FORMULATION, EVALUATION AND OPTIMIZATION OF TOPICAL SERTACONAZOLE NITRATE EMULGEL BY BOX-BEHNKEN DESIGN

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Abstact

The primary objective is to produce sertaconazole nitrate emulgel to enhance the topical release of the drug via Carbopol 934P. Different types and amounts of gelling agent, surfactant, and oil (stearyl alcohol) were used to investigate the drug release.

The manufactured emulgels were evaluated for their appearance, pH, viscosity, spreadability, extrudability, in vitro and in vivo drug release, and stability. Observations were discovered to fall within the acceptable range. The in-vitro release rate was measured using a diffusion cell with a dialysis membrane and phosphate buffer pH 7.4 as a medium. Box-Behnken design was used to optimise the system. Ex-vivo studies revealed that the EM (overlay plot) containing Carbopol 934P (1.8%), surfactant (2.62%), and oil (10%) released the least quantity of drug, 12.3%.

The globules have a diameter of 1714 d.nm and a polydispersity index of 0.638%. These figures indicate that the formulation is consistent. The zeta potential has been determined to be -11.1mv. The emulgels were found to be stable for a month at ambient temperature and in the refrigerator. Therefore, it was determined that EM was the finest, as it was stable and had an anamolous transport method. An emulgel containing sertaconazole nitrate has the potential to treat fungal infections, according to a study.

Keywords: Emulgel, Sertaconazole nitrate, Carbopol 934P, Box-Behnken design

INTRODUCTION

Locally administered medications can be administered via the epidermis, vaginal canal, rectal canal, or eyes[1]. It is possible to create topical medications from solids, liquids, and semisolids (such as granules, creams, and lotions). Depending on the nature and severity of the disease and when other methods of administering the medication are limited, topical administration is preferable. The majority of antiseptics, antifungals, and emollients are administered topically.

Several common topical formulations, such as creams, gels, ointments, powders, and lotions, have issues with stability, stickiness, inadequate permeability, irritation, and absorption of large molecules. The advantages of external drug administration are that patients are more likely to take their medication, that it is simple to initiate and discontinue, that first-pass metabolism is avoided, and that stomach discomfort is diminished[2,3]. In comparison to creams and ointments, gel formulations deliver medicine to the body more rapidly, are simpler to apply, and are more fluid. When it comes to administering hydrophobic drugs gels present a significant challenge[4,5].

A two-phase system consisting of two liquids that do not combine is an emulsion. In the second phase, one of the liquids is distributed consistently and precisely as globules[6]. Since emulsions are thermodynamically unstable, they must be stabilised by adding an emulsifier, which forms a thin membrane over the spread phase globules[7]. The majority of the time, the particulate sizes in the dispersed phase range from 0.1 to 100 m[8].

Emulgels are the most intriguing topical delivery options because they combine an emulsion and a gel with a dual release control system[9,10]. With topical treatment, the medication can be applied directly to the affected area and remain there for an extended period of time, which can be beneficial. In order to produce emulgels, an O/W or W/O emulsion is combined with a gelling agent. They create improved methods for administering medications that do not mix or dissolve well in fluids.

As an antifungal, sertaconazole nitrate is employed to treat dermatophytosis, tinea pedis, versicular dermatitis, and seborrheic dermatitis. As a BCS class II substance, it is nearly impossible to dissolve in water.

Sertaconazole nitrate is a medication used for the treatment of fungal infections. The Box-Behnken design, the gelling compound Carbopol 934p, and surfactants such as Tweens and Spans were utilised in this study to formulate an emulgel that would decrease drug release and increase skin retention. Physical, stability, and skin irritation tests were conducted on the manufactured emulgel.

MATERIALS AND METHODS

Sertaconazole nitrate originated from Varahi Chemicals. S.D.Fine Chemicals Ltd. was the supplier of Spans, Tweens, stearyl alcohol, methylparaben, and propyl paraben. Carbopol 934p was manufactured by Yarrow Chemicals Ltd.

EMULGEL PREPARATION

In this investigation, gel formulations were produced by dispersing carbopol 934P in purified water with constant, moderate-speed stirring. Using triethanolamine (TEA), the pH was then decreased to 6 to 6.5. To create the emulsion, the HLB technique was employed. Tween 20 was dissolved in purified water to create the water component of the emulsion, while Span 20 was dissolved in stearyl alcohol. Both methyl and propyl parabens were applied to the water and oil phases. The substance is added to the oil phase. The fluid phase and the oily phase were each heated to between 70 and 80 degrees Celsius separately. They were then combined and continuously agitated until they reached room temperature. According to Wesley Z. D'Souza et al. (2015), emulgel is created by combining gel and emulsion in a 1:1 ratio[11].

Calculations

Required HLB of Stearyl alcohol equals fourteen

Required components of the expression Span 20(8.6)=x

Essential components of Tween 20(16.7)=(100-x).

8.6(x)+16.7(100-x)=14 100

8.6x+1670-15x=1400

8.1x=270 X=33 (100-x)=67

Span 20

2.5% = 2.5(33/100) = 0.8gm

3.75% = 3.75(33/100) =1.23gm

5% = 5 (33/100) = 1.65gm

Tween 20

2.5% = 2.5(67/100) = 1.67gm

3.75% = 3.75(67/100) =2.51gm

5% = 5 (67/100) = 3.35gm

EXPERIMENTAL DESIGN

Box Behnken Design was used to make the emulgel better[12]. Using design expert 11, a three-factor, three-level design was chosen for optimisation in order to investigate the quadratic response surface (version 11; Stat-Ease Inc., Minneapolis, MN). Each independent variable or component is assigned one of three evenly distributed values, -1, 0, or +1. (low, average, or high). A quadratic model is one with squared terms, products of two components, linear terms, and an intercept. The plan should function well with a quadratic model[13]. **Table 1** displays the quantities of each factor. The results of formulas are shown in **Table 2**. In **Table 3**, the formulas utilise real numbers.

Table 1: Factors and factor levels of box-behnken design

Independent factors	**Levels**		
	Low	**Medium**	**High**
	-1	0	1
A= Oil	10	15	20
B=Surfactant	2.5	3.75	5
C=Gelling agent	1	1.5	2
Responses (Dependent factors)			
R1 - Drug release (%)			
R2 - Skin retention (%)			

Table 2: Formulation runs using Box-Behnken design

Std	**Runs**	**Factor A Oil**	**Factor B Surfactant**	**Factor C Gelling agent**	**R1-Drug release**	**R2-Skin retention**
13	1	0	0	0	19.7	80.3
3	2	-1	1	0	18.1	81.9
2	3	1	-1	0	21.3	78.7
4	4	1	1	0	21.8	78.2
7	5	-1	0	1	16.5	83.5
10	6	0	1	-1	20.7	79.3
1	7	-1	-1	0	15	85

6	8	1	0	-1	24.4	75.6
8	9	1	0	1	26	74
12	10	0	1	1	20.2	79.7
5	11	-1	0	-1	17.6	82.4
11	12	0	-1	1	18.9	81.1
9	13	0	-1	-1	19.4	80.6

Table 3: Actual values of formulation of sertaconazole nitrate emulgels using boxbehnken design

Formulation code	**Sertaconazole nitrate**	**Stearyl alcohol**	**Surfactants**	**Carbopol 934P**	**Methyl paraben**	**Propyl paraben**	**Water**
E1	2	15	3.75	1.5	0.03	0.02	q.s
E2	2	10	5	1.5	0.03	0.02	q.s
E3	2	20	2.5	1.5	0.03	0.02	q.s
E4	2	20	5	1.5	0.03	0.02	q.s
E5	2	10	3.75	2	0.03	0.02	q.s
E6	2	15	5	1	0.03	0.02	q.s
E7	2	10	2.5	1.5	0.03	0.02	q.s
E8	2	20	3.75	1	0.03	0.02	q.s
E9	2	20	3.75	2	0.03	0.02	q.s
E10	2	15	5	2	0.03	0.02	q.s
E11	2	10	3.75	1	0.03	0.02	q.s
E12	2	15	2.5	2	0.03	0.02	q.s
E13	2	15	2.5	1	0.03	0.02	q.s

EVALUATION OF EMULGELS

Fourier Transform Infrared (FTIR) Spectroscopy

Using Fourier Transform Infra-Red Spectroscopy (FTIR), the spectra of sertaconazole nitrate and excipients used to examine how well drug excipients function together and analysed. Potassium bromide (KBr) discs were manufactured at a Schimadzu Company facility in Kyoto, Japan, where FTIR spectra (model 8400S) were collected. A mixture of the sample and potassium bromide at a ratio of 1:10 was used to create KBr discs, which were then pressed through a hydraulic press at a pressure of 6-8 tonnes under a

vacuum. The manufactured disc was placed in the sample receptacle of an infrared spectrophotometer, and spectra were collected between 4000 cm-1 and 200 cm-1[14]. Compared the final spectrum to the mixture to determine if there were discernible differences. There were distinct peaks in the complex that indicated the location of the functional divisions. FTIR was used to ensure that the drug and excipients were compatible.

Assessing emulgels manufactured

The uniformity, pH, spreadability, extrudability, drug content, in vitro diffusion, ex vivo permeation, cutaneous irritation, drug excipient compatibility, and stability of emulgels were all evaluated in tests[15].

Homogeneity: The gel was visually examined to determine whether it was uniform and whether it contained any particles[16].

Spreadability

The emulgel (approximately 1 g) being examined is placed on a glass slide of the same dimensions as the first. For one minute, a 25-gram weight is placed on top of two slides to remove air and uniformly distribute the film between them. If there is excess emulgel, it is removed from the sides. The length of the emulgel that has been stretched out is recorded[17,18.]

Extrudability

Extrudable emulgel was tested by filling a collapsible lacquered aluminium tube until a minimum of 0.5 cm of emulgel ribbon could be extruded in 10 seconds. Larger quantities of extrusion are simpler to push out[19]. Measured each formulation's capacity to pass through a tube three times. The mean values are displayed in Table 19. This method is used to determine whether an object can pass through a hole:

$$Extrudbility = \frac{\text{Weight (in grammes) required to expel emulgel from the tube}}{\text{Area}}$$

Content analysis

1 g of emulgel is combined with the appropriate liquid. Filters are utilised to clarify the correct response. Using a UV spectrophotometer, absorbance is measured. The standard formulation of the drug is produced in the same liquid. Using the absorbance number, the same standard plot can be used to determine both the concentration and the quantity of drug present[20].

Studies on *in vitro* diffusion

To investigate how emulgels propagate, a modified KC diffusion cell was utilised. The cell was constructed nearby. The receptor compartment could contain 20 ml. Between the donor compartment and the receiver compartment was where the dialysis membrane mounting was located. On the clamped-together dialysis membrane and section, 0.5 mg of the medication was applied in emulgel form. The receptor section was filled with 7.4-pH phosphate buffer saline and stirred at 600 revolutions per minute with a magnetic stirrer. At predetermined intervals, five millilitres of material was removed and replaced with fresh buffer. Examined the samples for drug substance using spectrophotometry and the appropriate quantity of water[21].

Relase Rate of substance

The slope can be determined by plotting the quantity of medication consumed per square centimetre over time. The rate of release decreases. This quantity is A/cm2/hr1/2.

EVALUATION OF OPTIMIZED FORMULATION

Vesicle morphology, particle size and size distribution

Using scanning electron microscopy (SEM), the morphology of globules is examined. The Malvern Zetasizer Version 7.12 can be used to measure the size of globules. A Zetasizer can be used to determine the polydispersity index[22].

Zeta potential

Zetasizer (Version 7.12, Malvern Instruments) can examine zeta potential to determine the stability of an emulgel based on its colloidal property. Using zeta potential analysis, the net charge of each globule in an emulgel is determined. A greater surface charge repels the globules, which keeps them stable, prevents them from adhering together, and accelerates their settling, resulting in a more effective formulation[23].

Determining Viscosity

A Brookfield Viscometer was utilised to measure the thickness of the previously created samples. The viscosity-measuring formula was placed in the beaker and allowed to settle for 30 minutes at the test temperature (25±1℃) before the measurement was obtained. The spindle was placed in the centre of the emulgel without touching the bottom of the container. The object was then rotated at various velocities for 10 minutes. The viscosity was measured[24].

Determine the pH

A pH metre is utilised to determine the pH of emulgel mixtures. One gramme of gel is combined with one hundred millilitres of pure water for two hours. Each formula's pH is measured three times, and the average is then determined[25].

Ex-vivo skin penetration studies

a) Procedure for preparing rodent abdominal skin

For the permeation test (ID number: GPRCP/IEC/23/19/02/PCE/AE-4), male Wistar rats weighing between 150 and 200 g were used. After the animal was slain by cervical dislocation, its belly hair was removed using animal hair clippers. Cuts and wounds were examined on a sample of skin extracted from the abdomen. The surface fat of the epidermis was scraped off and washed with tap water. After a week of storage at -20°C, the skin was put to use.

b) Permeation Evaluation

The penetration experiments utilised modified KC diffusion cells with a surface area of 4.92 cm2 and a volume of 20 ml. After thawing the rat skin, the dermis was positioned facing the receptor solution. The donor compartment's stratum corneum received 500 mg of gel, while the hypodermis in the receiver compartment was stirred at 350 rpm using a magnetic stirrer. Over the course of eight hours, 1ml samples were taken at regular intervals and analysed with a UV-VIS double beam spectrophotometer at 261 nm to determine the amount of substance present.

c) Figuring out the permeability limits

In this section of the research, parameters were calculated in order to compare the drug transfer and penetration properties of the tested formulations. These parameters are discussed in terms of their skin content, permeability coefficient, and steady state flux.

1) Flux(µg/cm2/h)

Flux refers to the rate at which a substance moves through a porous membrane or the membrane itself. The rate of absorption is determined by plotting the total quantity of medicine absorbed over time in micrograms against hours. The latency time is indicated by the curve's X intercept.

The permeability coefficient (cm/hr)

The following equation was employed to calculate the permeability coefficient (Kp):

$$\mathbf{Kp = flux/cv}$$

where "cv" contributing volume represents the total quantity of contributor.

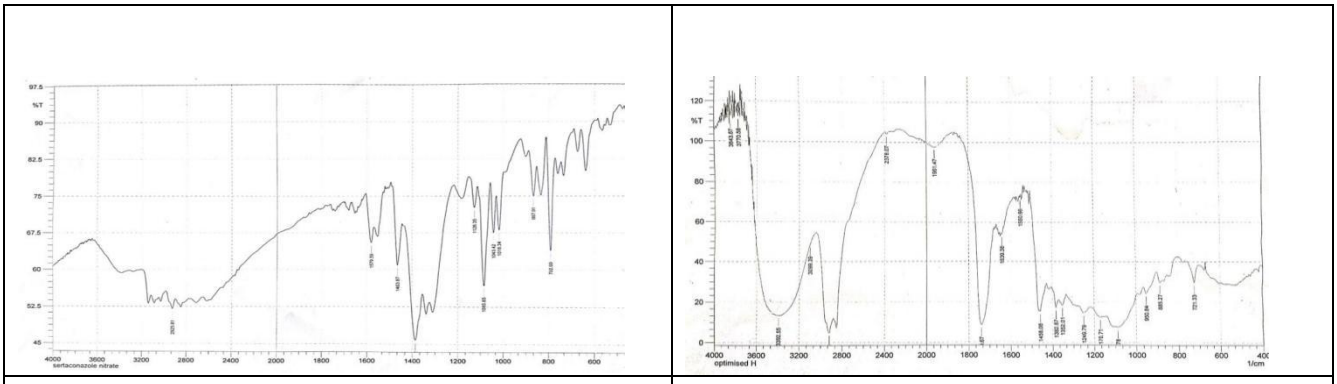
2) Drug content in epidermis

To determine how much sertaconazole nitrate was retained in the epidermis, "skin deposition tests" were conducted. After the 24-hour-long penetration experiments, the skin was cleansed with saline. A fragment of skin was weighed and mixed for 15 minutes with methanol using an electric mixer. Examined the supernatant at 261 nm using a UV-VIS double beam spectrophotometer[26] (Chemito Spectrascan UV2600, India).

RESULTS AND DISCUSSIONS

Fourier Transform Infra-Red (FTIR) Spectroscopy is utilised to determine the efficacy of a substance in conjunction with an excipient.

Utilising FTIR, the spectra of sertaconazole nitrate and its compatibility with the excipient were analysed. The FTIR spectra of products containing excipients demonstrate that the drug and excipient do not interact. The FTIR investigations of the spectra demonstrated that the drug and excipients did not react chemically. The FTIR spectra of the drug and formulation mixture are displayed in **Figure 1.**



a **b**

Figure 1: FTIR of a) Sertaconazole nitrate b) Formulation blend

Table 4: Evaluations for the formulation runs

Formulation	Spreadability(cm)	Drug content	Extrudability
E1	5.9±0.2	91.7±0.43	+++

E2	5.6±0.5	92.3±0.62	++
E3	4.8±0.3	94.3±0.25	++
E4	4.1±0.8	95.7±0.31	++
E5	3.8±0.1	98.9±0.58	+++
E6	5.2±0.6	92.9±0.81	++
E7	3.9±0.4	96.3±0.76	+++
E8	4.3±0.5	94.6±0.06	++
E9	6.1±0.8	90.2±0.92	++
E10	4.7±0.09	93.5±0.23	++
E11	4.5±0.1	95.9±0.68	+++
E12	4.2±0.4	97.2±0.59	+++
E13	5.7±0.7	94.1±0.33	++

Note: Values are expressed as Mean ± SD, n = 3; +++ = Excellent, ++ = Good, + = Satisfactory

Each formula was identical in appearance and was white. From 3.8 cm to 6.1 cm, the propensity to spread was observed. The drug concentration in emulgel was determined to be between 90,2 and 98,9. **(Table 4).**

Viscosity Calculation

Using a Brookfield viscometer with spindle number 64, the viscosity of the optimised emulsion was determined to be 6005 cps at 25°C. This indicates that the mélange is highly viscous.

pH calculation

The superior formulation had the appropriate pH and was simple to squeeze and spread, indicating that it could be used on skin.

In-vitro assays R1 stands for "response 1," which indicates the proportion of medication that is released. Since emulgel is administered to the skin, there should be decreased drug release and increased skin absorption. The amount of drug release was observed to range from 15.5% to 26.0%. The formulation with the lowest drug release was E7, which used the least quantity of oil and detergent. With a lower percentage of oil, more medication is deposited in the epidermis and less is released. With lower surfactant ratios, there may be less drug release and less dissemination. When penetration is minimal, there will be more skin deposition. By adding Carbopol 934P, an extremely water-loving polymer, the viscosity of the outer aqueous phase is increased. This reduces the likelihood that the dispersed globules

of the interior phase will collide and combine. After considering in-vitro drug release and additional investigations **(Figure 2),** the formulation (E7) with the lowest drug release was selected.

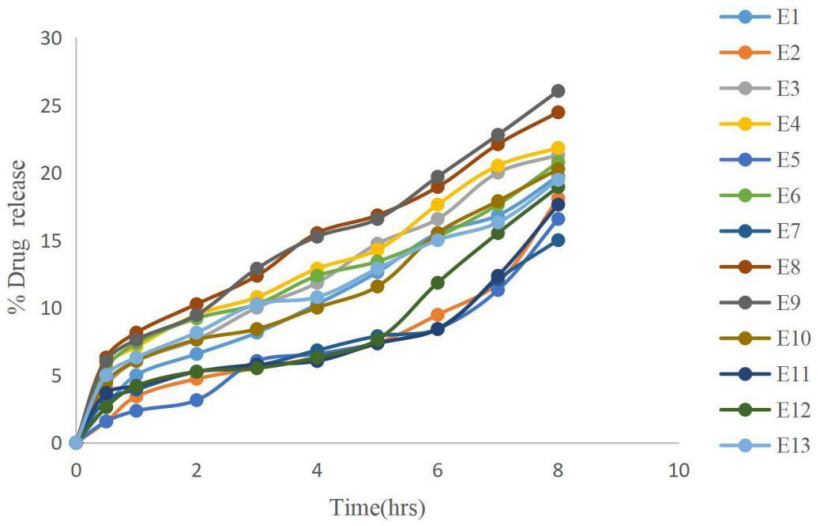


Figure 2:*In-vitro* diffusion studies of emulgel formulations

Analysis of responses

Fit summary:

The important statistics used to select the correct starting point for the final model were collected by fit summary

Table 6: Fit summary for responses

Response 1: Drug release					
Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	
Linear	**0.0007**		**0.7833**	**0.6420**	**Suggested**
2FI	0.6994		0.7393	0.2749	
Quadratic	0.3423		0.8046		
Cubic					Aliased
Response 2: Skin deposition					
Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R^2**	**Predicted R^2**	
Linear	**0.0007**		**0.7838**	**0.6427**	**Suggested**
2FI	0.6986		0.7401	0.2770	
Quadratic	0.3415		0.8055		
Cubic					Aliased

Fit summary: The suggested linear model has an adjusted R2 of 0.7833 and a predicted R2 of 0.6420 for response 1, i.e., drug release. The adjusted R2 value for response 2 (skin deposition) is 0.7838, while the predicted R2 value is 0.6427. There is no p-value for lack of fit, indicating that the model is significant and cubic model is aliased (Table 6).

Fit statistics

Table 7: Fit statistics for responses

Response 1: Drug release			
Std. Dev.	1.40	**R^2**	0.8375
Mean	19.97	**Adjusted R^2**	0.7833
C.V. %	7.03	**Predicted R^2**	0.6420
		Adeq Precision	10.4538
Response 2: Skin deposition			
Std. Dev.	1.40	**R^2**	0.8378
Mean	80.02	**Adjusted R^2**	0.7838
C.V. %	1.75	**Predicted R^2**	0.6427
		Adeq Precision	10.4768

The difference between the Predicted R2 of 0.6421 and the Adjusted R2 of 0.7833 is less than 0.2 for response 1. Regarding response 2, the difference between the Predicted R2 of 0.6427 and the Adjusted R2 of 0.7838 is less than 0.2. The signal-to-noise ratio is measured by Adeq Precision. A ratio greater than four is preferred. A sufficient signal is indicated by the ratios of 10,454 for response 1 and 10,477 for response 2. The design space was navigated using this model **(Table 7).**

ANOVA summary

ANOVA was performed by the design for the respomses and were shown in Table:8

Table 8: ANOVA for the responses

Response 1: Drug release						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	91.38	3	941.30	15.46	0.0007	significant
A-oil	86.46	1	2305.20	43.88	< 0.0001	

B-surfactan t	4.90	1	494.55	2.49	0.1493	
C-gel	0.0242	1	24.15	0.0123	0.9142	
Residual	17.73	9	21.41			
Cor Total	109.12	12				
Response 2: skin deposition						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	91.44	3	30.48	15.50	0.0007	significant
A-oil	86.46	1	86.46	43.96	< 0.0001	
B-surfactan t	4.96	1	4.96	2.52	0.1467	
C-gel	0.0200	1	0.0200	0.0102	0.9219	
Residual	17.70	9	1.97			
Cor Total	109.14	12				

Coding factors is Coded. The sum of squares is a Type III - Partial function. The Model F-value of 15.46 for response 1 indicates that the model is significant. The Model F-value of 15.50 for response 2 indicates that the model is significant. There is a 0.07% probability that an F-value of this magnitude could be caused by noise. Less than 0.0500P-values indicate significant model terms. In this case, A is a significant model term. Values exceeding 0.1000 indicate that the model terms are not significant. If your model contains numerous insignificant model terms (excluding those required to support hierarchy), model reduction may enhance it.

Predicted to Actual

The observed (actual) response values were plotted against the predicted response values on a graph. It was observed from the graph that the actual values are close to the predicted values.

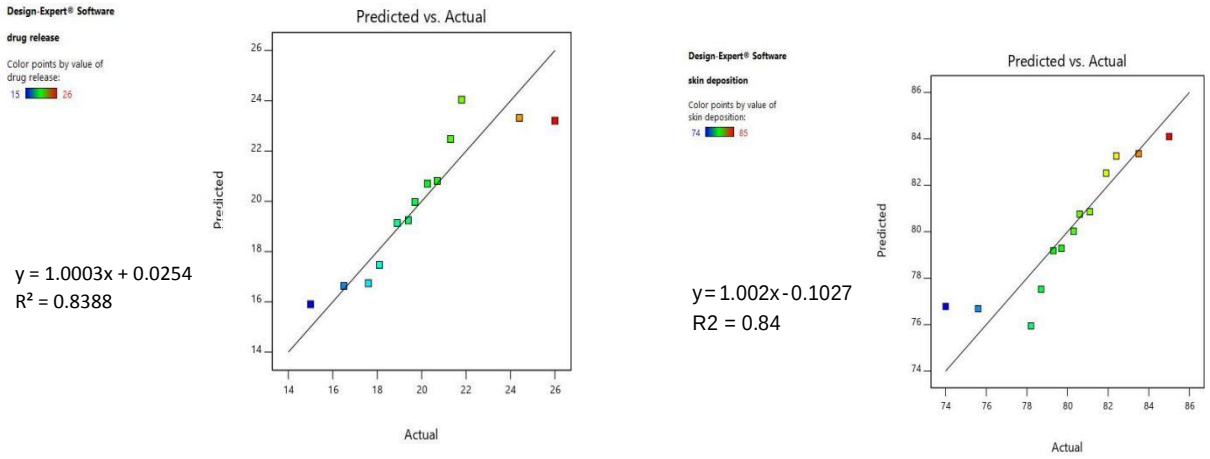
Table 9: Predicted vs. Actual values for the responses

Formulation code	Response 1: Drug release		Response 2: Skin deposition	
	Predicted	Actual	Predicted	Actual
E1	19.97	19.7	80.02	80.3
E2	17.46	18.1	82.52	81.9
E3	22.47	21.3	77.52	78.7
E4	24.04	21.8	75.94	78.2

E5	16.63	16.5	83.36	83.5
E6	20.81	20.7	79.18	79.3
E7	15.90	15	84.09	85
E8	23.31	24.4	76.68	75.6
E9	23.20	26	76.78	74
E10	20.70	20.2	79.28	79.7
E11	16.74	17.6	83.26	82.4
E12	19.13	18.9	8086	81.1
E13	19.24	19.4	80.76	80.6

Actual values are the values obtained in practise, whereas anticipated values are the values predicted by the design for the formulation runs based on the responses. The actual values should be close to those predicted. **(Table 9)**

Excel was used to plot a graph of predicted versus actual values to determine the R2 value, which should be close to 1 to indicate linearity of predicted versus actual values. R2 value for response 1 was found to be 0.838, and R2 value for response 2 was found to be 0.84; both values are close to 1 which shows the linearity of the values. **(Figure 3)**

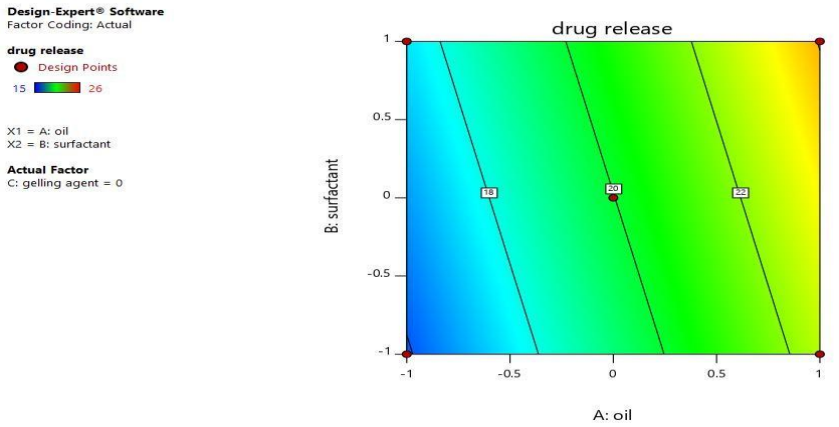


a **b**

Figure 3: Comparison of Predicted *vs* Actual values by design
a. Drug release B. Skin deposition

The predicted and actual values are depicted in Figure 3 by the coloured marks on either side of the straight line. In 2D and 3D graphs, the response to the effect of factors is reflected in the different colours of the points, which can be observed in a straightforward manner.

Two-Dimensional counter plots.



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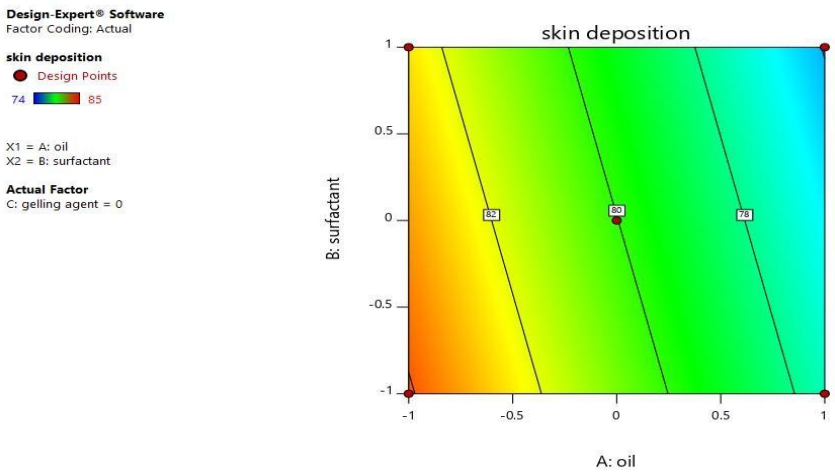
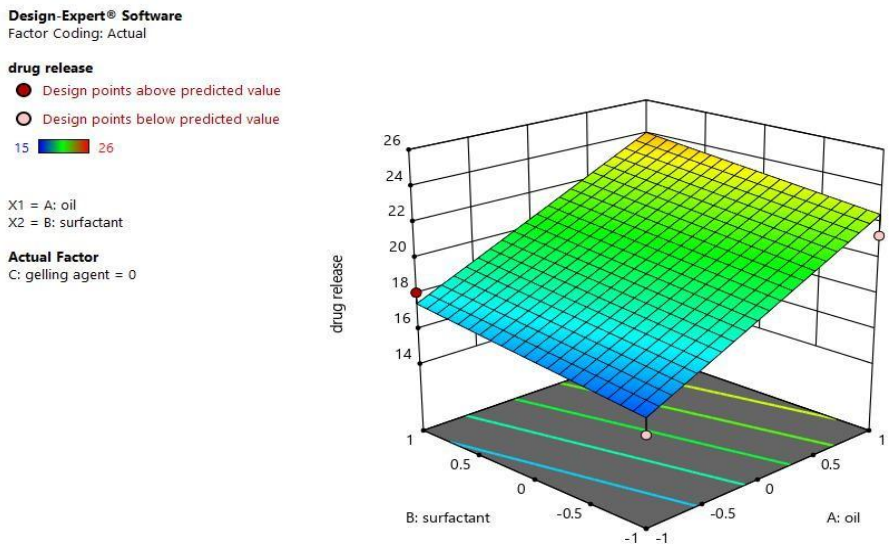


b

Figure 4: 2D counter plots a. Drug release b. Skin deposition

As shown in **Figure 4,** 2D Plots, the colour change is inverse, indicating that as drug release increased, epidermis deposition decreased and vice versa. This article demonstrates the effect of oil concentration and surfactant concentration on drug release and epidermis deposition. As shown in **Figure 4a,** drug release increases as the concentration of surfactants and oil increases. Minimum drug release was attained at lower surfactant and oil concentrations. The lowest drug discharge was observed near the colour blue. **Figure 4b** demonstrates that greater skin deposition was attained at lower surfactant and oil concentrations. As the oil and surfactant concentrations increased, epidermis deposition decreased. On the 2D plot, maximum epidermis deposition was observed near the colour red.

Three-Dimensional plots



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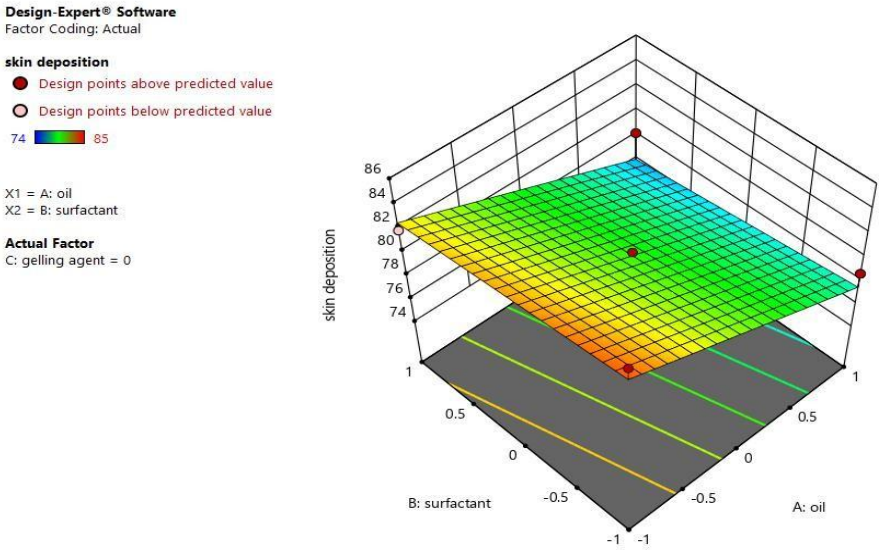


b

Figure 5: 3D Plot a. Drug release b. Skin deposition

From **Figure 5a's** 3D plots, we can observe that the area with the lowest drug release (lower oil and surfactant concentration) is coloured blue. As the drug discharge increased, a gradual transformation to a red hue was observed. From **Figure 5b**, we can see that the maximum amount of skin deposition was attained, which is depicted as a red hue. As oil and surfactant concentrations decreased, drug release increased and skin deposition decreased. Increased oil and surfactant concentration will increase drug release and permeation.

Color-coded 2D and 3D diagrams illustrate the relationship between the responses and factors. The 3D Surface plot is a projection of the 2D (contour) plot that adds shape to the colour and contour.

Optimization

The design was optimised based on the obtained responses, which were represented numerically and graphically.

Numerical optimization

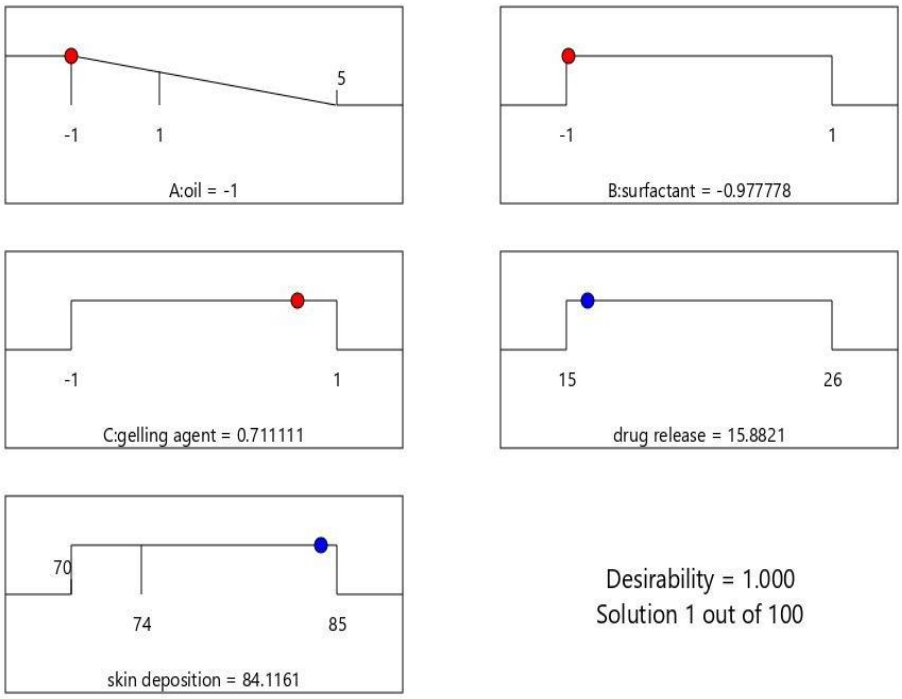


Figure 6: Numerical optimization

Optimisation is performed numerically based on the acquired responses and minimum and maximum criteria for drug release and skin deposition. On the basis of specified criteria, solutions will be found between the maximum and minimum limits. After providing numerical criteria, the solution or result will fall between the maximum and minimum limits. One hundred solutions with varying concentrations of oil, surfactant, and gelling agent were obtained, with little variation in concentration range. Based on the concentrations of the first solution out of a hundred, an emulgel was prepared and evaluated. The obtained concentrations are encoded numerically, which is decoded before the emulgel is prepared. The optimal factor parameters are represented by red dots. The optimal values for response prediction are depicted in blue **(Figure 6).**

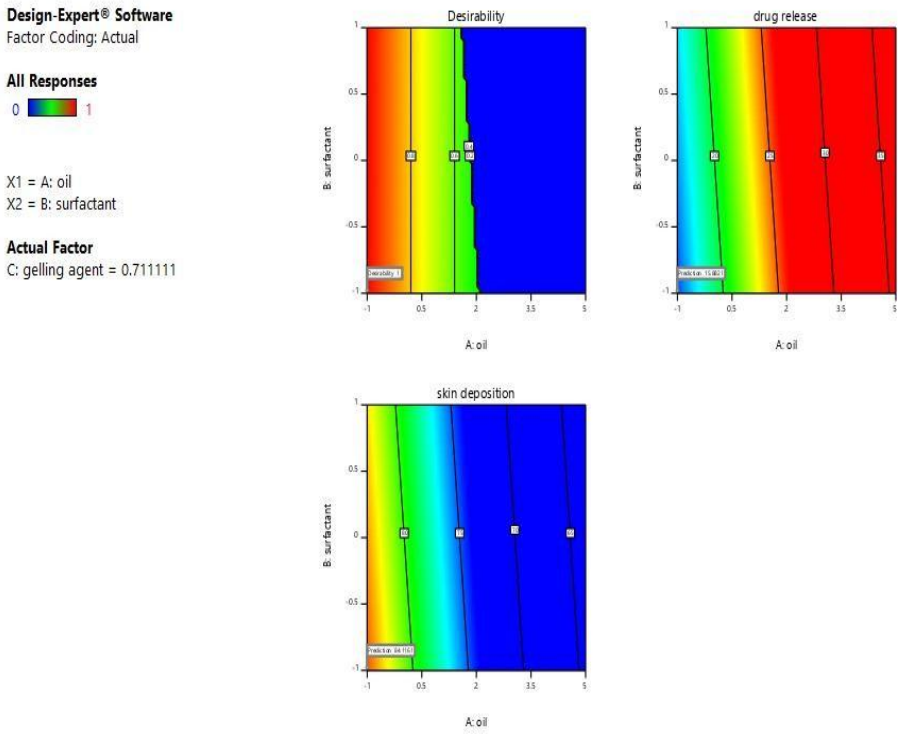


Figure 7: Desirability graphs

Figure 7 illustrates global 2D plots of responses, specifically drug release and skin deposition. Desirability is greater than 1, indicating that the predicted concentrations of factors will produce a superior response.

Graphical representation

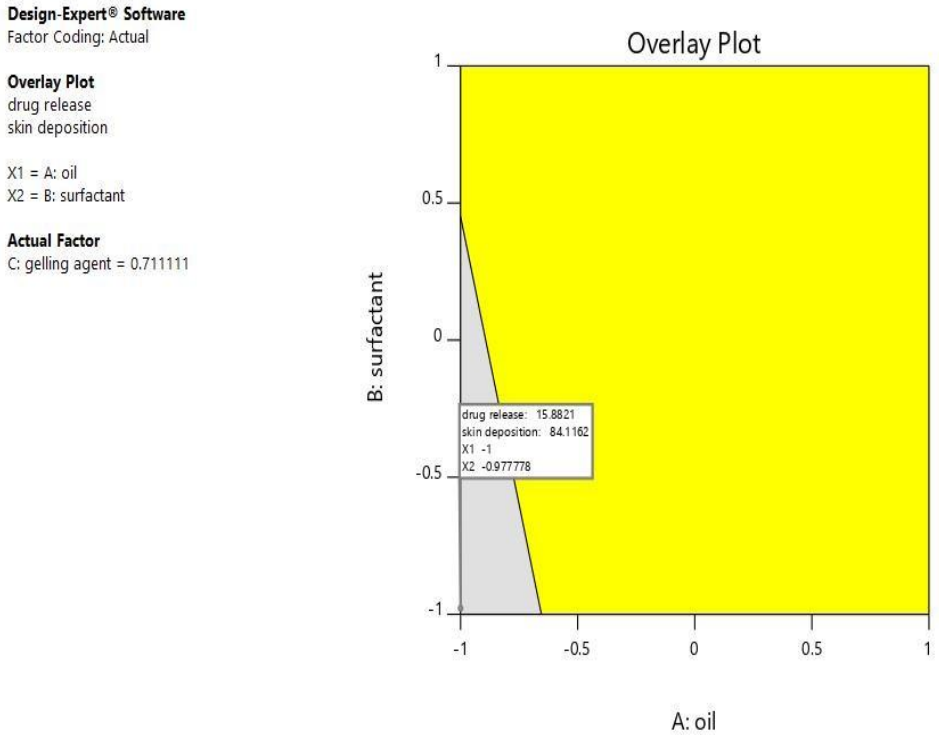


Figure 8: Overlay plot

Overlay plot generates a singular plot emphasising the "sweet spot" where response criteria can be satisfied. In figure 8, default settings define acceptable criteria as vibrant yellow and unacceptable criteria as grey.In the graph, the numerical optimisation solutions (flags) are displayed. Here, the predicted concentrations of the factors match the numerical solution.

THE OPTIMISED FORMULA ANALYSIS

The optimal composition was determined using ex-vivo skin penetration experiments, vesicle shape, particle size and size distribution, zeta potential, and viscosity. These are the outcomes that were achieved.

Vesicle morphology, particle size and size distribution

SEM (scanning electron microscopy) can be used to examine the composition's morphology. Globules can be observed in three dimensions using SEM. The magnification was performed at distances of 2 m, 3 m, and 5 m. The **Figure 9** depicts SEM images.

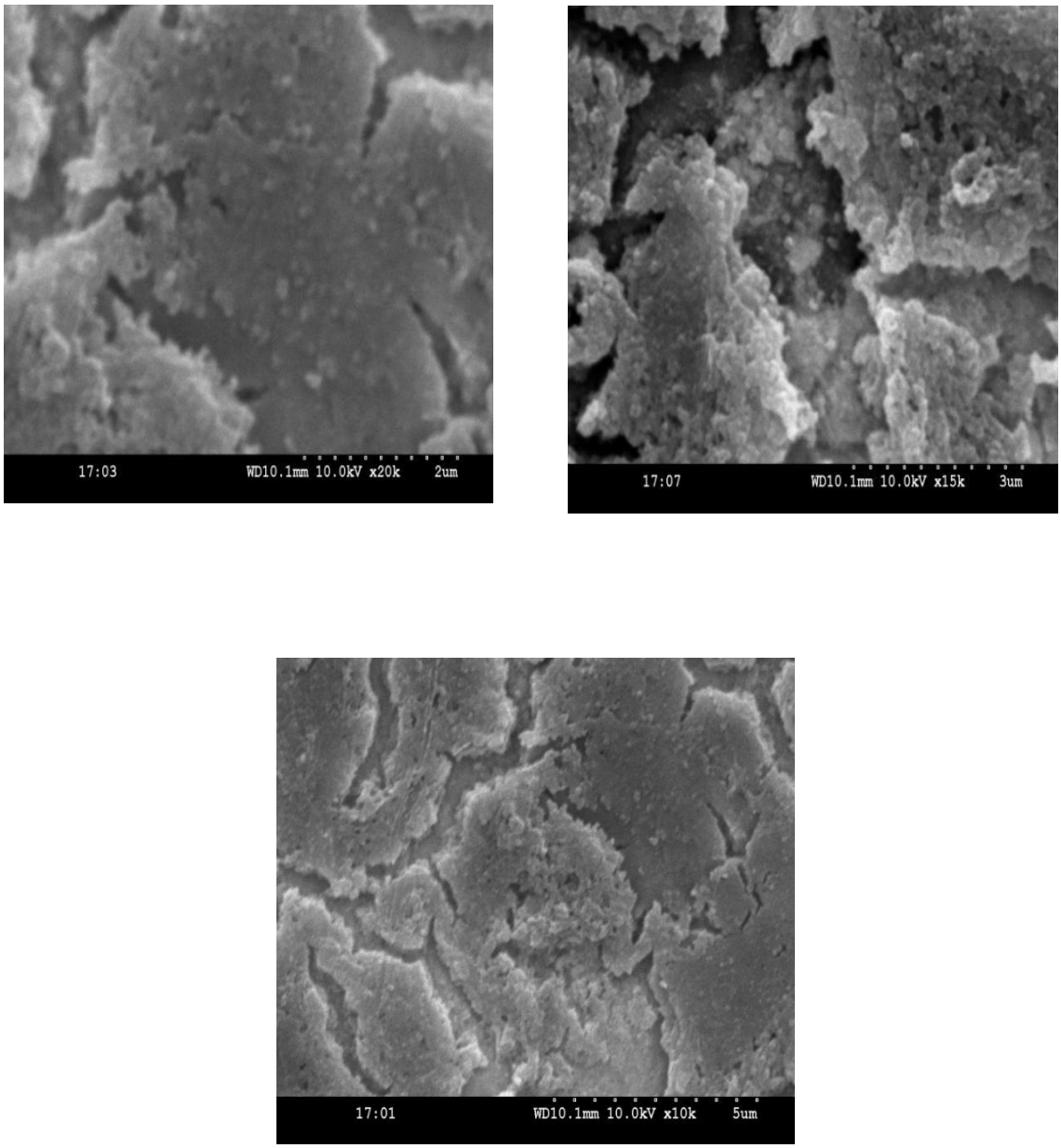
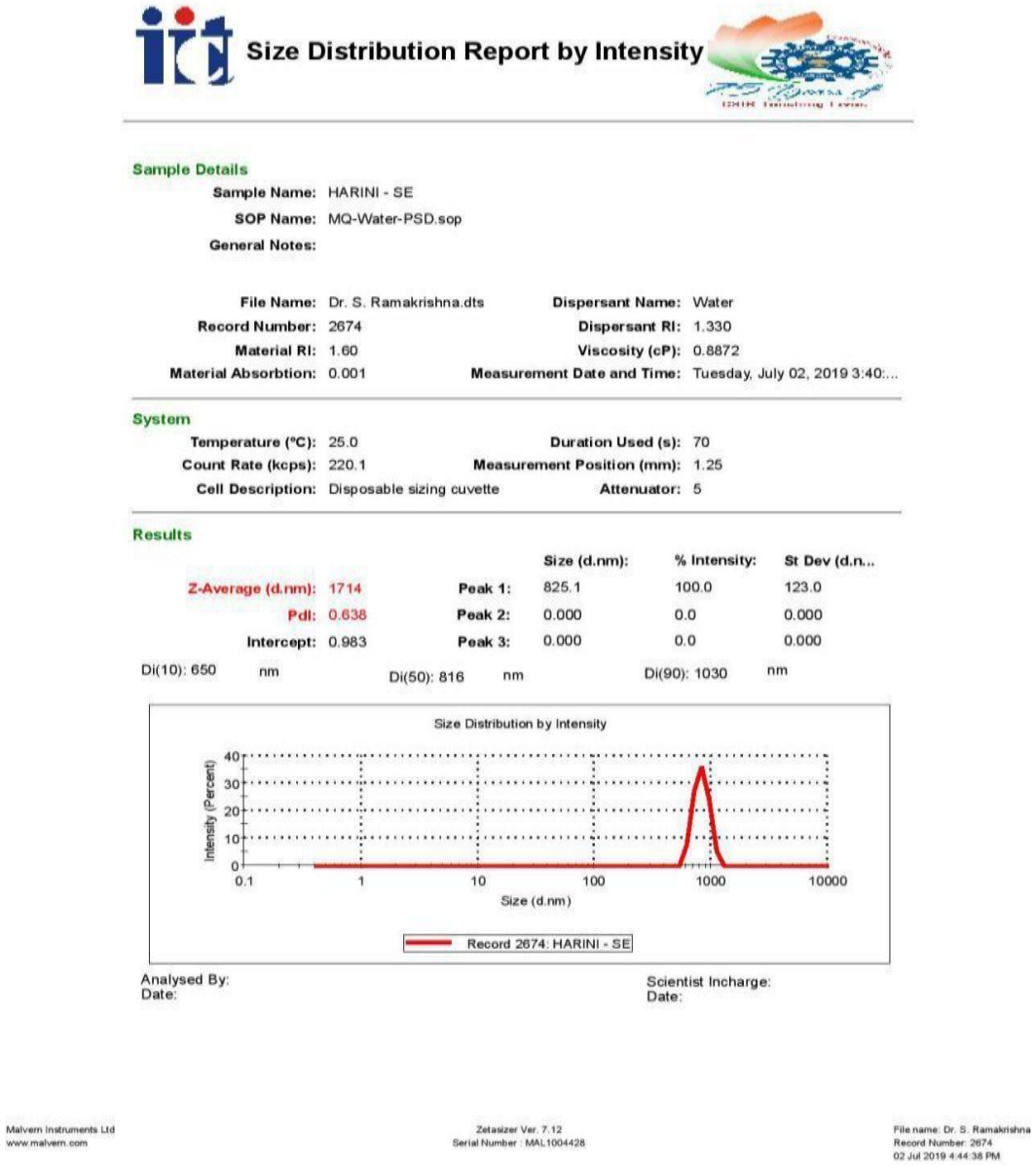


Figure 9: SEM images of EM formulation



Size Distribution Report by Intensity

Sample Details

Sample Name:	HARINI - SE		
SOP Name:	MQ-Water-PSD.sop		
General Notes:			
File Name:	Dr. S. Ramakrishna.dts	**Dispersant Name:**	Water
Record Number:	2674	**Dispersant RI:**	1.330
Material RI:	1.60	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.001	**Measurement Date and Time:**	Tuesday, July 02, 2019 3:40:...

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	220.1	**Measurement Position (mm):**	1.25
Cell Description:	Disposable sizing cuvette	**Attenuator:**	5

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	1714	**Peak 1:**	825.1	100.0	123.0
PdI:	0.638	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.983	**Peak 3:**	0.000	0.0	0.000

Di(10): 650 nm Di(50): 816 nm Di(90): 1030 nm

Analysed By:
Date:

Scientist Incharge:
Date:

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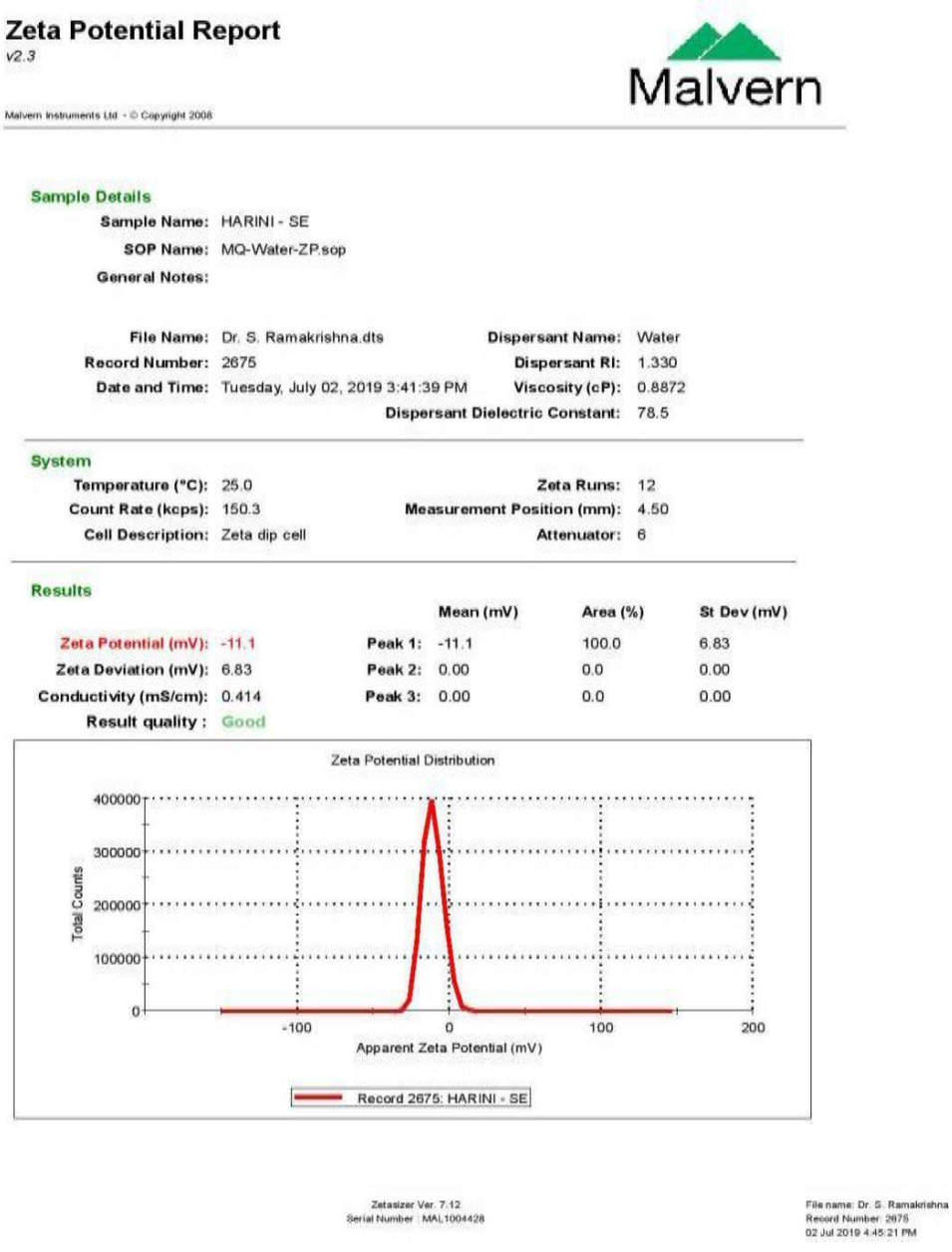
Serial Number : MAL1004428

File name: Dr. S. Ramakrishna
Record Number: 2674
02 Jul 2019 4:44:38 PM

Figure 10: Size distribution of EM formulation

The 1714 d.nm diameter of the globules in the obtained emulgel and the 0.638% polydispersity index indicate that the screened formulation is polydisperse. The particle size analysis revealed that the emulgel's globules were microscopic **(Figure 10).**

The zeta potential was measured to be -11.1 mV using a Malvern zeta sizer at the Indian Institute of Chemical Technology in Hyderabad, India. **(Figure 11)**



Zeta Potential Report
v2.3

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Malvern

Sample Details

Sample Name:	HARINI - SE		
SOP Name:	MQ-Water-ZP.sop		
General Notes:			
File Name:	Dr. S. Ramakrishna.dts	**Dispersant Name:**	Water
Record Number:	2675	**Dispersant RI:**	1.330
Date and Time:	Tuesday, July 02, 2019 3:41:39 PM	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	150.3	**Measurement Position (mm):**	4.50
Cell Description:	Zeta dip cell	**Attenuator:**	6

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-11.1	**Peak 1:**	-11.1	100.0	6.83
Zeta Deviation (mV):	6.83	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.414	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				

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Serial Number : MAL1004428

File name: Dr. S. Ramakrishna
Record Number: 2675
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Figure 11: Zeta potential report of EM formulation

Determining Viscosity

Using a Brookfield viscometer, the optimised emulsion's viscosity was determined to be 6005 cps at 25 °C with spindle number 64, demonstrating the formulation's high viscosity.

***Ex-vivo* skin permeation studies**

Table 10: *Ex-vivo* comparison between optimised and other formulations

Flux (µg/cm²/hr)	EM	Marketed	Pure drug	Control gel
	27.28	270.55	432.33	839.65

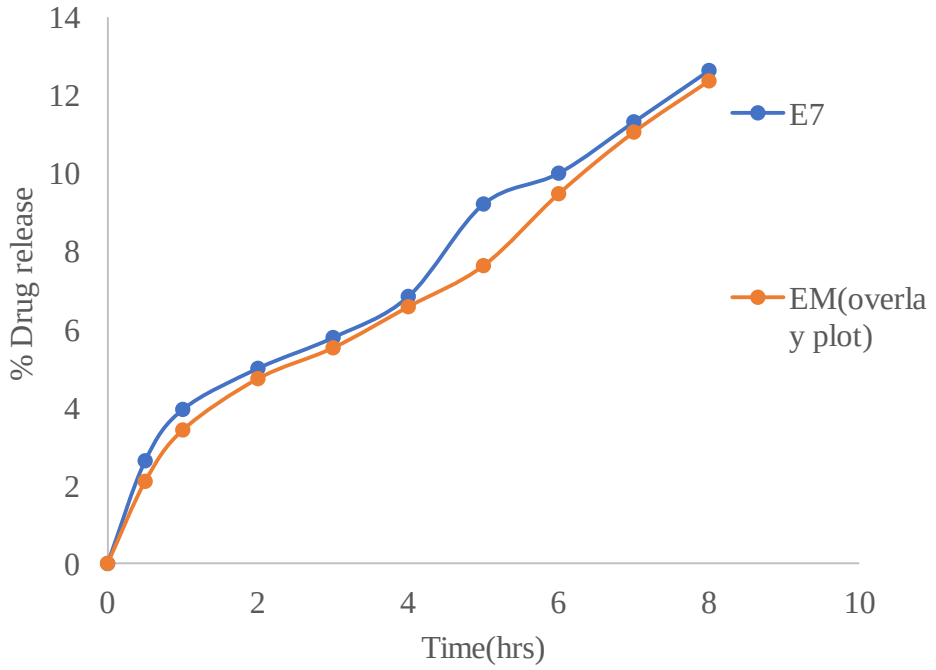

Figure 12: *Ex-vivo* drug release through rat skin

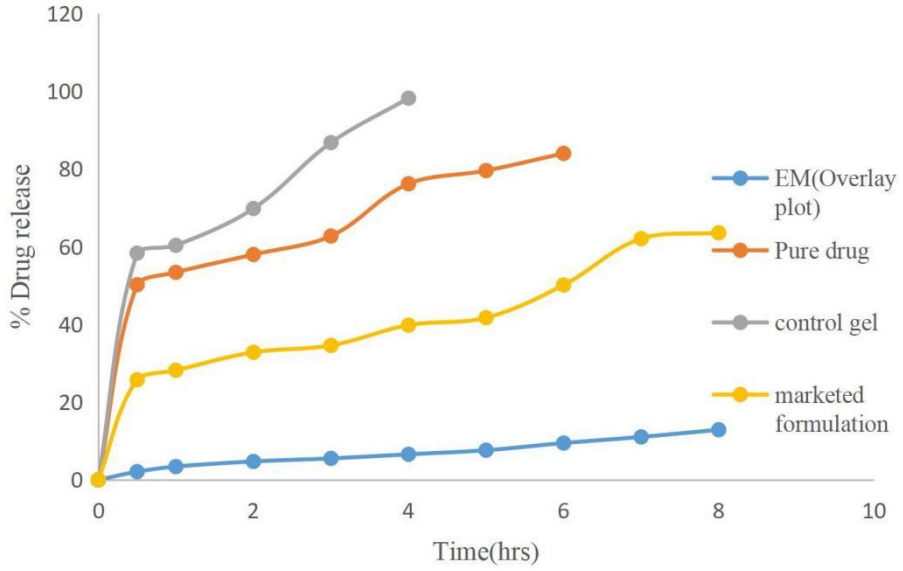

Figure 13: Comparision of optimised emulgel with marketed formulation

On performing ex-vivo studies on rat epidermis, it was observed from the results **(Table 11)** that the emulgel formulation EM with an overlay plot yielded the desired lower percentage of drug release, which was 12.89%. **(Figure 12). Figure 13** compares the permeability parameters of optimised emulgels to those of a commercial formulation.

Upon comparing the ratio of drug permeability, the EM formulation containing Carbopol 934P (1.85%), Surfactant (2.62%), and Stearyl alcohol (10%) was found to be optimal due to its high skin deposition and excellent permeability. **(Table 12)**

Table 11: Skin permeability parameters of optimized emulgels

Permeability parameters	**E7**	**EM**
Flux(µg/cm^2/hr)	27.28	26.53

Permeability coefficient (cm/hr×10^{-3})	1.36	1.32
Lag time(hr)	0.4	0.3
Skin content(mg/g)	75.62	72.41

Model-dependent kinetics emulgels

Attempts were made to incorporate the **ex-vivo** release investigation results of the improved batch into various mathematical models. The regression coefficient (r2) values of the formulations for zero order, first order, Higuchi matrices, and Peppas are presented in **Table 12 (Figures 14,15).** Comparing the regression coefficient "r" values of the zero order and first order plots of the optimised formulation EM revealed that the zero order plots' "r" values were 0.989 and 0.987, respectively, indicating that the optimised formulation's drug release followed zero order release kinetics. The "r" value of Higuchi kinetics was determined to be 0.943%. The Korsemeyer-Peppas equation was fitted to the ex-vivo dissolution data as log cumulative percent drug release versus log time, and the value of the exponent 'n' was found to be 0.478, demonstrating drug release by anomalous transport.

Table 12: Model dependent kinetic analysis of the *ex-vivo* permeation studies.

FC	**Zero order release**	**First order release**	**Higuchi release**	**Korsemeyer-Peppas release**		**Release mechanism**
	r^2	**r^2**	**r^2**	**r 2**	**N**	
EM (Overlay plot)	0.989	0.9881	0.9546	0.9794	0.610	Anomalous transport
E7	0.99	0.989	0.96	0.9694	0.548	Anomalous transport

Note: FC- Formulation Code

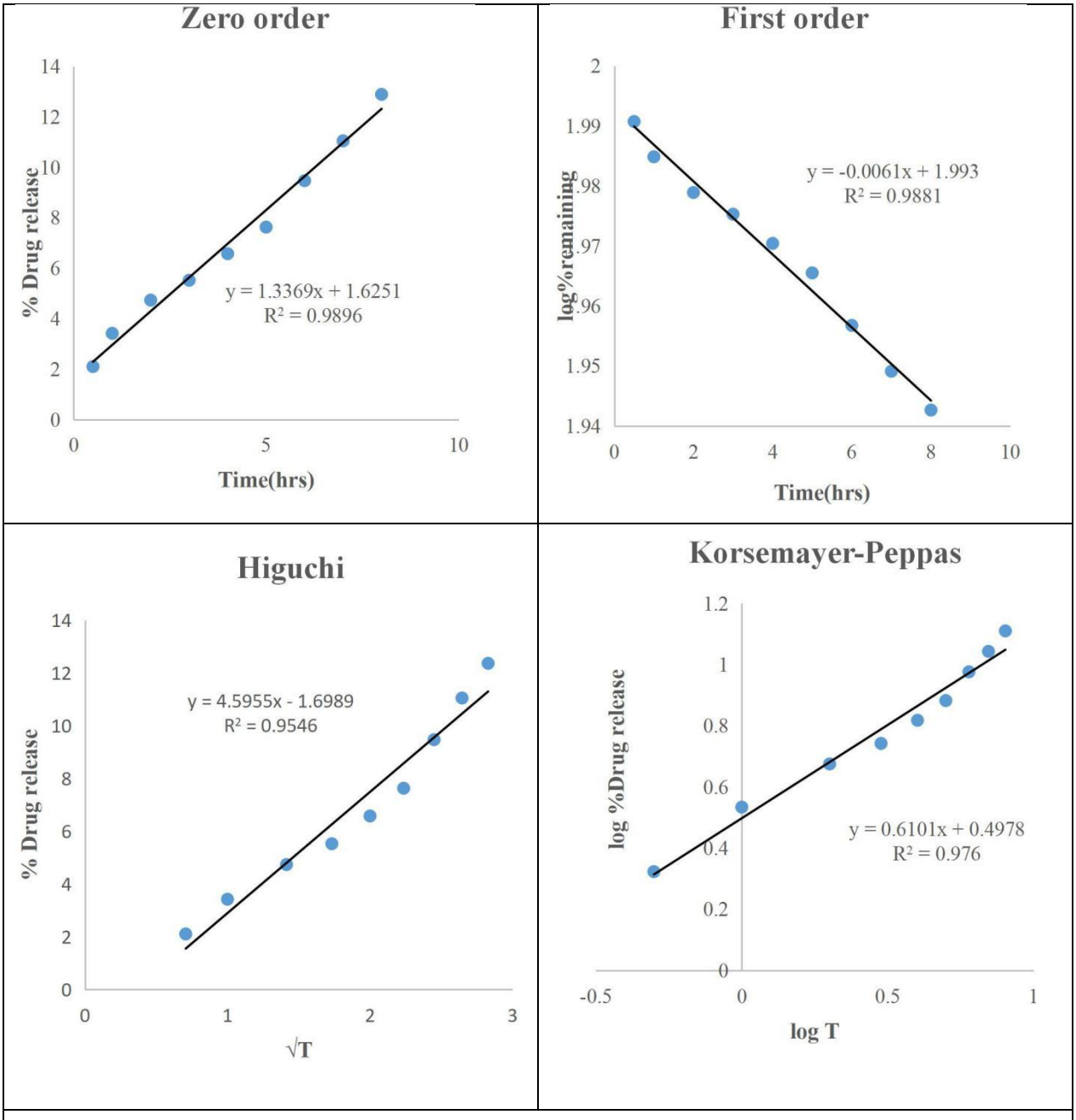

Figure 14: Model dependent kinetics for optimised EM formulation

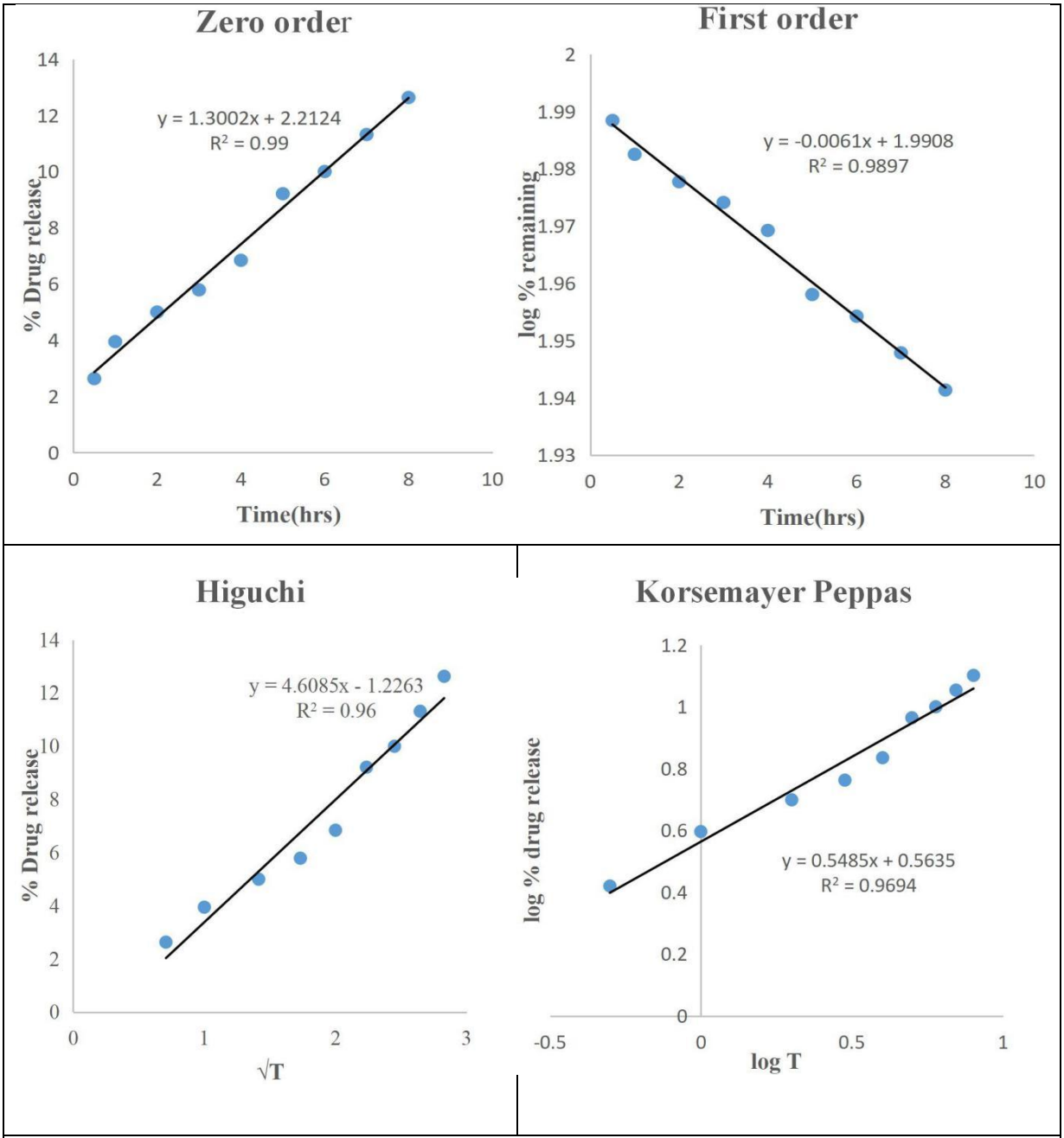

Figure 15: Model dependent kinetics for E7 formulation

Skin irritation study

Control and test substances were applied to the dorsal surface of the rabbit's epidermis, and the animal was observed for eight hours. The severity of erythema and edoema was determined using the Primary Dermal Irritation Index (PDII) classification system.

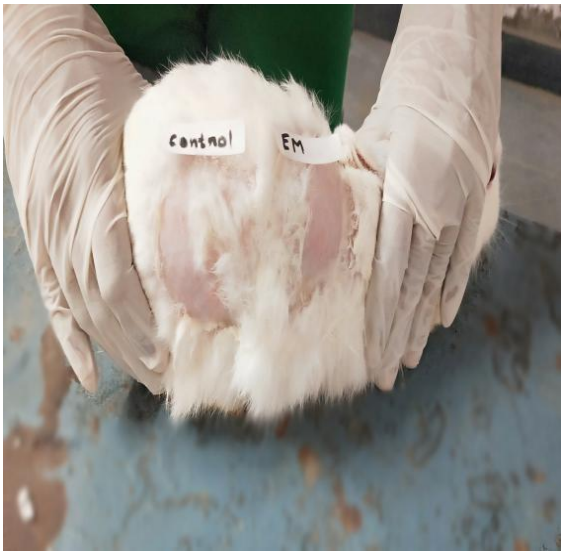

Figure 16: Skin irritation studies

The formulations of EM and Control demonstrated a '0' irritation potential, indicating that they are non-irritant. The value '0' in an irritancy test signifies that the applied formulations are typically non-irritating to human skin. After 8 hours of application of the optimised formulations to rabbit skin **(Figure 16)**, neither erythema nor edoema were observed.

Stability studies

The stability of this optimised formulation was determined through one month of stability testing at ambient temperature.

Table 13: Stability study of optimized formulation EM

Time in weeks for EM				
Parameters	**O (Initial)**	**1st week**	**2nd week**	**4th week**
Appearance	+++	+++	+++	+++
Drug content (%)	99.24±1.43	98.98±1.22	98.28±1.23	98.12±1.74
Viscosity (cps)	6005±180	60005±120	60003±150	60003±140
pH	6.23±0.24	6.08±1.23	6.06±1.24	5.94±1.23

Note: All values are expressed as Mean ± SD, n = 3; +++ = Excellent, ++ = Good, + = Satisfactory

The formulation was found to be stable, with insignificant changes in appearance, drug content, viscosity, and pH, as shown in **Table 13.**

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