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Development and Evaluation of Naringenin Loaded Niosomal gel

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ABSTRACT

The pathophysiology of some chronic diseases, such as viral and autoimmune diseases, is increasingly thought to involve inflammation. Interest in plant-derived products has increased again because of the negative effects and resistance linked to traditional treatments like glucocorticoids and antibiotics. Large amounts of naringenin, a Phyto flavonoid present in citrus fruits, have demonstrated significant anti-inflammatory and anti-infective properties. However, its limited clinical applicability is caused by its poor water solubility and poor absorption. As a result, niosomes containing naringenin were optimized using Box-Behnken design and made using a thin-film hydration technique. Important formulation parameters, such as Span 60 concentration, cholesterol level, and hydration duration, were examined to determine how they affected particle size and drug entrapment effectiveness. The particle size of the improved niosomal preparation was around 310 nm, and its entrapment effectiveness was approximately 82%. A stable, skin-compatible topical system was produced by dispersing the niosomal dispersion in a Carbopol 934 gel. According to diffusion-controlled mechanisms and zero-order kinetics, in vitro permeation tests via Strat-M® membrane demonstrated a controlled release of the medication. Overall, the naringenin-loaded niosomal gel presents a viable strategy to improve topical administration, therapeutic efficacy, and patient adherence.

Highlight

1. **Need for Innovation:**

Topical delivery of bioactive compounds like **naringenin** is limited by its **poor water solubility and low skin permeability,** reducing its therapeutic efficacy. Naringenin control formulations fail to provide sustained action and optimal skin retention. Therefore, there's a need for an innovative **carrier system that enhances skin permeation, entrapment, and controlled release**.

2. **Formulation Plan:** Develop a niosomal gel formulation incorporating naringenin to improve its topical bioavailability. The plan involves:
    - Preparing niosomes using the thin-film hydration method
    - Optimizing for particle size, entrapment efficiency
    - Incorporating niosomes into a Carbopol 934 gel base for topical application.
3. **Formulation Strategy**:
    - **Step 1**: Thin Film Hydration ➔ Naringenin-loaded Niosomes (Optimized via Box-Behnken Design)
    - **Step 2**: Incorporation into Carbopol Gel.
4. **Key Results** :

Particle size: ~310 nm ➔Entrapment Efficiency: ~82% ➔ Controlled Drug Release (24 hours) ➔ Zero-order kinetics & diffusion-controlled release.

1. INTRODUCTION

In the last few years, more research has linked inflammation to the pathogenesis of many diseases. Inflammation is the body's immediate, non-specified response to infection or injury. In reaction to injury or external stimuli, the body initiates the migration of white blood cells like neutrophils, monocytes, eosinophils, and basophils into the blood.Inflammation is a natural physiologic reaction to tissue damage, microbial pathogen invasion, and chemical irritation. Inflammation begins with the release of chemical mediators at the injury site and the movement of immune cells from the bloodstream into the affected tissue.. Following this procedure, inflammatory cells are recruited, proinflammatory cytokines, ROS, and RNS are released to get rid of foreign pathogens, and damaged tissues are repaired. Normal inflammation often resolves quickly and goes away on its own, but continuous inflammation can lead to a number of chronic illnesses It also brings about the formation of inflammatory signaling molecules such as , pro-inflammatory cytokines, serotonin, and thrombo modulators, among others, by tissue-resident mast macrophages. These mediators then become incorporated into the bloodstream and increase leukocyte and acute phase protein concentrations. Antibiotics and glucocorticoids, commonly prescribed drugs for infection and inflammation treatment, are not without risk. Orally taken glucocorticoids enhance the risk of adrenal insufficiency, Cushing syndrome, and mortality, as per a cohort study. Furthermore, the misuse of antibiotics has decreased their efficacy by promoting the development of bacteria and viruses that are resistant to them. Human health is increasingly threatened by these resistant viruses and microbes Discovering natural drugs to cure inflammatory condition and infectious condition is thus important as plant constituents especially flavonoids, which gives numerous health benefits in humans being have a number of benefits, such as multi-targeting, affordable, relative less reactive,and high availability Naringenin is a naturally occurring flavonoid whose pharmacological advantage, among others, including its anti-inflammatory effects, has been demonstrated in various studies. We believe that naringenin is very promising for the treatment of inflammatory condition and infectious condition since much research has established its potential anti-inflammatory and anti-infective activities. (1)

Naringenin (molecular formula: $C_{15}H_{12}O_5$) is the monomer naringin glycoside component name, which features an interesting chemical structure. Its two benzene rings, connecting the three carbon chains, and its third ring comprise its flavonoid skeleton of basic 15 carbon atoms. 4′,5, 7-trihydroxyflavone is its chemical name. It possesses a molecular weight of 272.3 and a melting point between 245 -250 degree celsius. Naringenin occurs naturally in solid state dissolved into organic solvents like ethanol, ether, dimethylformamide, and dimethyl sulfoxide but practically insoluble in water. The primary source of naringenin is citrus fruits like grapes, oranges, blood oranges, lemons, and grapefruit. Grapefruit peel has also been found to contain a high concentration of this compound according to some studies.

The inflammation-inhibiting and pathogen-inhibiting properties of naringenin are the primary targets of ongoing research, particularly against autoimmune inflammatory conditions and diseases induced by a variety of bacterial and viral infections. Naringenin's limited water solubility (5.81%) have a very significant impact on its clinical relevance and may led to the drug's poor duration of action at the absorption site. Additionally, the substance's poor bioavailability may be linked to high first-pass excretion. The GI tract is where naringenin is absorbed quickly, binding to serum albumin and quickly forming glucoside or Thio glucoside. After being delivered to critical organs like the kidney, spleen, heart, liver, and brain, the metabolites of serum albumin are eliminated by the urine and biliary systems.

Naringenin has been demonstrated to exert inflammation-inhibitary activity in a variety of inflammatory diseases, and it has already been postulated that inflammation-inhibitary could diminish inflammation as a whole by modifying proinflammatory mediators produced by several inflammatory cells. Inflammation is the immune system's second line of defense against infection.(2) Especially in autoimmune neurological diseases such as multiple sclerosis (MS) and immune-induced diabetes mellitus (DM) and immune-mediated inflammatory diseases such as RA , IBD , and so on, the molecular mechanism behind its inflammation-inhibitary action is yet unknown

2. MATERIALS AND METHODS

2.1. Materials

Naringenin was obtained from Dhamtec pharma Navi Mumbai, India. Span60, cholesterol, methanol, chloroform, carbapol 940, triethanolamine, potassium dihydrogen orthophosphate, sodium hydroxide, were obtained from loba chemicals pvt. Mumbai, India.

2.2. Development and optimization of Naringenin loaded Niosomes

Thin film hydration was used to prepare niosomes loaded with naringenin, and Design Expert® (Version 7.1.6, StatEase In c., Minneapolis, MN) was used to optimise the process utilising the 3-factors, 3-levels BBD. The 15 formulations were prepared using the independent variables Span60 (X1), cholesterol (X2), and hydration time (X3) at low, medium, and high amounts. Particle size (Y1) and entrapment efficiency (Y2) were the dependent variables. **As shown in Table no.1 and 2.**

2.3. Thin Film Hydration method

The surfactant and cholesterol were dissolved in 10ml chloroform: methanol(1:2)and the Naringenin(5mg) was added in that mixture, solvent under reduced pressure using rotary vacuum evaporator at 150 rpm for 50 min at 60 °C. A thin film of the solid mixture was formed on the inner walls of the flask. Hydration of the dried film was carried out using 10 ml of phosphate-buffered saline (PBS). The resulting suspension was vortexed and subsequently evapourated at 60 °C for 30, 45,60 minutes, depending on the medicinal preparation requirements. After heating, the MLV formed were cooled in an ice bath and then subjected to sonication for three minutes at 150 V. (3)This process produced ULV niosomes.

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

Variables	Levels		
Independent Variable	**-1**	**0**	**+1**
Span 60	**70**	**80**	**90**
Cholesterol	**10**	**20**	**30**
Hydration time	**30**	**45**	**60**
Dependent Variables			
Particle Size			
Entrapment Efficiency			

Table no.2. Formulation table of Naringenin loaded Niosomes

Formulation code	Independent variables			Dependent variables	
	Span60 (x_1)	Cholesterol (x_2)	Hydration time (x_3)	Particle size (Y_1)	Entrapment efficiency (Y_2)
F1	80	10	30	295.03±2.05	60.8 ± 0.87
F2	90	20	60	290.15±1.56	84.4 ± 0.80
F3	80	30	60	330.21±3.11	83.5 ± 0.60
F4	70	10	45	320.27±2.76	73.6 ± 1.01
F5	90	20	30	295.06±1.49	62.4 ± 0.70
F6	80	20	45	310.3± 3.53	81.8 ± 1.21
F7	90	30	45	310.09±1.98	80.2 ± 0.60
F8	90	10	45	270.39±5.49	77.3 ± 1.01
F9	80	30	30	335.03±3.47	63.3 ± 0.7
F10	70	20	60	340.12±4.31	79.2 ± 0.96
F11	80	10	60	300.15±1.34	83.2 ± 0.7
F12	80	20	45	315.24±3.18	82 ± 1
F13	70	20	30	345.39±3.12	64.1 ± 0.34
F14	70	30	45	360.03±2.89	74.8 ± 1.55
F15	80	20	45	310 ± 1.27	80.7 ± 0.7

2.4. Particle size

(Zetasizer, HAS 3000; Malvern Instruments) with a scattering angle of 90° and at 25 ± 1 °C. The samples were negatively stained after drying on a carbon-coated grid with an aqueous phosphotungstic acid solution. By utilizing an accelerating voltage of 100 kV, the sample was observed under the microscope at a magnification of 10–100 k after drying.(4)

2.5. Determination of entrapment efficiency

To separate the niosomes from the unentrapped medication, formulations of naringenin niosomes were centrifuged at 10,000 rpm for 30 minutes at 4 °C using a cooling centrifuge (C-24, Remi instruments, India). A UV-Visible spectrophotometer (Shimadzu, Japan) was used to measure absorption at 288 nm in order to determine the concentration of the free medication in the supernatant .The following formula was used to determine the proportion of drug entrapment in niosomes. To make sure that all of the free drug was eliminated, this procedure was carried out three times.

EE% = Total drug – drug supernant/ Total drug *100

2.6. Selection of optimised niosomal formulation.

On the basis of particle size, entrapment efficiency parameters of all 15 niosomal formulation prepared as per 3 factors,3 levels BBD using DOE, where the values corresponding to F1-F15 batches represented particle size and entrapment efficiency distinctly showed that the formulation significantly differed from each other.

2.7. Zeta Potential

To ascertain whether the optimized formulation of niosome (F6) are colloidal, zeta potential analysis is performed. Using a zeta potential analyser, the suitably diluted niosomes from the proniosome dispersion were determined using the laser Doppler velocimetry method and electrophoretic light scattering. A temperature adjustment of 25°C was made. Vesicle charge, mean zeta potential values, and measurement standard deviation were all obtained directly from the measurements.

2.8. Scanning electron microscopy.

Niosome particle size is a very important characteristic.Scanning electron microscopy (SEM) optimized formulation of niosomes (F6) was used to determine surface morphology (smoothness, roundness, aggregate formation) of niosomes. The double-sided tape that was adhered to aluminium stubs was covered in niosomes. A scanning electron microscope's vacuum chamber was used to inject the aluminium stub. The samples' morphological characteristics were assessed through the use of a gaseous secondary electron detector.

2.9. Invitro Drug Permeation of Naringenin incorporated Niosomes Formulation(F6)

The optimized Naringenin incorporated niosomal formulation was used for in vitro permeation 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.
The donor compartment was precisely filled with 2.5 mL of the niosomes formulation, which contained an equivalent of 5 mg of medication. At 37 ± 0.5°C, phosphate-buffered saline (pH 7.4) was added to the receptor compartment as the dissolution medium. To ensure sinking conditions and even mixing, the mixture was constantly stirred with a magnetic stirrer. At intervals of 0, 1, 2, 4, 6, 8, 12, and 24 hours, 1 mL samples were taken out of the receptor compartment and immediately replaced with an equivalent volume of brand-new PBS to maintain a consistent volume. The amount of drug released cumulatively was determined by UV spectroscopy analysing the extracted samples at the drug's λmax.

2.10. Preparation Naringenin incorporated niosomal gel

For the formulation of naringenin-loaded niosomal gel , the niosomal suspension of optimised formulation (F6) was incorporated into a Carbopol 934 gel base. In order to ensure complete swelling of the polymer, Carbopol 934 (0.5% w/v, 1% w/v, 1.5% w/v) was mixed in distilled water under constant stirring and allowed to hydrate overnight. 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 niosomes were uniformly dispersed within the gel matrix, the prepared naringenin-loaded niosomal suspension was added gradually to the Carbopol gel under stirring conditions. Stirring was continued until a uniform niosomal gel was obtained Until it was required again.(5)

2.11. Evaluation of niosomal gel(6)

2.11.1. Organoleptic characteristics :

The formulated niosomal gel was visually determined for colour, consistency and particle presence.

2.11.2. Viscosity:

Viscosity of formulated niosomal gel was determined using Brookfield viscometer (Model DV-II+ Viscometer, India). The dial reading was recorded at 25 °C and 100 rpm after the gel sample was placed in a beaker.

2.11.3. PH Measurement:

A calibrated pH meter, was utilized to find the pH of NG (F6). The electrode was dipped in the formulation and three individual measurements of the pH of niosomal gel were taken, and average results were calculated.

2.11.4. Spreadability :

The Spreadability of Niosomal gel (F6) was assessed by putting required amount of the corresponding gel inside a 1 cm-diameter circle that had been previously marked on a glass plate, which was then placed on top of another glass plate. For roughly five minutes, a required weight was left to rest on the upper glass plate.

2.11.5. In vitro Permeation of Naringenin incorporated Niosomal gel

In vitro permeation study was performed to assess the topical delivery potential of the Naringenin loaded niosomal gel formulation by utilizing a Franz diffusion cell system. Strat-M® synthetic membrane was employed as the diffusion barrier based on its multilayered structure and permeability profile closely resembling human skin, rendering it suitable for modeling in vivo conditions.

An amount of 1 g of the Naringenin loaded niosomal gel preparation, containing 5 mg of drug, was cast evenly on the donor compartment of the Franz diffusion cell. The receptor compartment was loaded with phosphate-buffered saline (PBS, pH 7.4) at a temperature of 37 ± 0.5°C to mimic skin surface conditions. The receptor fluid was stirred constantly with the aid of a magnetic stirrer to ensure homogeneity and sink conditions throughout the experiment.

1-mL samples were removed from the receptor compartment at preselected time intervals (e.g., 0, 1, 2, 4, 6, 8, 12, upto 24 hours) and refilled instantly with fresh PBS to provide a constant volume and concentration gradient. The harvested samples were measured using a validated UV-Visible spectrophotometric assay to quantify the quantity of drug permeated through the membrane.(7)

3. RESULTS AND DISCUSSION

We can demonstrate how various parameters impact a formulation's performance using the knowledge gathered from the most recent testing data and building design specialist software. As an alternative, the empirical workplace values of drug entrapment efficacy (X2) and particle size (X1) were computed using Design-Expert13 version software **(Table 2).** 3-D surface graphs, contour plots, and mathematical models were used to illustrate each reaction. The analysis of variance (ANOVA) results and the statistical variables that are linked to the responses are shown in **Tables no.3.** Once irrelevant components have been eliminated,

Table no.3.ANOVA for Quadratic model for particle size of naringenin loaded niosomes

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	**7901.06**	**9**	**877.90**	**103.88**	**< 0.0001**
A-Span 60	**5009.00**	**1**	**5009.00**	**592.72**	**< 0.0001**
B-Cholesterol	**2796.77**	**1**	**2796.77**	**330.94**	**< 0.0001**
C-Hydration time	**12.20**	**1**	**12.20**	**1.44**	**0.2833**
AB	**0.0000**	**1**	**0.0000**	**0.0000**	**1.0000**
AC	**0.0324**	**1**	**0.0324**	**0.0038**	**0.9530**
BC	**24.70**	**1**	**24.70**	**2.92**	**0.1480**
A^2	**32.22**	**1**	**32.22**	**3.81**	**0.1083**
B^2	**0.5308**	**1**	**0.5308**	**0.0628**	**0.8121**
C^2	**30.61**	**1**	**30.61**	**3.62**	**0.1154**
Residual	**42.25**	**5**	**8.45**		

Lack of Fit	**24.94**	**3**	**8.31**	**0.9600**	**0.5466**
Pure Error	**17.32**	**2**	**8.66**		
Cor Total	**7943.32**	**14**			

3.1.1. Impact of Independent variable on Particle size Niosomes(5)

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

- Where Span 60 gives significant effect (p-value less than 0.0001, sum of square=5009.00) which implies negative effect, where Span 60 increases the particle size of vesicle decreases.
- Cholesterol gives significant positive effect (p value less than 0.0001, sum of square = 2796.77) it states that as cholesterol concentration increases the particle size of vesicle increases.
- Hydration time exhibit significant negative effect (p value =0.2833,sum of square= 12.20)it shows that as hydration time changes the particle size of vesicles is affected moderately

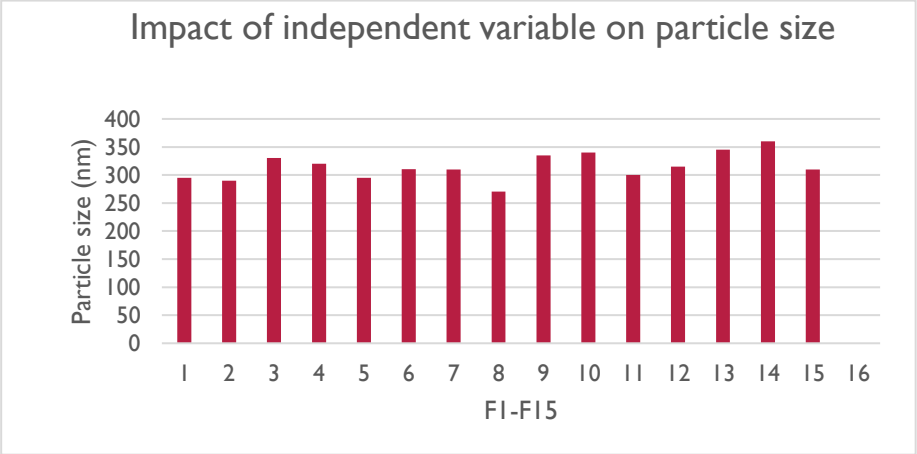

Fig.1. Impact of independent variable on particle size

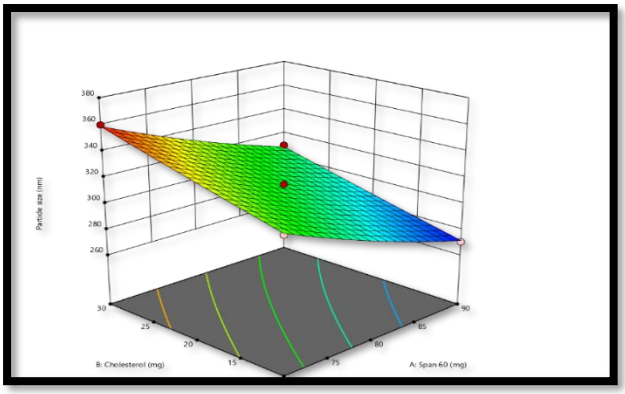

Fig.no.2: Effect of Cholesterol and Span 60 on particle size

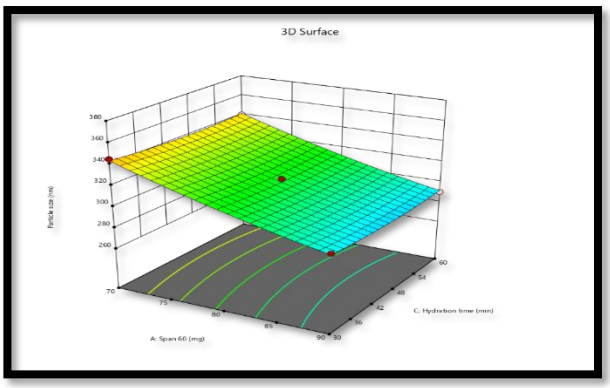

Fig.no.3: Effect of Span 60 and Hydration time on Particle size

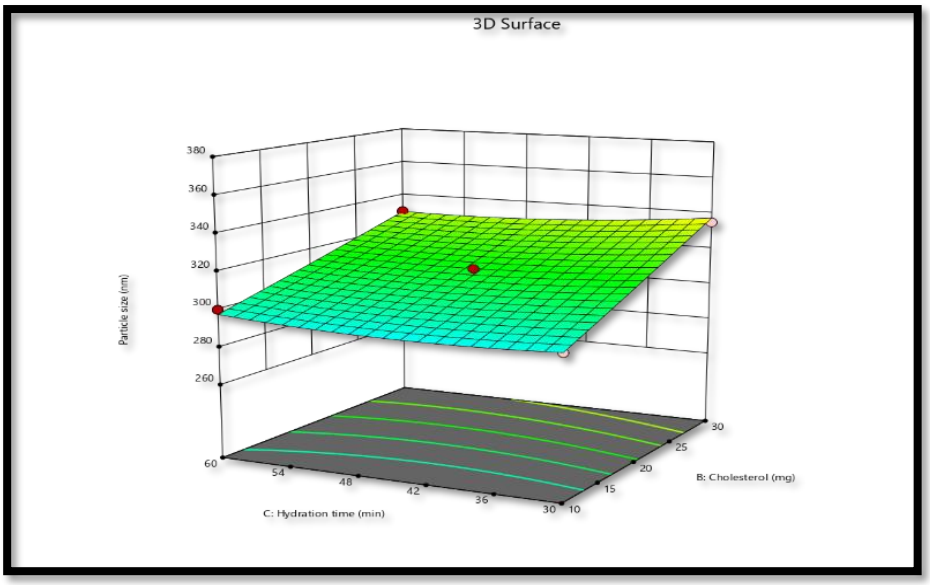

Fig .4.Effect of Hydration time and Cholesterol on Particle size

Response 1: Particle size

Particle size =+311.85-25.02A+18.70B-1.23C+0.0000 AB+0.0900AC-2.48BC+2.95A^2+0.3792 B^2+2.88C^2

The equation obtained in codes of factors allows prediction of the response based on specific levels of each variable. Typically, the highest level of the variables are represented as +1, while the lowest levels are denoted as -1. This codes form of the equation is particularly helpful for assessing the comparative influence of each factor by comparing their respective coefficients.

This formula shows the relationship between three variables (A, B, and C) and particle size. The formula includes: When all variables are at their base (coded zero), the particle size is known as the base value. Linear terms in A, B, and C show how particle size is affected by changes in only one of the factors. Particle size increases when B grows, but reduces when A increases. C has very little effect on particle size. Interaction words (AB, AC, and BC) that indicate the manner in which pairs of factors in conjunction might affect particle size. In this case, the interactions between A and B are ineffective, whereas those between A and C and B and C are weak. A, B, and C are squared terms that represent the relationship's curvature, meaning that each factor's effect is not linear but rather changes with its level. Overall, the model shows that factor interactions and nonlinear (curved) behavior have small effects, while A has a big negative influence, B has a positive effect, and C has a very small effect.

In this analysis, the variables were coded, and the variation or sum of squares was calculated using a technique known as Type III-Partial. With an F-value of 103.88, the model is excellent at explaining results, and there is only a 0.01% chance that this significant effect is the result of random noise alone. Any p-value < 0.0500 indicates that the factor is significant for the model. Factors A and B are significant in this instance. A factor is often not significant if its p-value is greater than 0.1000. Removing unnecessary elements from the model could make it better if there are a lot of them (apart from those required for its structure).

Given that we want the model to match the data well, the F-value of Lack of match is 0.96, which shows that there aren't any major problems with the model's ability to do so. The likelihood that the observed lack of fit is the result of random variation alone is about 54.66%.

The model's quality is demonstrated by the extremely low coefficient of variation (C.V.) = 0.9224%, the average (mean) of 315.16, and the standard deviation (which shows how dispersed the data values are) of 2.91. R2 = 0.9947 indicates that 99% of the variation in results can be explained by the model. The model predicts well because the difference between the adjusted R2 (0.9851) and the projected R2 (0.9449) is less than 0.2. Finally, the model has a strong and distinct signal, as evidenced by the Adequate Precision (scale of signal strength in respect to noise) of 36.839, which is significantly higher than the minimal value of 4. In general, the model is quite good and reliable for searching and analysing the design space.

3.1.2 Impact of Independent variable on Entrapment Efficiency(8)

Table no.4. Shows that ANOVA results of Entrapment Efficiency of naringenin loaded niosomes;

Table no.4.ANOVA for Quadratic model for EE

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1018.00	9	113.11	64.29	0.0001
A-Span 60	19.53	1	19.53	11.10	0.0207
B-Cholesterol	5.78	1	5.78	3.29	0.1297
C-Hydration time	802.00	1	802.00	455.81	< 0.0001
AB	0.7225	1	0.7225	0.4106	0.5499
AC	11.56	1	11.56	6.57	0.0504
BC	1.10	1	1.10	0.6266	0.4645
A^2	24.01	1	24.01	13.65	0.0141
B^2	22.62	1	22.62	12.85	0.0158
C^2	148.88	1	148.88	84.62	0.0003
Residual	8.80	5	1.76		
Lack of Fit	7.82	3	2.61	5.32	0.1623
Pure Error	0.9800	2	0.4900		
Cor Total	1026.79	14			

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

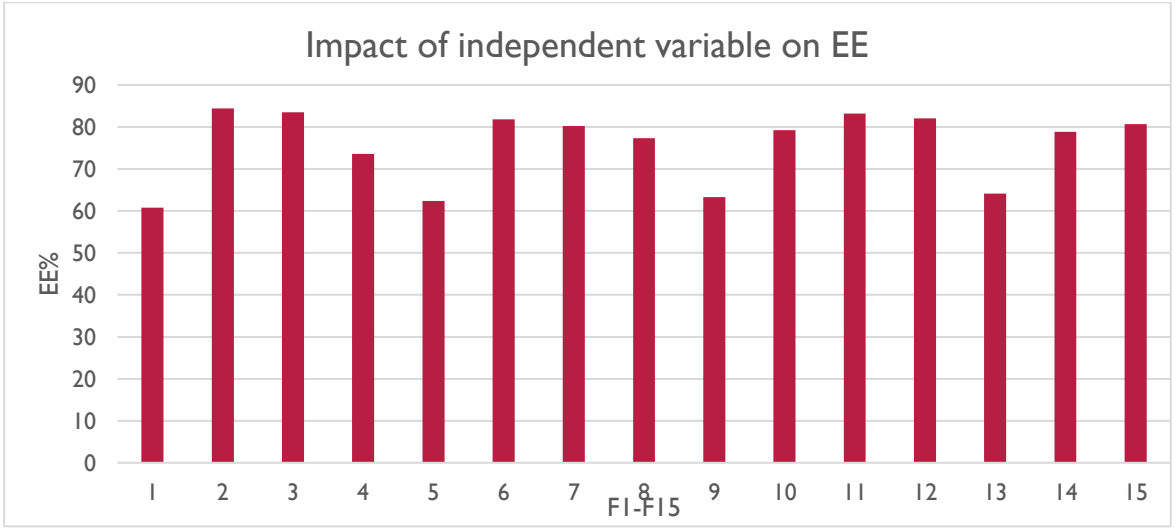

Fig.5.Impact of independent variable on EE

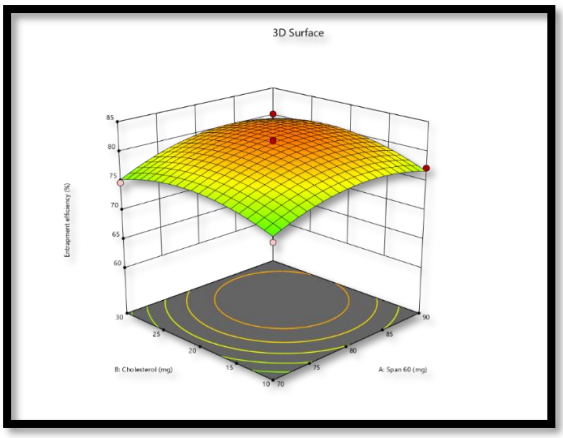

Fig.6. Effect of Cholesterol and Span60 on EE

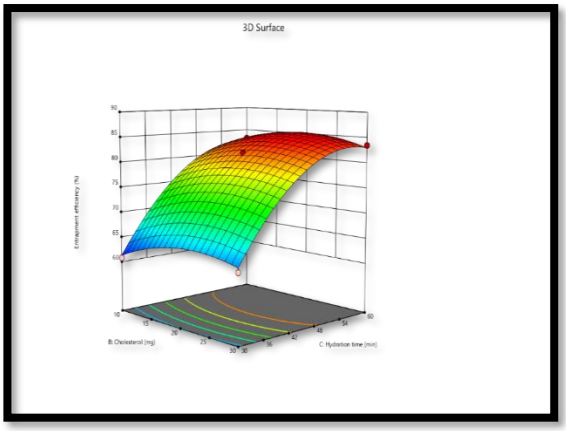

Fig .7. Effect of Cholesterol and Hydration time on EE

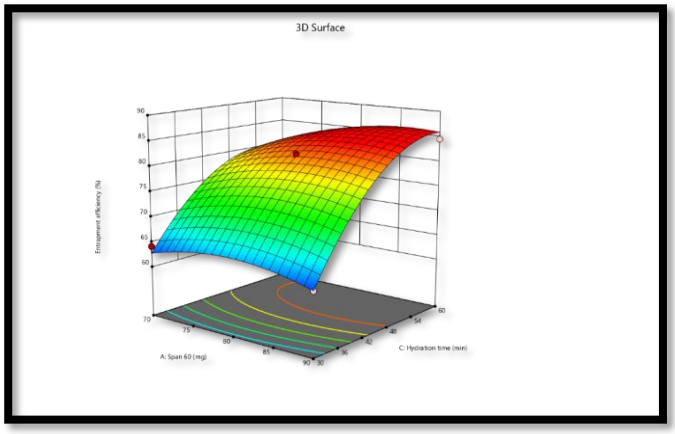

Fig.8. Effect of Span60 and Hydration time on EE

- Span 60 gives significant effect (p-value= 0.0207, sum of square=19.53) which implies Span 60 increases the entrapment efficiency of niosomal vesicle gives positive effect.
- Cholesterol gives significant positive effect (p value =0.1297, sum of square =5.78) it states that as cholesterol concentration increases the entrapment efficiency of vesicle increases moderately.
- Hydration time exhibit significant positive effect (p value less than 0.0001,sum of square=802.00)it shows that as hydration time increases the entrapment efficiency of vesicles is increases

Response 2: Entrapment efficiency

$EE\%=+81.50+1.56\ A+0.8500B+10.01\ C+0.4250AB+1.70\ AC-0.5250BC-2.55\ A^2-2.47\ B^2-6.35C^2$

The equation obtained in codes of factors allows prediction of the response based on specific levels of each variable. Typically, the highest level of the variables are represented as +1, while the lowest levels are denoted as -1. This codes form of the equation is particularly helpful for assessing the comparative influence of each factor by comparing their respective coefficients.

This formula shows how the three variables A, B, and C affect the Entrapment Efficiency (EE%). When A, B, and C are at their middle (zero-coded) positions, the EE% number remains constant. The independent effects of each variable on EE% are described by the linear terms for A, B, and C. EE% tends to rise when A, B, or C are raised, with factor C having the biggest beneficial impact. The interaction terms (AB, AC, and BC) show how two factors work together to influence EE%. Some interactions result in a minor rise in EE%, while others result in a slight decrease.

The effect between each element and EE% is curved rather than a straight line, as seen by the squared terms (A2, B, and C).

These terms decrease EE%, meaning that increases in A, B, or C may eventually cause EE% to decrease. Factor C generally has the most noticeable effect on EE%, while the model also accounts for interactions and nonlinear response, which have smaller effects.

The factors in this model were identified, and Type III-Partial was used to calculate the variation (sum of squares). The model has a statistically significant F-value of 64.29, meaning that there is only a 0.01% chance that such a strong outcome would have happened by chance. The corresponding terms are significant for the model if the P-value is less than 0.0500. Important terms in this context are A, C, A^2, B^2, and C^2. Model terms are usually not significant if their p-values are greater than 0.1000. Eliminating these unnecessary terms can enhance the model if there are many of them (aside from those needed for the model structure).

In comparison to pure error, the Lack of Fit F-value of 5.32 is not significant. The likelihood that this degree of lack of fit would be caused by random noise is 16.23%. Since it shows that the model fits the data well, a non-significant lack of fit is desirable.

According to the fit statistics, the data points are highly consistent, with the standard deviation = 1.33, average (mean) = 75.43, and coefficient of variation (C.V.) = 1.76%. R2 is 0.9914, meaning that 99% of the variation can be explained by the model. With a difference of less than 0.2, the adjusted R2 and anticipated R2 are sufficiently close to show that the model makes accurate predictions (0.9760 and 0.8760, respectively).

The signal is quite strong since the Adequate Precision, or signal relative to noise, is 22.7021, which is significantly higher than the minimum threshold of 4. Overall, the model is reliable and strong enough to analyze the design space.

3.2. Optimize formulation of Naringenin loaded niosomes

According to the statistical program's modelling and a 95% desirability factor, the software recommended the following factors for creating the best possible formulation: 80mg of span60, 20mg of cholesterol, and 45minutes of hydration time.

The experimental response values were compared with predicted values to calculate the percentage prediction error, as shown in **Table no.5**. The optimized niosomes formulation (F6) gives good particle size 310.3nm and EE 81.8% . This formulation is considered the best when compared to the optimized formula obtained by Box- Behnken Design (BBD) in Design of Experiments (DOE) with 3 factors at 3 levels **As shown in in fig no.9**(9)

Table no.5.Optimize Formula of Naringenin loaded niosomes

Coded no.	Optimized formula composition	Response Y	Predicted value	Observed value
X1	Span60 (80mg)	Particle size (nm)	313.526	310.3
X2	Cholesterol (20mg)	Entrapment efficiency	81.76	81.8
X3	Hydration time (45min)			

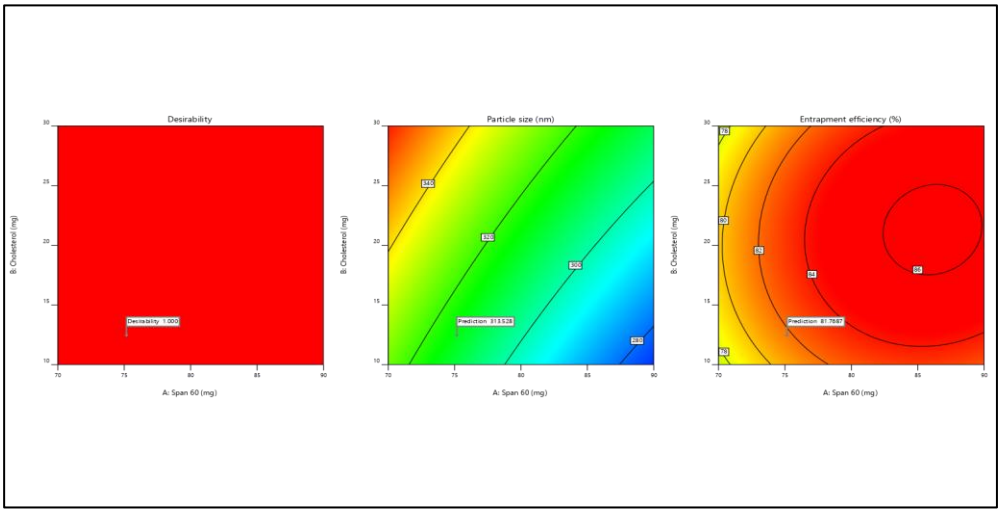

Fig.9.Counter plot of optimized formulation as a function of X1 X2 , A is overall desirability, B is Particle Size, C is Entrapment efficiency.

3.3.1. Zeta Potential (10)

The optimized Naringenin-loaded niosomal formulation had a zeta potential of −24.0 ± 0.6 mV, which represents moderate negative surface charge. This degree of charge is sufficient to create enough electrostatic repulsion among the vesicles, leading to the physical stability of the formulation through avoidance of aggregation. 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.

Table no.6. Drug release from Naringenin loaded niosomes (F6) (12)

Time (hr)	**% Drug Permeation of Naringenin incorporated Niosome formulaion(F6)**
0	0.00 ± 0.00
1	6.05 ± 0.07
2	11.00 ± 0.28
4	14.90 ± 0.14
6	21.10 ± 1.28
8	29.95 ± 0.21
10	38.05 ± 0.21
12	46.05 ± 0.49
14	51.00 ± 1.28
16	58.15 ± 0.21
18	67.20 ± 1.42
20	79.00 ± 0.28
22	84.90 ± 2.28
24	97.00 ± 0.42

3.3.2. Scanning Electron Microscopy

The SEM **fig no.10**. shows an assembly of round or nearly round particles, which are typical morphologies for niosomes. The particles are of varying sizes and seem to be aggregated or clustered in groups in some regions. The niosomes 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.(11)

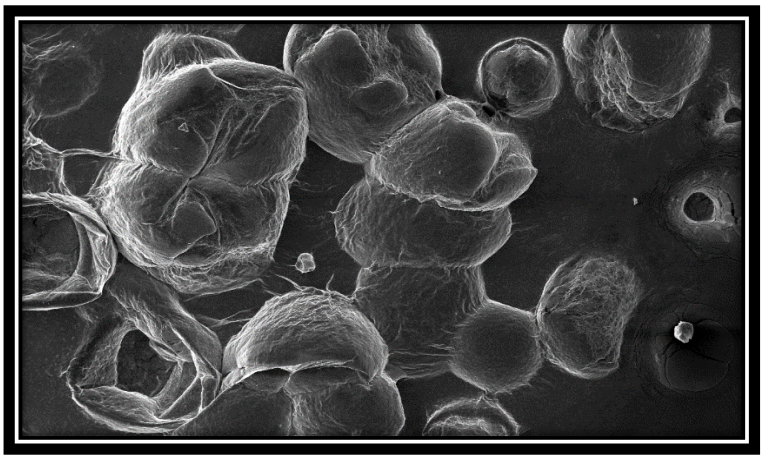

Fig no.10: Surface Morphology of Naringenin loaded Niosomes (F6)

3.3.3. Invitro Drug Permeation of Naringenin incorporated Niosomes Formulation(F6)

The permeation profile was biphasic in nature **as shown in fig 11**: a slow permeation phase in the beginning, followed by a controlled and sustained permeation over 24 hours. Lack of the initial burst effect proved the absence of free drug, and release observed was a result of diffusion only from inside the niosomal vesicles through the Strat-M® membrane.

This research proved that the niosomal system was able to sustain drug release from the entrapped drug over an extended duration, and as such, it is a potential carrier for use in topical drug delivery.

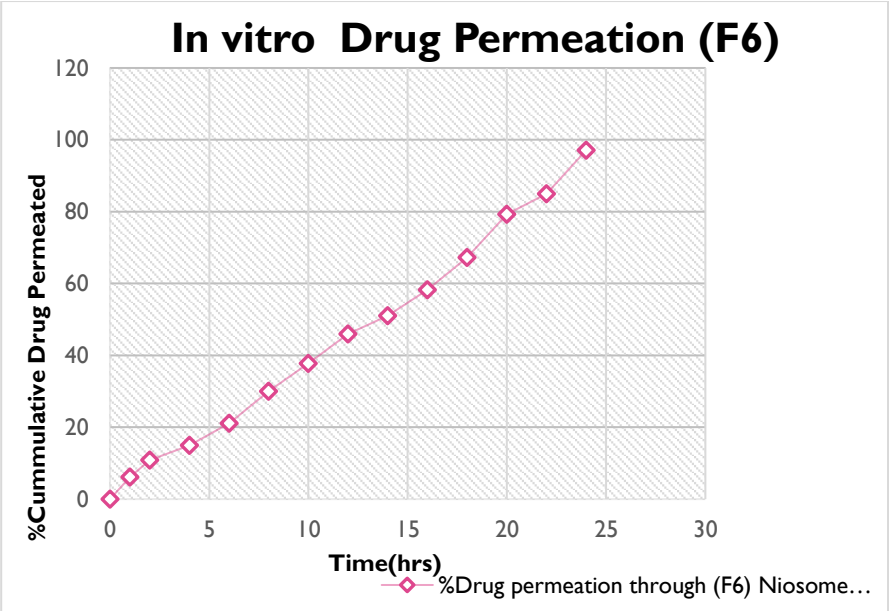

Fig11: % Cummulative Drug (Naringenin) permeated from niosome F6 Formulation

4.1 Organoleptic characteristics :(13)

As shown in **table no.7**

Table no.7. Conclusion of organoleptic properties

Viscosity	**14541±0.33**
PH Measurement	**6.5 ± 0.10**
Spreadability	**6.37 ± 0.15**

4.2 Niosomal Gel Parameters(14)

As shown in **table no.8**

Table no.8.Naringenin loaded Niosomal gel parameters

Parameter	Observation
Appearance	**Smooth, homogeneous, semi-solid, no phase separation**
Colour	**Pale yellow to yellowish-orange (due to aringenin)**
Consistency	**Medium-thick gel, spreads easily on the skin**
Texture	**Non-gritty, soft, smooth on application**
Stickiness	**Non-sticky or slightly tacky, easily washable**

4.3. In vitro Permeation of Naringenin incorporated Niosomal gel

As shown in **table no 9**

Table no.9. Invitro Drug Permeation from Niosomal gel and Plain gel

Time (hours)	% Cumulative of Drug Permeated from Niosomal gel	% Cumulative of Drug Permeated from Plain gel
0	0.00 ± 0.00	0.00 ±0.00
1	2.99 ± 0.14	0.90±0.14
2	3.64± 0.28	2.00±0.28
4	5.46± 0.42	3.70±0.28
6	10.66± 0.28	6.32±0.26
8	14.27± 1.57	8.77±0.35
10	22.19± 0.57	11.21±1.29
12	30.97± 2.85	15.00±0.42
14	39.38± 0.71	19.45±0.35
16	51.42± 1.13	23.00±0.71
18	57.4± 1.27	27.00±0.73
20	63.36± 1.99	31.00±0.69
22	75.11± 0.71	35.00±0.43
24	82.13± 1.13	39.00±0.70

To understand the mechanism of drug permeation from the naringenin-loaded niosomal gel, the in vitro release data were fitted to various mathematical models including **Zero-order**, **First-order**, **Higuchi**, and **Korsmeyer–Peppas** models in **fig.no 12,13,14** and **15** respectively. The regression coefficients (**R^2 values**) and kinetic parameters were used to evaluate the best-fitting model, and the results are summarized in the **table no 10.**

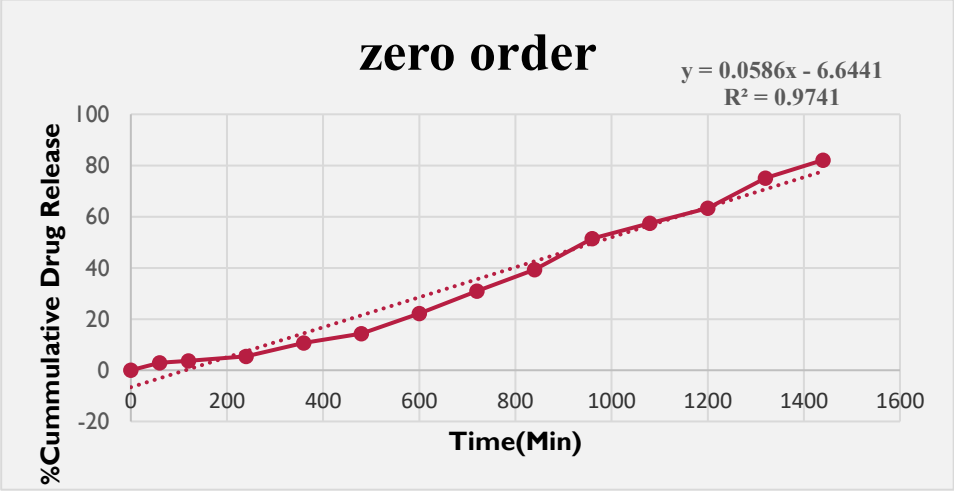

Fig no.12: Zero order

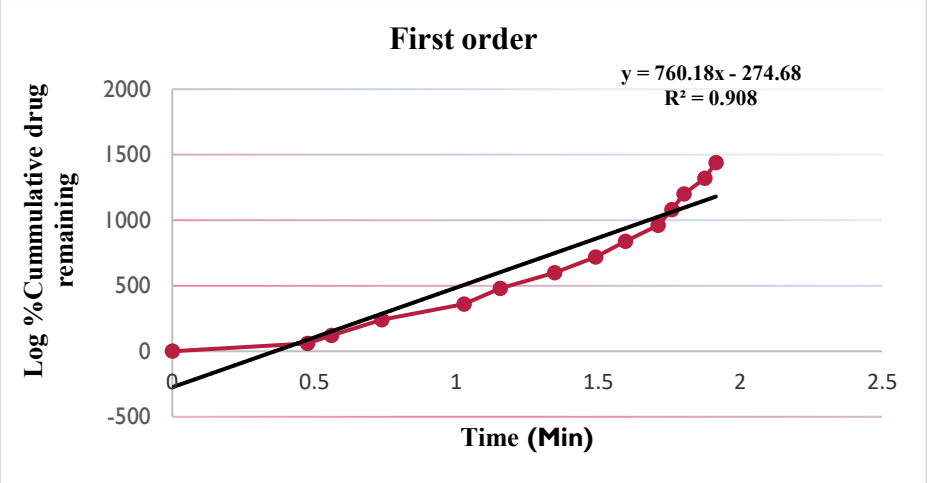

Fig no.13: First order

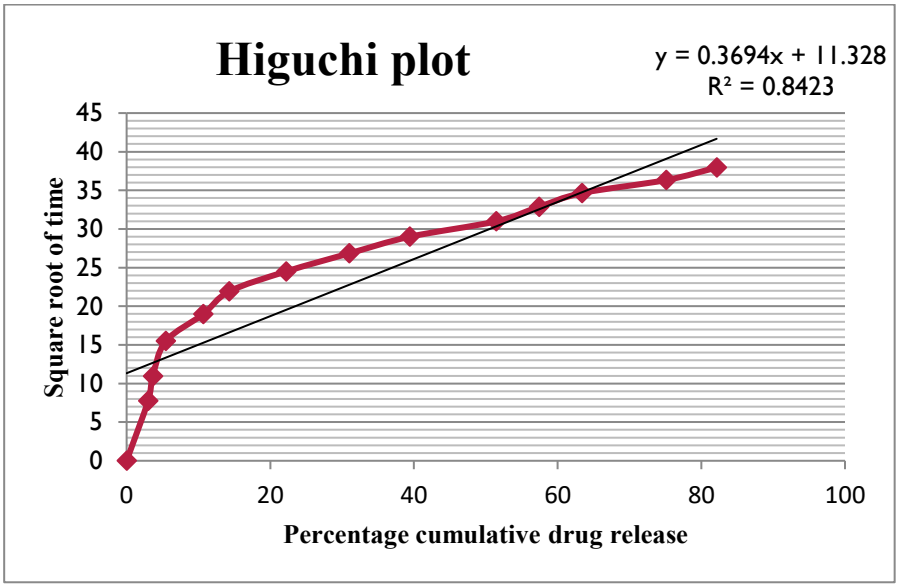

Fig no.14: Higuchi model

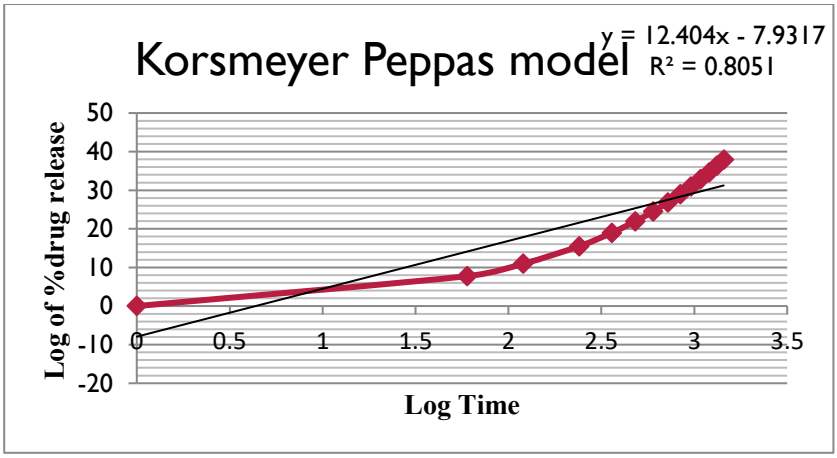

Fig no.15. Korsmeyer Peppas model

Table no.10. The table interpreting drug permeation kinetics of F6 formulation formulated in niosomal gel(15)

Kinetics Model	Equation	R^2 Value	Fit Quality	Mechanism Type
Zero-Order Kinetics	Qt=k0 .t	0.9741	Excellent fit	Constant release
First Order Kinetics	$Qt=Q\infty\cdot(1-e^{-k\cdot t})$	0.908	Good fit	Concentration-dependent
Higuchi Model	$Qt=kH\,.\sqrt{t}$	0.8423	Moderate fit	Diffusion-controlled
Korsmeyer- Peppas Model	$Qt= kKP\,.t^n$	0.8051	Moderate	Super Case-II

- Among all models, the **Zero-order model best describes** the drug release from the niosomal gel, confirming a **sustained and concentration-independent mechanism,** making it highly suitable for prolonged therapeutic effect(16)

As shown in fig no 16

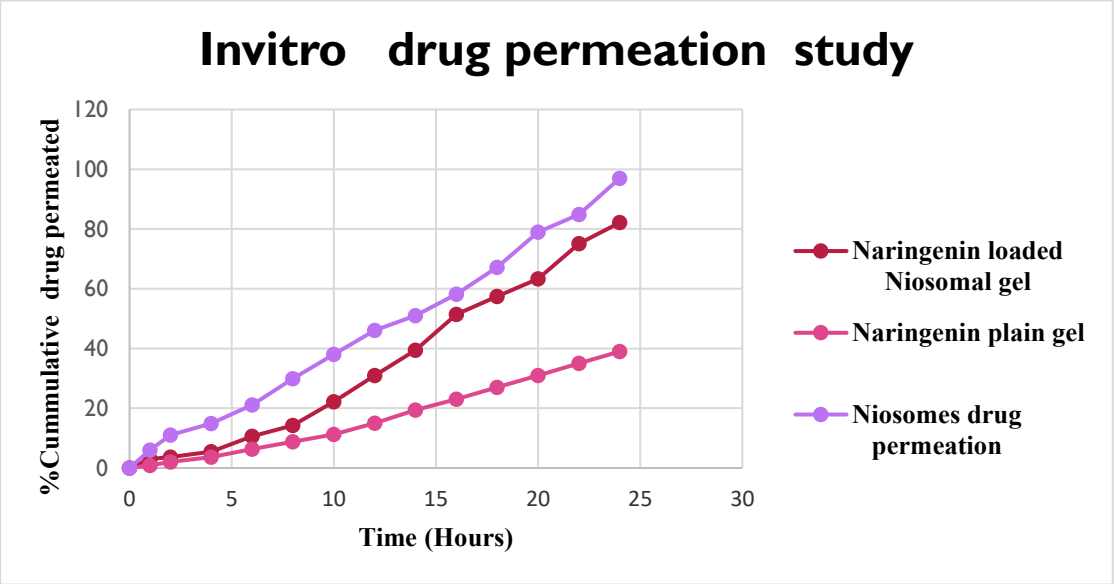

Fig no.16. Invitro drug permeation study

- Niosomal gel enhanced the release compared to plain gel, indicating better permeation and sustained delivery.
- Niosome suspension (F6) alone showed maximum release, suggesting faster release .
- Thus, niosomal gel provides controlled release and improves the delivery profile of Naringenin for topical application.

4. DISCUSSION

The current research was able to develop and optimize naringenin-loaded niosomes in a topical gel with a Box-Behnken design, which had accurate control over important formulation factors like Span 60, cholesterol, and hydration time to meet optimal particle size (310.3 nm) and excellent entrapment efficiency (81.8%). The optimized formulation showed good physical stability with zeta potential −24.0 ± 0.6 mV and typical spherical morphology. In addition, in vitro drug release studies validated a sustained and controlled release pattern over 24 hours, according to zero-order kinetics, pointing to the promise of this new niosomal gel as a suitable and improved topical delivery vehicle for naringenin with increased therapeutic efficacy and patient compliance.

5. CONCLUSION

Improved Bioavailability: The study shows that naringenin, a Phyto flavonoid with significant anti-inflammatory and anti-infective action, lacks proper water solubility and absorption. Niosomes' formulation rectifies this drawback and improves the bioavailability of naringenin for topical use

Optimization of Formulation Parameters: Key formulation parameters like Span 60 concentration, cholesterol content, and hydration time were systematically analysed. The optimized niosomal formulation obtained a particle size around 310 nm and an entrapment efficacy 81.8%, reflecting a successful formulation

Controlled Release Mechanism: The niosomal gel exhibited controlled release of naringenin, obeying zero-order kinetics and diffusion-controlled mechanisms. This indicates that the formulation would be capable of delivering sustained therapeutic effects, which is vital for effective topical administration

Model Reliability: The statistical evaluation of the model utilized in optimization revealed a good signal having an Adequate Precision of 36.839, which signifies that the model is trustworthy for examining the design space. The R^2 value of 0.9947 is an indication that 99% of results variation can be explained by the model, ensuring its predictability

Practical Implications: The results indicate that naringenin-loaded niosomal gel is an effective method of enhancing topical delivery, therapeutic response, and patient compliance. This would result in improved clinical outcomes for patients who need anti-inflammatory drugs

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