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Original Article

Formulation, Characterization, and Evaluation of Naringin-Loaded Phytosomal Gel and it's *in vivo* Anti-Inflammatory Activity by Carrageenan Animal Model

Satish Kavatagimath[1,*], Aqeeb Mevegar[1], Nayeem Khatib[2]

[1]Department of Pharmacognosy, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, INDIA.
[2]Department of Pharmacology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, INDIA.

ABSTRACT

Objective: The present study aimed to formulate, optimize, and evaluate a Naringin-loaded phytosomal gel using a 3^2 factorial design and to assess its anti-inflammatory activity. **Materials and Methods:** Phytosomes were prepared via the thin-film hydration method using soya lecithin and cholesterol and were characterized for vesicle size, Polydispersity Index (PDI), and entrapment efficiency. **Results:** Vesicle sizes ranged from 126 to 207.4 nm, with PDI values between 0.119 and 0.312, indicating uniform particle distribution. Entrapment efficiency ranged from 80.08% to 90.66%, with the optimized formulation (F6) exhibiting a vesicle size of 198.9 nm, PDI of 0.236, and the highest entrapment efficiency of 90.66%. This optimized formulation was incorporated into a gel base and evaluated for physicochemical properties, including pH, spreadability, viscosity, and drug content. The Naringin Phytosomal Gel (NG-PH gel) showed a viscosity of 1462±12 cPs and a drug content of 97.18±0.94%. The phytosomal gel demonstrated superior drug diffusion and permeation compared to the plain and standard gels. Skin irritancy tests revealed no erythema or edema, confirming its safety for topical application. **Conclusion:** Moreover, the phytosomal gel exhibited significant anti-inflammatory activity and maintained stability throughout the study period, highlighting its potential as an effective transdermal delivery system for targeted anti-inflammatory therapy.

Keywords: Naringin, Phytosomes, Anti-inflammatory activity, Quality by Design.

Correspondence:
Prof (Dr.) Satish A Kavatagimath
Department of Pharmacognosy, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, INDIA.
Email: satishak123@rediffmail.com
ORCID: 0000-0003-4114-4396

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INTRODUCTION

Inflammation is a vital biological response to harmful stimuli such as pathogens, damaged cells, or irritants, playing a key role in tissue repair and defense mechanisms.[1] However, when inflammation becomes chronic or excessive, it contributes to the development of various acute and chronic conditions, including arthritis, dermatitis, cardiovascular diseases, and autoimmune disorders.[2] To manage these conditions, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) like ibuprofen, diclofenac, naproxen, and indomethacin are commonly prescribed due to their strong analgesic and anti-inflammatory properties.[3] Despite their effectiveness, prolonged or high-dose use of NSAIDs is often linked to serious side effects, such as gastrointestinal irritation, peptic ulcers, kidney damage, and an elevated risk of cardiovascular complications, which limit their long-term clinical application.[4] In recent years, natural compounds with anti-inflammatory potential have gained attention as safer alternatives to conventional therapy.[5] Among them, Naringin, a flavonoid glycoside found predominantly in citrus fruits like grapefruits and oranges, has shown considerable promise.[5] It demonstrates a wide range of biological activities, including antioxidant, anti-inflammatory, anticancer, and hepatoprotective effects. However, its therapeutic potential is limited by poor water solubility, low permeability, and reduced bioavailability, particularly when administered via oral or topical routes.[6]

To overcome these challenges, innovative drug delivery systems such as phytosomes have been developed. Phytosomes are advanced nanocarriers formed by complexing phytoconstituents with phospholipids, enhancing their lipophilicity and membrane permeability.[7] This technology improves drug solubility, stability, and skin absorption, making it particularly beneficial for topical applications. When formulated into a gel, phytosomes offer additional benefits including ease of application, localized effect,

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and sustained drug release, which together enhance patient compliance and therapeutic outcomes.[7]

The present study focuses on the formulation and evaluation of a Naringin-loaded phytosomal gel for topical use in managing inflammation.[8] The phytosomal complex is prepared and integrated into a gel base, followed by assessment of its physicochemical characteristics, drug release behavior, and anti-inflammatory potential through established *in vitro* and *in vivo* models.[9] This strategy leverages the synergistic benefits of a plant-derived bioactive compound combined with an advanced lipid-based delivery system, providing a promising and safer alternative to conventional anti-inflammatory treatments with reduced risk of side effects.[10]

MATERIALS AND METHODS

Materials

Naringin was purchased from Sisco Research Laboratory, Mumbai. Soya lecithin was obtained from Molychem, Mumbai. Cholesterol and Carbopol were procured from Hi-media Pvt. Ltd., Mumbai. Solvent methanol and chloroform were obtained from Fisher Scientific, Mumbai.

Methods

Preparation of Naringin-loaded Phytosomes

Experimental design

Na-Ph phytosomes were formulated using the thin-film hydration method. Accurately weighed quantities of phospholipid (soya lecithin), cholesterol, and the lipophilic drug naringin were dissolved in 20 mL of a chloroform: methanol mixture (2:1 v/v) within a Round-Bottom Flask (RBF). The solvent was then evaporated using a rotary evaporator maintained at 60ºC and a speed of 72-80 rpm for 1 hr, resulting in the formation of a thin lipid film on the inner surface of the flask. To ensure complete removal of residual solvents, the film was placed in a desiccator and left undisturbed overnight. The following day, the dried lipid film was hydrated using a water-ethanol mixture under the same rotary conditions (60ºC, 72-80 rpm) for 1 hr. This hydration step resulted in the formation of a milky white suspension containing Multilamellar Vesicles (MLVs) of phytosomes. To reduce the vesicle size and achieve small Unilamellar Vesicles (SUVs), the suspension was subjected to probe sonication. The final Na-Ph formulation was stored in an airtight container at 4ºC until further use.[11]

Experimental design

A Design of Experiments (DOE) approach was employed using a 3^2 (three-level, two-factor) factorial design to optimize the formulation batches. The independent variables selected were the concentrations of lecithin and cholesterol, each evaluated at three levels: low, medium, and high. The impact of these variables was studied on two key dependent responses-vesicle size and entrapment efficiency. To assess the statistical significance of variations in particle size and entrapment efficiency, the data were analyzed using one-way Analysis of Variance (ANOVA), followed by the generation of a polynomial equation.[12] The formulation table for the preparation of Naringin-loaded Phytosomes (Na-Ph) is depicted in Table 1.

Characterization of Naringin-loaded Phytosomes

Particle size and poly-dispersity index

The particle size and Polydispersity Index (PDI) of Na-Ph were analyzed using a Malvern Zetasizer (Malvern Instruments Ltd., Malvern, UK). Prior to measurement, all formulation batches were diluted with Milli-Q water to ensure accurate analysis.[13]

Entrapment efficiency

The Entrapment Efficiency (EE%) of Na-Ph was evaluated using the ultracentrifugation method. Formulation aliquots were centrifuged at 15,000 rpm for 2 hr at 4ºC using an ultracentrifuge. Following centrifugation, the supernatant containing the unencapsulated (free) drug was collected, appropriately diluted, and analyzed at 283 nm using a UV spectrophotometer. The entrapment efficiency was then calculated using a standard equation.[14]

$$EE\% = \frac{\text{total drug} - \text{free drug}}{\text{total drug}} \text{ x } 100$$

Transmission Electron Microscopy (TEM)

The vesicular morphology of the optimized Naringin-loaded Phytosomes (OPT-Na-Ph) was examined using Transmission Electron Microscopy (TEM; Tecnai G20, Philips Scientific, Netherlands). A small quantity of the sample was placed onto a carbon-coated copper grid and allowed to air-dry. To enhance contrast, the sample was negatively stained with phosphotungstic acid. Imaging was conducted at an accelerating voltage of 200 kV, with magnifications ranging from 10,000× to 100,000×.[15]

Preparation of Naringin-loaded Phytosomal Gel (Na-PG)

For the topical application, OPT-Na-Ph formulation was converted into a gel dosage form. A precisely weighed amount of Carbopol 934 (1% w/v) was incorporated into the Na-Ph dispersion to achieve a uniform consistency. The mixture was kept overnight in a cool, dark environment to facilitate complete gelling. To obtain a clear, viscous gel with the desired pH, triethanolamine was carefully added. Finally, sodium benzoate was added as a preservative.[16]

Evaluation of Naringin-loaded phytosomal gel

Physical appearance

The physical appearance of Na plain gel and Na-PG was determined for its color, appearance and homogeneity.

pH determination

The pH of Na plain gel and Na-PG was measured using a digital pH meter. For the analysis, 1 g of gel was dissolved in 10 mL of distilled water. Prior to measurement, the pH meter was calibrated using phosphate buffer solutions of pH 4 and pH 9.[17]

Viscosity

The viscosity of the plain Naringin gel (Na plain gel) and the Naringin phytosomal gel (Na-PG) was assessed using a Brookfield viscometer (CAP 2000+, Brookfield Engineering Laboratories, MA, USA) fitted with a cone and plate system. The measurements were carried out at 25°C using disc spindle No. 1 at a rotational speed of 50 rpm.[17]

Spreadability

The spreadability of the plain Naringin gel (Na plain gel) and Naringin Phytosomal Gel (Na-PG) was assessed using the parallel plate method. An accurately weighed 0.5 g of gel was placed at the center of a glass petri plate, forming an initial spot of 1 cm in diameter. A second glass petri plate was gently placed over it, followed by the application of a 50 g weight. The setup was left undisturbed for 5 min, after which the final diameter of the spread gel was measured. A larger diameter and shorter spreading time were indicative of excellent spreadability.[17]

$$\text{Spreadability} = \frac{\text{Wx L}}{\text{T}}$$

W = A weight was attached to the upper slide (2 slides),

L = length of slide,

T = The time required for the slides to separate.

Drug content

The drug content of Na plain gel and Na-PG was determined using UV spectrophotometry. 1 g of gel was dissolved in 10 mL of methanol, followed by filtration of the solution. The drug concentration was then measured at 283 nm.[17]

In vitro diffusion study

The drug release profile of Na plain gel, Na-PG, and standard Diclofenac gel was evaluated using a Franz diffusion cell equipped with a dialysis membrane. The membrane was pre-soaked overnight in Phosphate Buffer Solution (PBS) at pH 5.5. It was then positioned between the donor and receptor compartments of the diffusion cell. Precisely 1 g of each gel formulation was placed in the donor compartment, which was sealed to prevent evaporation. The receptor compartment was filled with 20 mL of PBS, maintained at 32±0.5°C, and stirred continuously at 100 rpm to ensure uniformity. At predetermined time intervals (1st, 2nd, 3rd, 4th, 5th, and 6th hr), 1 mL samples were withdrawn and immediately replaced with fresh PBS to maintain sink conditions. The concentration of Naringin in the collected samples was determined using UV spectrophotometry at 283 nm.[18]

Ex vivo permeation study

The permeation profiles of Na plain gel, Na-PG, and standard Diclofenac gel were assessed using a Franz diffusion cell equipped with excised rat skin as the membrane. Abdominal skin was obtained from Wistar albino rats for the experiment. The

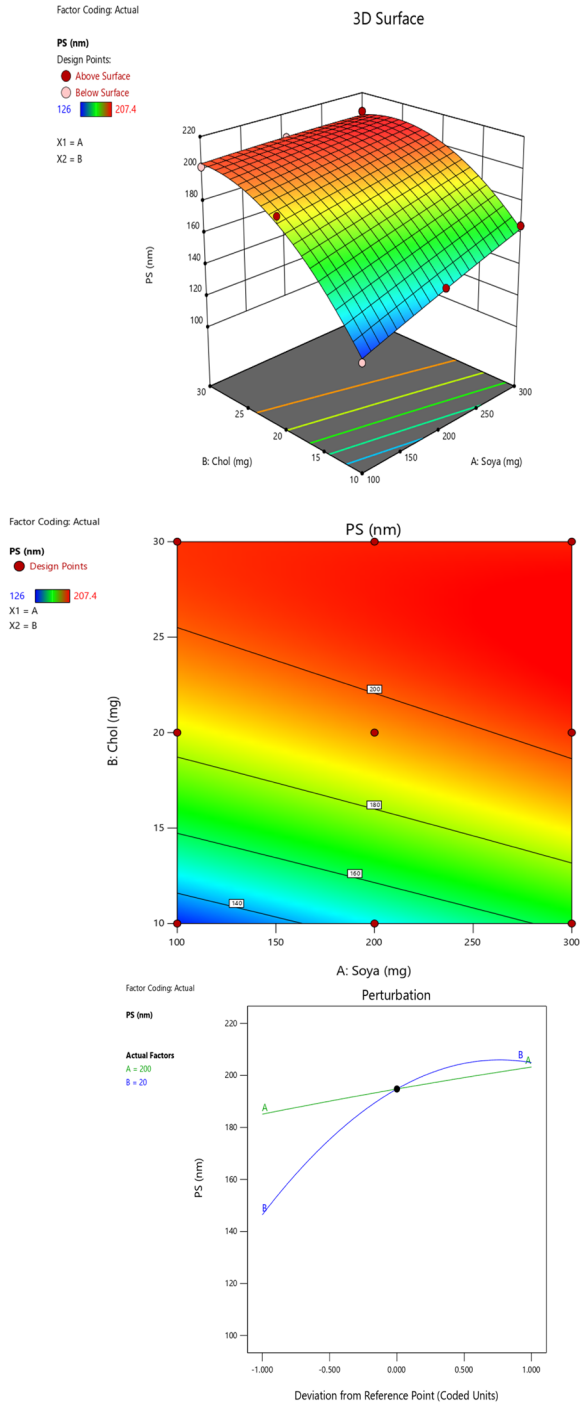

Figure 1: The 3D response surface plot (A), 2D contour plot (B), and perturbation plot (C) illustrate the influence of phospholipid and cholesterol concentrations on the vesicle size of Na-P.

Table 1: Formulation composition table of Naringin-loaded phytosomes.

Formulation code	Drug (mg)	Lecithin (mg)	Cholesterol (mg)
Na-Ph 1	10	100	10
Na-Ph 2	10	100	20
Na-Ph 3	10	100	30
Na-Ph 4	10	200	10
Na-Ph 5	10	200	20
Na-Ph 6	10	200	30
Na-Ph 7	10	300	10
Na-Ph 8	10	300	20
Na-Ph 9	10	300	30

abdominal hair was initially trimmed with an electric clipper, followed by the application of a depilatory cream to ensure complete hair removal. The skin was then carefully excised using forceps, and any remaining subcutaneous tissue was removed. The cleaned skin was subsequently shaved, rinsed, and soaked overnight in Phosphate-Buffered Saline (PBS, pH 5.5) prior to use in the study.[19]

During the experiment, the prepared skin membrane was positioned between the donor and receptor compartments of the Franz diffusion cell, with the stratum corneum (epidermal side) facing the donor compartment. An accurately weighed amount (1 g) of each gel formulation was applied to the donor compartment. The receptor compartment was filled with 12 mL of Phosphate-Buffered Saline (PBS, pH 5.5), maintained at 32±0.5°C, and continuously stirred at 100 rpm to ensure uniform mixing. Samples (1 mL) were withdrawn from the receptor compartment at predetermined time intervals (1st to 6th hr) and immediately replaced with an equal volume of fresh PBS to maintain sink conditions. The amount of Naringin permeated through the skin was quantified using UV spectrophotometry at 283 nm.[19]

Skin irritancy test

To evaluate potential skin irritation, a study was conducted using 18 albino rats, divided into three groups of six. The animals were housed in cages under controlled conditions of temperature, lighting, and free access to food and water. 24 hr prior to the experiment, the dorsal area of each rat was shaved and cleaned with alcohol.

Group I (Control): received plain gel without any active ingredient.

Group II (Test Group 1): received Naringin plain gel.

Group III (Test Group 2): received the experimental gel containing Na-PG.

Following application, the rats were monitored for signs of skin irritation, such as redness (erythema) and swelling (edema), at specified time intervals: 1 hr, 24 hr, 48 hr, 72 hr, and on the 7th day.[20]

Anti-inflammatory activity

The anti-inflammatory potential of the formulations was evaluated using the carrageenan-induced paw edema model in Wistar albino rats. A total of 18 rats were randomly divided into three groups, each consisting of six animals.

All formulations were topically applied to the right hind paw 30 min after the administration of carrageenan. Edema was induced by subplantar injection of 0.1 mL of 1% w/v carrageenan solution into the right hind paw of each rat. Paw volume was measured at intervals of 1, 1.5, 2, 2.5, 3, and 3.5 hr using a plethysmometer. The extent of paw edema inhibition in the treated groups was compared to the negative control group and the standard treatment group.[21]

Stability study

The stability studies were conducted in accordance with the ICH Q1A(R2) guidelines for stability testing of new dosage forms and stability was evaluated by monitoring the particle size and entrapment efficiency of the optimized Na-Ph, along with the physical appearance, pH, viscosity, and drug content of the Naringin-loaded phytosomal gel over a three-month period. The formulations were stored in airtight containers at two different temperatures: 4±0.5°C and 25±0.5°C. To assess any changes over time, samples were collected in triplicate at four-time intervals: 0, 1, 2, and 3 months.[22,23]

Statistical analysis

The experimental data were statistically analyzed using one-way Analysis of Variance (ANOVA) with GraphPad Prism software, version 9.0 (GraphPad Software Inc., CA, USA). Statistical significance was determined at *p*-values of <0.05, <0.01, and <0.0001.

RESULTS AND DISCUSSION

Formulation of Naringin-loaded phytosomes

The study employed the thin-layer method, a well-established technique known for its suitability with water-insoluble drugs like Naringin. This method offers several advantages: it is relatively simple to implement, produces stable vesicles for storage, and allows for control over certain product characteristics. It is previously investigated several factors that influence the final properties of the Naringin vesicles. These factors include: evaporation time which can impact the thickness and uniformity of the initial lipid film. The rotation speed of the flask during evaporation was found to significantly affect these aspects. The choice of solvent for hydrating the lipid film is crucial and depends on the intended use of the final formulation (dosage form, route of administration). In this study, Phosphate-Buffered Saline (PBS) at pH 7.4 was selected. Notably, the presence of salts in PBS also aids the self-assembly process of phospholipids into vesicles

Effect of vesicle size on the independent variable

The vesicle size of Naringin-loaded phytosomes ranged from 126 to 207.4 nm, as presented in Table 2. Among the formulations, Na-Ph 9 exhibited the largest vesicle size, whereas Na-Ph 1 showed the smallest.

The effect of independent variables on vesicle size is described by a quadratic equation:

The vesicle size (Y_1) is represented by the polynomial equation:

$$Y_1 = 194.78 + 90.5A + 29.25B - 8.33AB - 0.6167A^2 - 19.12B^2$$

where A is the amount of lecithin and B is the amount of cholesterol.

This equation indicates that both soya-lecithin and cholesterol have a positive and synergistic effect on vesicle size. The high positive coefficient of A suggests that lecithin has a more pronounced influence on vesicle size compared to cholesterol in the Na-Ph formulation.

ANOVA results support the statistical significance of the quadratic model, with an F-value of 69.36. The model exhibits strong predictive capability, with an R^2 value of 0.9917, an adjusted R^2 of 0.9771, and a predicted R^2 of 0.8954. The model also demonstrates high adequacy with a precision value of 21.449, confirming its reliability (Table 3).

The influence of the independent variables on vesicle size was visualized using 3D response surface plots, 2D contour plots, and a perturbation plot (Figure 1). These graphical representations show a marked increase in vesicle size with rising concentrations of lecithin and cholesterol. The perturbation plot displays a linear response for factor A (lecithin), while factor B (cholesterol)

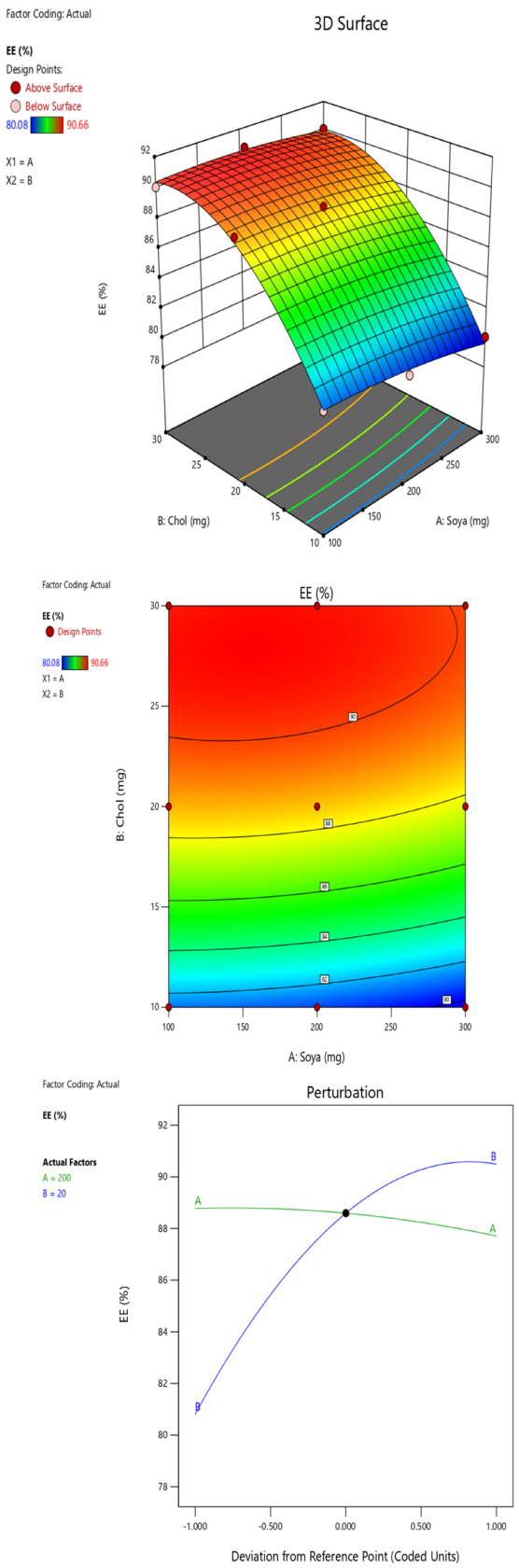

Figure 2: 3D response surface plot (A), 2D contour plot (B), and perturbation plot (C) illustrating the effect of phospholipid and cholesterol concentrations on the entrapment efficiency of Na-Ph.

exhibits a slight curvature, suggesting that cholesterol plays a critical role in controlling vesicle size.

Additionally, the study evaluated the impact of phospholipid complex concentration on vesicle size. Formulations were prepared with concentrations ranging from 100 mg to 300 mg, all producing vesicles below 200 nm. A gradual increase in vesicle size was observed with higher phospholipid concentrations, attributed to enhanced vesicle-vesicle interactions-such as collisions or electrostatic attractions-that hinder free movement and promote aggregation.

Moreover, formulations with elevated lipid content showed a greater tendency to agglomerate, further contributing to increased vesicle size. Despite this, all formulations maintained a low Polydispersity Index (PDI <0.5), indicating a high degree of uniformity and homogeneity in vesicle size distribution.

Effect of entrapment efficiency on the independent variable

As shown in Table 2, the entrapment efficiency of Naringin-loaded phytosomes ranged from 80.08% to 90.66%. The highest vesicle size was observed in formulation Na-Ph 6, whereas the lowest was recorded in Na-Ph 7. The influence of independent variables on entrapment efficiency is represented by a quadratic equation.

Entrapment efficiency = 67.9800 + 0.002867 A + 1.60200 B + 0.000290 AB - 0.000035

Where Y2 represents entrapment efficiency, A denotes the amount of lecithin, and B represents the amount of cholesterol. According to the equation, both soya-lecithin and cholesterol exhibit a positive or synergistic effect on entrapment efficiency. The high coefficient of B indicates that cholesterol has a more pronounced impact on the vesicle size of Na-Ph. ANOVA results confirm the significance of the quadratic model, with an F-value of 90.53. The model shows strong statistical validity, as evidenced by an R^2 value of 0.9934, an adjusted R^2 of 0.9824, and a predicted R^2 of 0.9231. Additionally, an adequate precision value of 22.3482 further supports the model's reliability (Table 3). The effect of independent variables on vesicle size was depicted by the graphs of 3D response surface graph, 2D contour plot and perturbation plot in Figure 2. The graph confirms that there is a substantial increase in vesicle size with an increase in the amount of lecithin and cholesterol. The success of naringin entrapment within the phytosome form, essentially a vesicular system, is heavily dependent on the composition of the surrounding membrane. The ratio of phospholipid to Naringin significantly influences the amount of naringin incorporated. A higher phospholipid ratio within the vesicle membrane enhances its ability to encapsulate

Table 2: Formulation codes along with independent variables, dependent variables, and Polydispersity Index (PDI) of Naringin-loaded phytosomes based on a 3^2 factorial design.

Formulation code	Lecithin	Cholesterol	Particle size (nm)	EE%	PDI
Na-Ph 1	100	10	126	81.24	0.145
Na-Ph 2	100	20	189.5	89.15	0.119
Na-Ph 3	100	30	201.6	90.06	0.198
Na-Ph 4	200	10	146.9	80.42	0.219
Na-Ph 5	200	20	194.7	88.82	0.139
Na-Ph 6	200	30	204.5	90.66	0.220
Na-Ph 7	300	10	165.1	80.08	0.243
Na-Ph 8	300	20	198.9	87.11	0.121
Na-Ph 9	300	30	207.4	90.06	0.208

Table 3: Analysis of Variance (ANOVA) results and adequate precision values for each response of Naringin-loaded phytosomes based on quadratic models.

Source	Responses					
	Y_1 (Vesicle Size)			Y_2 Entrapment Efficiency		
	F-Value	*p*-Value Probe >F	Adequacy Precision	F-value	*p*-value Probe >F	Adequacy Precision
Model	69.36	0.0027	21.449	90.53	0.0018	22.3482
A	25.69	0.0148		4.82	0.1156	
B	268.35	0.0005		397.29	0.0003	
AB	14.49	0.0319		0.9509	0.4014	
A^2	0.0398	0.8547		0.6925	0.4664	
B^2	38.21	0.0085		48.86	0.0060	

water-insoluble drugs like naringin. While the initial study using a rotary evaporator provided qualitative information through visual evaluation, it highlights the crucial role of phospholipid content in entrapment efficiency.

Optimization of Naringin-loaded phytosomes

Using the point prediction tool in Design-Expert® software based on the 3^2 factorial design. was utilized to identify the optimal formulation among the nine developed batches. The selection criteria focused on achieving maximum entrapment efficiency and minimum vesicle size. After comprehensive analysis, formulation Na-Ph5, comprising 200 mg of soya lecithin and 20 mg of cholesterol, was identified as the optimal batch (Figure 3). The optimized Na-Ph5 formulation exhibited a vesicle size of 194.5 nm, a Polydispersity Index (PDI) of 0.139 (Figure 4), and an entrapment efficiency of 88.82%.

Polydispersity Index (PDI)

The uniformity of the vesicles' size distribution was assessed by Polydispersity Index (PDI). As shown in Table 2, the PDI of Na-Ph batches ranged from 0.119 to 0.312. These low values indicate a narrow size distribution, meaning the vesicles are all relatively similar in size. In the context of lipid-based drug delivery systems, PDI values below 0.3 generally signify a homogenous population.

TEM analysis

The Transmission Electron Microscopy (TEM) image (Figure 5) revealed that the optimized Naringin-loaded phytosomes exhibited a predominantly spherical morphology with a smooth and well-defined surface, indicating successful vesicle formation. The vesicle sizes were observed in the range of 122.85-141.77 nm, which closely corresponded with the particle size data obtained from the Malvern Zetasizer analysis, confirming the consistency between both characterization methods. The phytosomes appeared as unilamellar vesicles, with no evident multilamellar structures, suggesting uniform lipid arrangement around the Naringin molecules. Furthermore, the image showed only minimal aggregation, implying effective dispersion

Table 4: Evaluation of gels.

Parameters	Na gel	Na-PG gel
Color	White	White
Homogeneity	Good	Good
pH	5.85±0.21	6.0±0.10
Viscosity (cP)	1452±23	1465±14
Spreadability (g.cm/sec)	5.21±0.40	6.21±0.13
Drug content (%)	96.45±1.12	97.13±0.83

Means±SD, *n*=3.

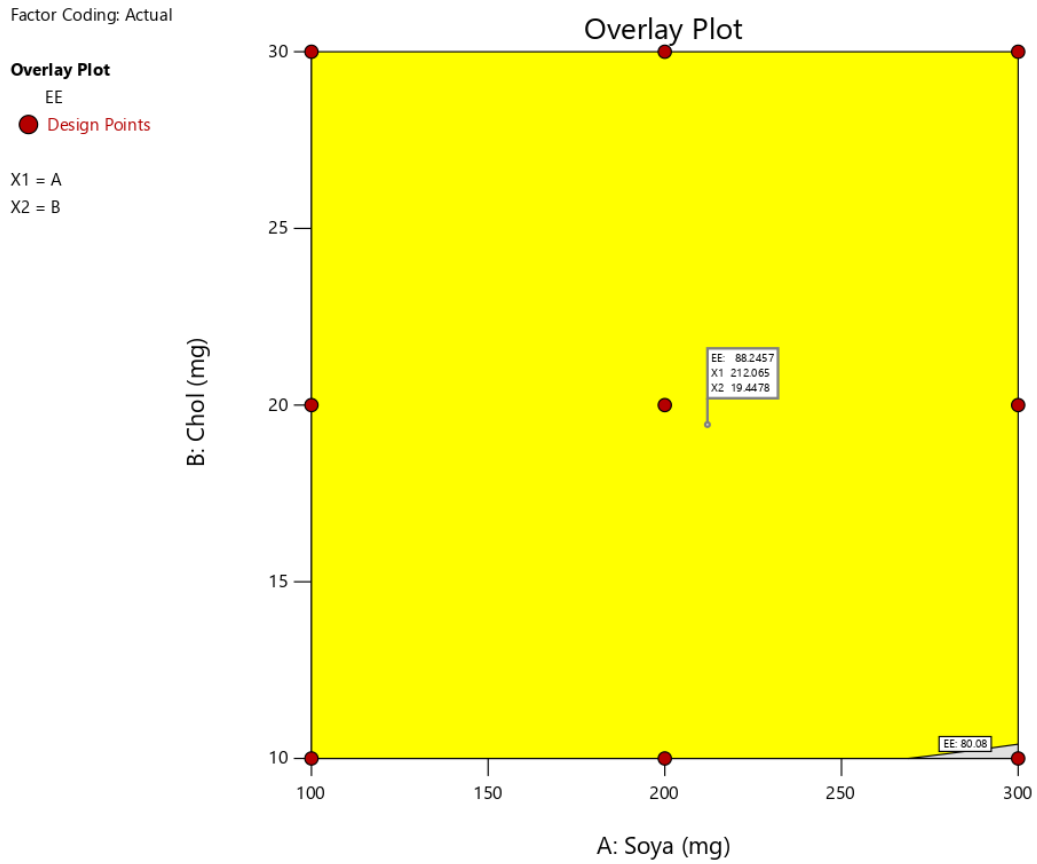

Figure 3: Overlay Plot.

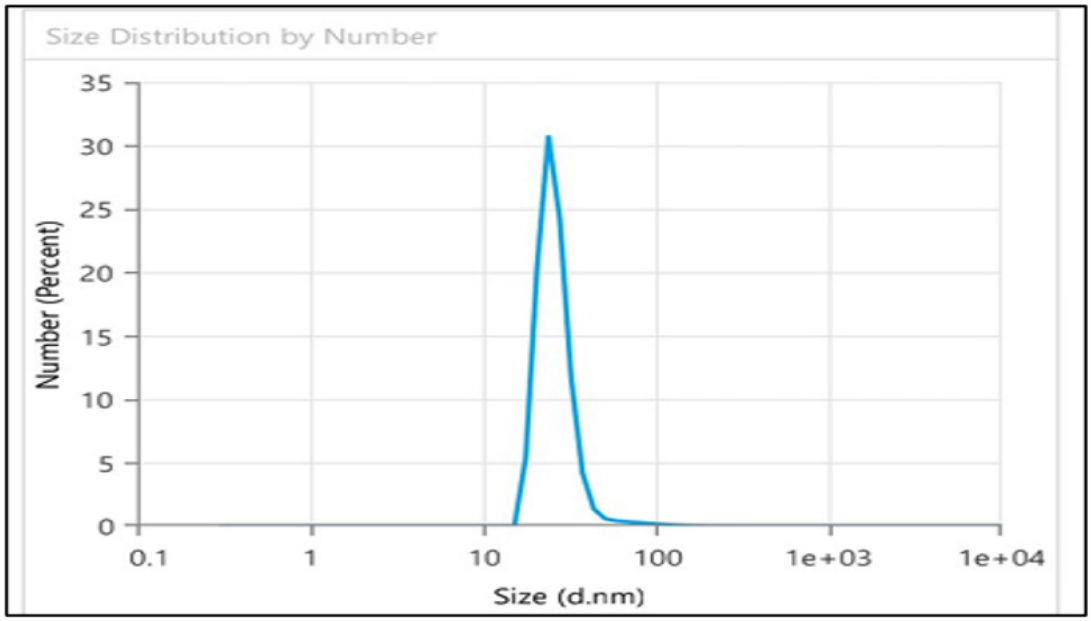

Figure 4: Vesicle size of optimize batch Na-Ph 5.

stability and uniformity of the vesicular system. The absence of significant clumping or deformation further supports the good colloidal stability and homogeneity of the optimized phytosomal formulation.

Evaluation of gels

The evaluation parameters of the Naringin plain gel (Na plain gel) and the optimized Naringin-loaded Phytosomal Gel (Na-PG), as summarized in Table 4, indicate that both formulations displayed an aesthetically pleasing appearance with suitable color, smooth texture, and uniform consistency, free from any lumps. The pH values recorded for Na plain gel and Na-PG were 5.85±0.21 and 6.00±0.10, respectively-falling within the ideal range for topical application and closely matching the skin's natural pH. The viscosity values for the Na plain gel and Na-PG were found to be 1452±23 cP and 1465±14 cP, respectively, indicating similar rheological properties. The spreadability of the plain gel was 5.21±0.40 g·cm/sec, while the phytosomal gel exhibited a slightly higher value of 6.21±0.13 g·cm/sec, demonstrating that both formulations can be effortlessly applied to the skin. Furthermore, the drug content was notably high in both cases 96.45±1.12% for the Na plain gel and 97.13±0.83% for Na-PG-indicating effective and uniform drug distribution throughout the formulations

In vitro diffusion study

Figure 6 illustrates the release profiles of Na-PG compared to plain Na gel and a standard gel. The plain Na gel released 66.53±4.11% of the drug within 6 hr, while the standard gel released 78.9±3.12%. Notably, the Na-PG formulation exhibited a significantly enhanced and sustained release profile, reaching

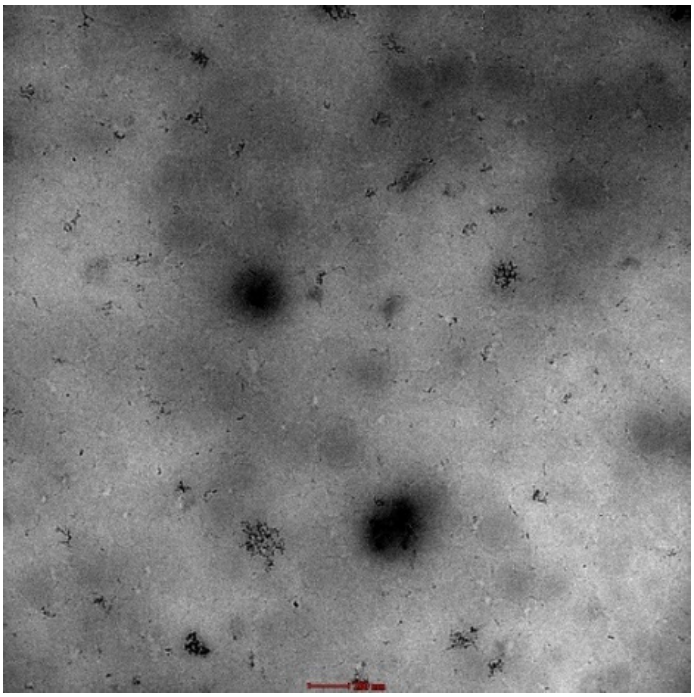

Figure 5: Transmission Electron Microscopy of optimized Naringin-loaded phytosomes.

84.26±5.20% release over the same timeframe. The Na-PG demonstrated an initial burst release within the first hour, likely due to the presence of drug deposited on the surface of the phytosomal particles. This initial release could potentially help achieve therapeutic drug concentration rapidly. The entrapped extract within the lipid content of the phytosomes facilitated a sustained release, which is an ideal characteristic for topical formulations. This sustained release helps to maintain therapeutic drug concentration over a longer period.

Ex vivo permeation study

The permeation of the drug Naringin was studied over 24 hr for Na-PG, Na plain gel and standard gel. As illustrated in Figure 7, Na-PG demonstrated superior permeation with 80.54±1.21% of the drug permeating compared to 71.85±2.51% for the standard gel and 63.61±1.42% for the Na plain gel. This enhanced permeation in the Na-PG is likely attributed to the presence of phospholipids. When these phospholipids come into contact with the surrounding medium, they self-assemble into bilayer structures, ultimately forming lipid vesicles. These vesicles act as a reservoir, slowly releasing the entrapped Naringin over time. This sustained release mechanism contributes to the higher permeation observed with the Na-PG compared to Na plain gel and standard gel which lack this controlled release feature.

Skin irritancy test

The skin irritancy potential of the formulations was assessed over a 7-day period using Wistar rats. The animals were randomly assigned to three groups:

Group 1: Received the plain gel base (drug-free),

Group 2: Was treated with the Naringin plain gel, and

Group 3: Received the experimental gel containing Naringin-loaded Phytosomes (Na-PG).

Throughout the study duration, all groups were monitored daily for any visible signs of erythema (redness) and edema (swelling). No observable changes in skin color, texture, or morphology were detected in any of the groups, indicating the absence of

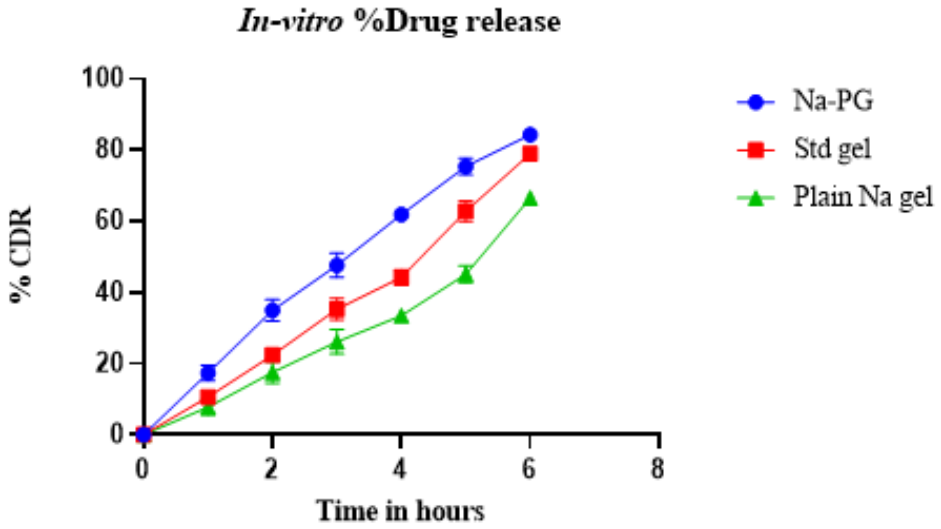

Figure 6: *In vitro* drug release profile of Naringin gel and Naringin loaded phytosomes gel.

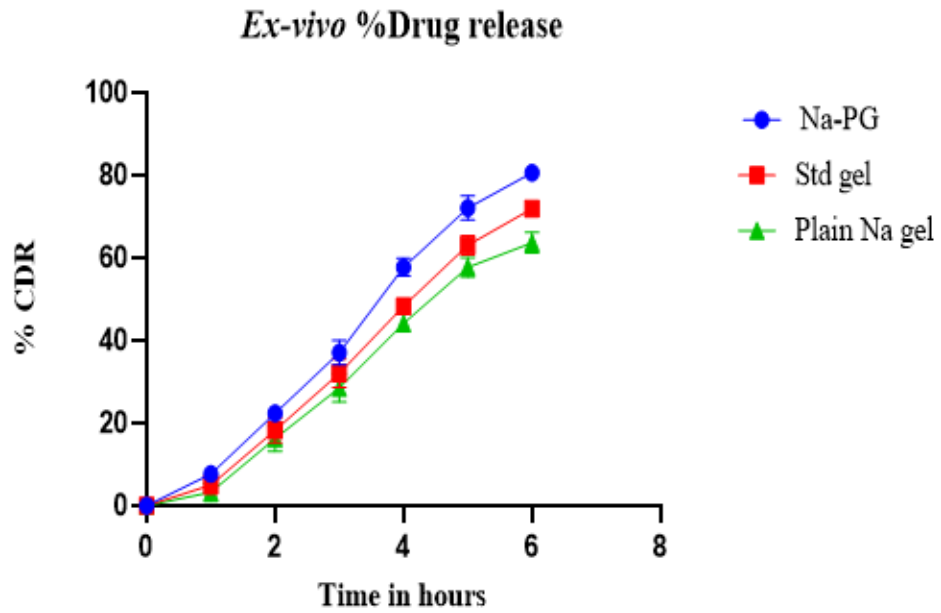

Figure 7: *Ex vivo* permeation studies of Naringin gel and Naringin loaded phytosomes gel across rat skin.

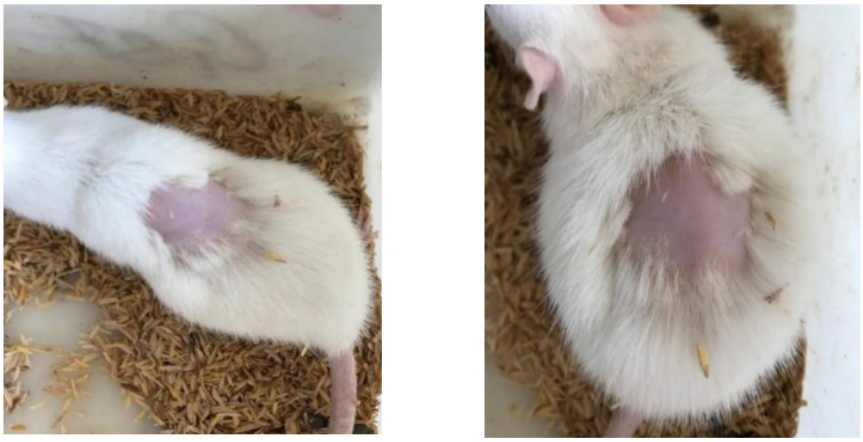

Figure 8: Results of the skin irritancy test.

Table 5: Effect of Naringin phytosomal gel on carrageenan-induced paw edema in Wister rats.

Time (min)	0	30th min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Group Control 1	1.67±0.25	2.47±0.29	2.56±0.28	2.67±0.26	2.79±0.31	2.83±0.32	2.94±0.34	2.90±0.38
Groups Standard (Diclofenac gel) 2	1.65±0.41	2.31±0.45	2.40±0.39	2.49±0.28	2.44±0.43	2.28±0.35	2.12±0.26	1.76±0.32
Group 3 (Naringin phytosomal gel)	1.66±0.20	2.38±0.4s4	2.47±0.38	2.58±0.42	2.65±0.37	2.60±0.25	1.99±0.19	1.70±0.23
Group 4 (Naringin plain gel)	1.65±0.53	2.43±0.64	2.51±0.62	2.62±0.72	2.71±0.96	2.72±0.29	2.24±0.83	1.86±0.38

Table 6: Stability of optimized formulation Naringin phytosomal gel at different storage condition.

Temp		4°C			25°C	
Time	Day 1	Day 30	Day 90	Day 1	Day 30	Day 90
pH	5.96±0.201	5.98±0.30	5.97±0.12	5.96±0.201	5.97±0.14	5.98±0.34
Viscosity	1327±12	1326 13	1325±11	1327±12	1322±14	1323±16
Drug content (%)	97.18±0.94	97.23±0.90	97.25±0.88	97.1±0.94	97.31±0.72	97.14±0.92

skin irritation. Thus, all tested formulations were found to be non-irritant and safe for topical application. The results of the skin irritancy test are illustrated in Figure 8.

Anti-inflammatory activity

The anti-inflammatory effect of Naringin phytosomal gel was evaluated using the carrageenan-induced paw edema model in Wistar rats, and the results are presented in Table 5 and Figure 9. In the control group, a progressive increase in paw edema was observed from 0 min (1.67±0.25 mm) to 6 hr (2.90±0.38 mm), indicating significant inflammation throughout the study period. Treatment with the standard diclofenac gel significantly reduced paw edema from 3 hr onward, showing a steady decline from 2.44±0.43 mm at 3 hr to 1.76±0.32 mm at 6 hr. Similarly, animals treated with Naringin phytosomal gel demonstrated a marked reduction in paw thickness, with edema peaking at 3 hr (2.65±0.37 mm) and then decreasing sharply to 1.70±0.23 mm by 6 hr. The phytosomal formulation exhibited a higher anti-inflammatory response compared to the plain Naringin gel, which showed moderate inhibition with a reduction to 1.86±0.38 mm at 6 hr. The enhanced effect of the Naringin phytosomal gel may be

Prior to the carrageenan injection. Following the carrageenan injection

Subsequent to the application of the Na-Ph gel. Instrument- Plethysmometers

Figure 9: Anti-inflammatory activity by carrageenan-induced paw edema method on Wister rats.

attributed to improved drug penetration and bioavailability through the phytosomal carrier system.

Stability studies

Stability is crucial for any formulation's success. To ensure this, a stability study was conducted. As detailed in Table 6, all parameters assessed for both the phytosomal formulation and phytosomal gel remained within acceptable limits throughout the three-month study period at both temperatures. This confirms the stability of both formulations.

CONCLUSION

Naringin-loaded phytosomes were successfully developed and optimized using a 3^2 factorial design approach with the assistance of Design-Expert® software. The optimized formulation exhibited nanoscale vesicle size and high entrapment efficiency, confirming the efficient encapsulation of Naringin within the lipid-based phytosomal matrix. Both *in vitro* and *ex vivo* evaluations demonstrated significantly improved drug release from the phytosomal gel in comparison to the plain Naringin gel and standard diclofenac gel.

Dermal safety was confirmed through skin irritancy studies, with no signs of erythema or edema observed during the assessment period. Additionally, anti-inflammatory evaluations showed a marked reduction in carrageenan-induced paw edema, underscoring the formulation's strong therapeutic efficacy. Stability studies supported the formulation's robustness, indicating no significant alterations in physicochemical characteristics over time.

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ABBREVIATIONS

ANOVA: Analysis of Variance; **BCS:** Biopharmaceutics Classification System; **cP:** Centipoise; **DOE:** Design of Experiments; **EE%:** Entrapment Efficiency Percentage; **FDA:** Food and Drug Administration; **ICH:** International Council for Harmonisation; **MLVs:** Multilamellar Vesicles; **Na-Ph:** Naringin-loaded Phytosomes; **Na-PG:** Naringin-loaded

Phytosomal Gel; **NG-PH gel:** Naringin Phytosomal Gel; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **OPT-Na-Ph:** Optimized Naringin-loaded Phytosomes; **PBS:** Phosphate Buffer Solution; **PDI:** Polydispersity Index; **RBF:** Round-Bottom Flask; **rpm:** Revolutions per minute; **SUVs:** Small Unilamellar Vesicles; **TEM:** Transmission Electron Microscopy; **UV:** Ultraviolet; **KAHER:** KLE Academy of Higher Education and Research; **KLE:** Karnataka Lingayat Education; **QbD:** Quality by Design; **HPTLC:** High Performance Thin Layer Chromatography; **INDIA:** Republic of India; **w/v:** Weight by Volume; **v/v:** Volume by Volume; **nm:** Nanometer; **mg:** Milligram; **mL:** Milliliter; **hr:** Hour; **min:** Minutes; **sec:** Seconds; **cm:** Centimeter; **g:** Gram; **µg:** Microgram; **°C:** Degree Celsius; **kV:** Kilovolt; **g·cm/sec:** Gram Centimeter per Second.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The study aimed to formulate and evaluate a Naringin-loaded phytosomal gel using a 3^2 factorial design to enhance anti-inflammatory efficacy. Phytosomes were prepared by the thin-film hydration method with soya lecithin and cholesterol and characterized for vesicle size, PDI, and entrapment efficiency. The optimized formulation (F6) showed a vesicle size of 198.9 nm, PDI of 0.236, and 90.66% entrapment efficiency. The gel exhibited suitable pH, viscosity (1462 cPs), and high drug content (97.18%). It showed superior diffusion, permeation, and significant anti-inflammatory activity without skin irritation, indicating its potential as a safe and effective transdermal delivery system.

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