research is advised.

Table no.8: % Drug release from Ferulic acid loaded Transethosomes F4 Formulation

Time (hr)	% Drug Release from Ferulic acid loaded Transethosomes(Calculated)
0	0.00 ± 0.00
1	21.8 ± 0.05
2	34.6 ± 0.26
4	47.9 ± 0.34
6	53.1 ± 1.28
8	58.5 ± 0.21
10	65.5 ± 0.11
12	76.1 ± 0.39
14	79.0 ± 1.14
16	83.2 ± 0.21
18	85.4 ± 1.22
20	89.9 ± 0.18
22	93.3 ± 1.08
24	96.7 ± 0.42

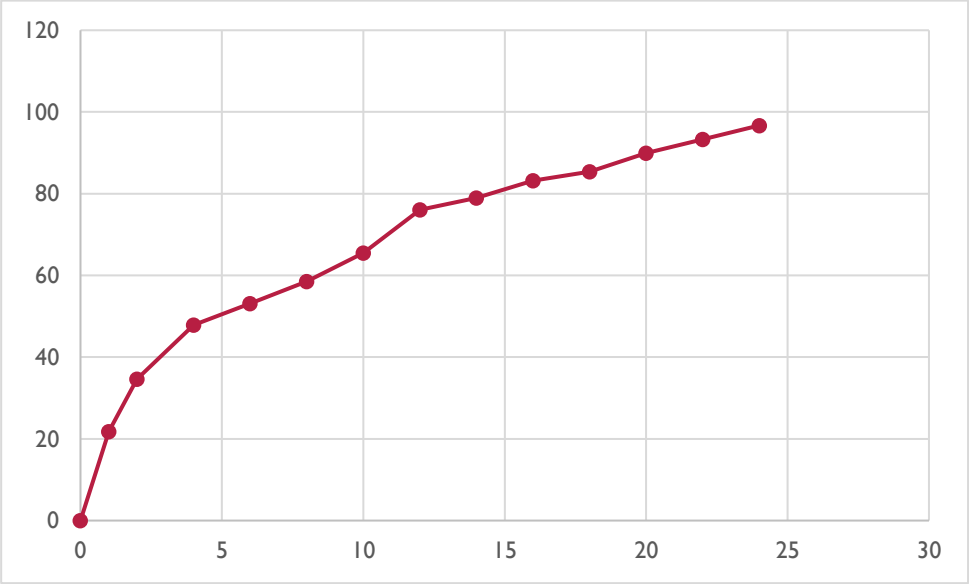


Fig No.12: Cumulative % Drug release from Ferulic acid loaded Transethosomes F4 Formulation

4.1 Organoleptic characteristics: (16)

As shown in Table no. 9.

Table no. 9. Conclusion of organoleptic properties

Parameter	Observation
Appearance	Smooth, homogeneous, semi-solid, no phase separation
Colour	Clear, Transparent
Consistency	Thick gel, spreads easily on the skin
Texture	Non-gritty, soft, smooth on application
Stickiness	Non-sticky or slightly tacky, easily washable

4.2. Gel Parameters: (17)

As shown in Table no.10

Table no.10 Ferulic acid loaded Transethosomal gel parameters

PH Measurement	6.5 ± 0.10
Viscosity	4990 ±33.5 cP
Spreadability	11.56 ± 0.4 g.cm/sec

4.3. In vitro Permeation study

As shown in Table no.11.

The in vitro release data were fitted to a number of mathematical models, including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models, in order to comprehend the process of drug release from the transethosomal gel filled with Ferulic acid. The table below summarizes the findings of the evaluation of the best-fitting model using the kinetic parameters and regression coefficients (R2 values). (18)

Table no.11. Drug permeated from Transethosomal gel and Plain gel

Time (hours)	Drug released from Transethosomal gel	Drug released from plain gel
0	0.00 ± 0.00	0.00 ±0.00
1	9.2 ± 0.14	4.1±0.14
2	15.4 ± 0.28	6.7±0.28
4	24.4 ± 0.42	11.2±0.28
6	32.3 ± 0.28	14.7±0.26
8	41.6 ± 1.57	18.1±0.35

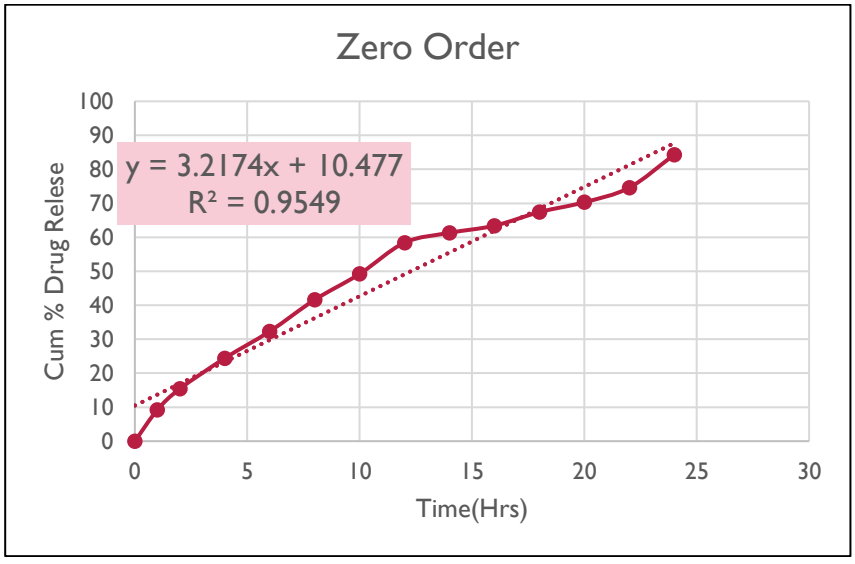
10	49.2 ± 0.57	23.4±1.29
12	58.4 ± 0.85	28.2±0.42
14	61.3 ± 0.71	29.5±0.35
16	63.4 ± 0.13	32.0±0.71
18	67.4 ± 0.27	34.0±0.73
20	70.3 ± 0.99	36.0±0.69
22	74.6 ± 0.71	37.8±0.43
24	84.3± 1.13	39.0±0.70

As shown in Table no.12 and 13

The drug release by the Transethosomal gel is best described by the Higuchi model out of all of them; it shows efficient diffusion-based sustained release from the gel-Transethosomes matrix. It is ideal for topical delivery, where steady release and prolonged skin contact are needed for therapeutic effect. (19)

Table no.12. The table interpreting drug release kinetics of F4 formulation formulated in transethosomal gel

Kinetics Model	Equation	R^2 Value	Fit Quality	Mechanism Type
Zero-Order Kinetics	Qt=k0 .t	0.9549	Good fit	Constant release
First Order Kinetics	Qt=Q∞·(1−e−k·t)	0.9724	Good fit	Concentration-dependent
Higuchi Model	*Qt=kH .√t*	0.9905	Excellent fit	Diffusion-controlled
Korsmeyer- Peppas Model	*Qt= kKP .tn*	0.6692	Moderate	Super Case-II



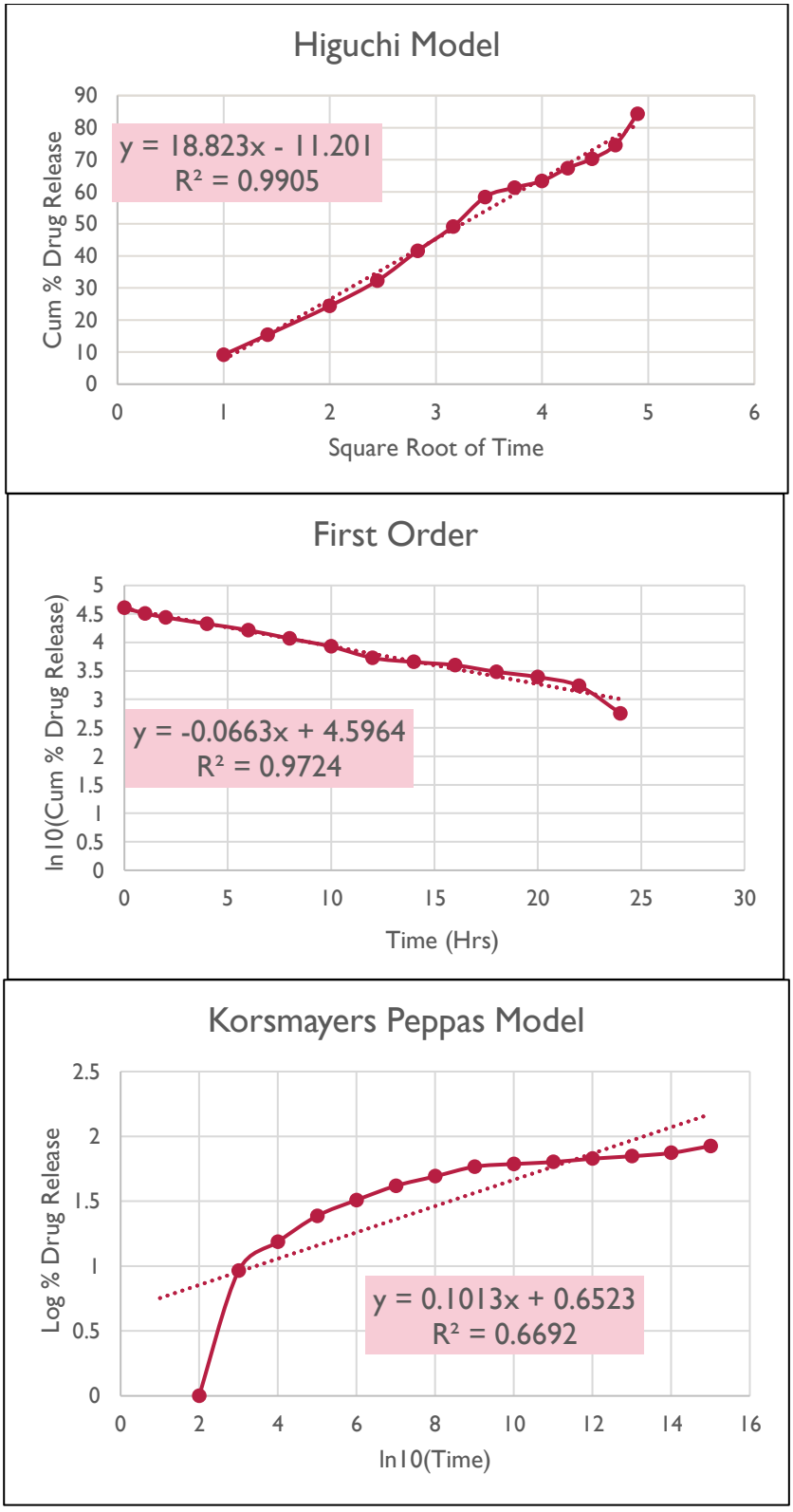


Fig NO. 13 Fitting model Kinetics

As shown in Fig no.14. Comparison of drug release

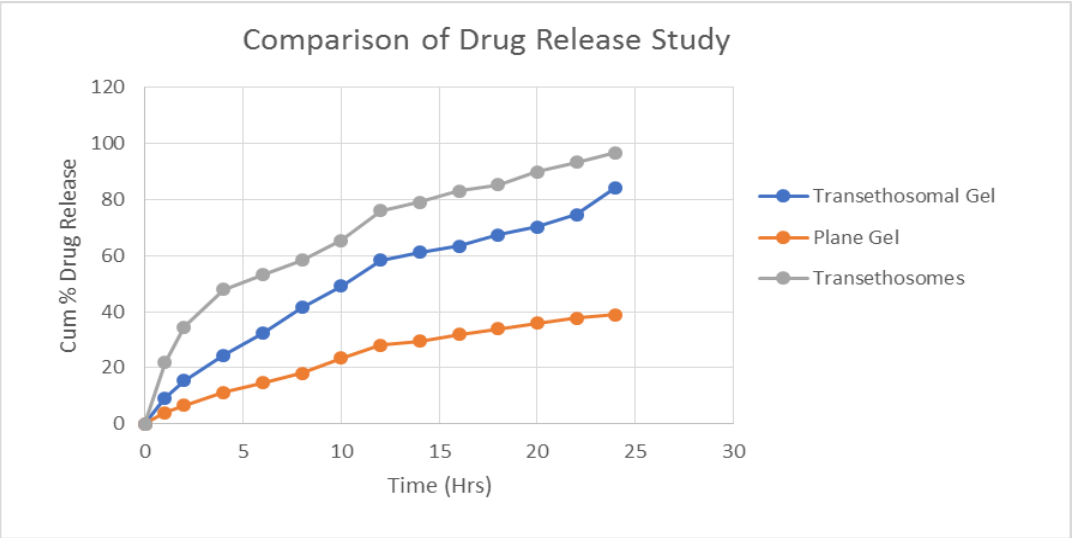


Fig no.14. Comparison of drug release

- Transethosomal gel enhanced the release compared to plain gel, indicating better permeation and sustained delivery.
- Transethosomes suspension (F4) alone showed maximum release, suggesting faster release.
- Thus, transethosomal gel provides controlled release and improves the delivery profile of Ferulic acid for topical application.

Conclusion

- The **optimized transethosomal gel** of Ferulic acid was successfully formulated using the **Box–Behnken design**.
- It showed:
  - **Entrapment efficiency**: **78.98 ± 0.85%**
  - **Vesicle size**: **181.28 ± 0.41 nm**
  - **Zeta potential**: **–22.91 ± 0.6 mV** (indicating good stability)
- **In vitro drug release** was **96.7% over 24 hours**, following **Higuchi diffusion model**, ensuring **sustained release**.
- The gel showed **better skin permeation** (**84.3%**) than the plain gel (**39.0%**) across 24 hours.
- **Physicochemical properties** (pH, viscosity, spreadability) were found suitable for topical application.

According to the study, transethosomal gel filled with ferulic acid is an impactful topical drug delivery method that offers improved penetration, controlled release, and possible anti-inflammatory advantages.

This study successfully demonstrated that the Box–Behnken Design (BBD) is a reliable appliance for optimizing formulation variables in transethosomal systems. The concentration of phospholipids (soya lecithin) and surfactant (Span 60) significantly influenced vesicle size and entrapment efficiency. Ethanol concentration had a minor effect but supported membrane fluidity and penetration enhancement.

The optimized batch (F4) produced stable vesicles with a favorable zeta potential, as confirmed by SEM analysis showing spherical, smooth particles with minimal aggregation. The gel's intended topical application was supported by the in vitro drug release, which followed Higuchi kinetics and showed diffusion as the main release mechanism.

Furthermore, the transethosomal gel outperformed the plain gel in in vitro permeation studies, highlighting its superior drug delivery potential. These findings underscore the system's efficiency in enhancing skin penetration and sustaining drug release — crucial for managing inflammatory skin conditions.

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