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Journal of Drug Delivery and Therapeutics

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Research Article

Formulation and Evaluation of Phytosomal Gel of Hesperidin

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Article Info:

Article History:

Received 10 Oct 2025
Reviewed 22 Nov 2025
Accepted 19 Dec 2025
Published 15 Jan 2026

Cite this article as:

Mannan A, Naila R, Formulation and Evaluation of Phytosomal Gel of Hesperidin, Journal of Drug Delivery and Therapeutics. 2026; 16(1):59-72 *DOI: http://dx.doi.org/10.22270/jddt.v16i1.7510*

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Abstract

Background: Hesperidin possesses potent antioxidant and anti-inflammatory activity but exhibits limited topical efficacy due to poor solubility and low skin permeability. Phytosomal systems can enhance dermal delivery of such phytoconstituents.

Materials and Methods: Hesperidin–phospholipid phytosomes were prepared by the thin-film hydration method and optimized based on particle size, zeta potential, and entrapment efficiency. The optimized formulation (F6) was incorporated into a Carbopol 934 gel and evaluated for physicochemical properties. In-vitro diffusion studies were performed using Franz diffusion cells, while FTIR analysis and release kinetics were also assessed.

Results: The optimized phytosomes showed nanosized vesicles (168 nm), stable zeta potential, and high entrapment efficiency (85.52%). The phytosomal gel exhibited acceptable physicochemical characteristics and sustained drug release, achieving 95.6% release within 8 h. FTIR confirmed compatibility, and drug release followed the Higuchi model (R^2 = 0.985).

Conclusion: The hesperidin phytosomal gel enhanced solubility, skin permeation, and sustained drug release, demonstrating its potential for effective topical antioxidant and anti-inflammatory therapy.

Keywords: Hesperidin; Phospholipid; Thin-film hydration method; Phytosomes; Topical gel; In-vitro drug release studies.

1. INTRODUCTION

Hesperidin is a citrus bioflavonoid reported to possess strong antioxidant, anti-inflammatory, vasoprotective, and wound-healing activities[1,2]. Despite its wide therapeutic potential, its clinical utility in topical applications remains limited due to poor aqueous solubility, low permeability, and inadequate bioavailability[3]. These biopharmaceutical challenges necessitate the development of delivery systems capable of enhancing its solubility and dermal penetration.

Phytosomes, which are molecular complexes of phytoconstituents and phospholipids, are known to enhance lipophilicity, membrane affinity, and dermal absorption of poorly soluble plant-derived molecules[4,5]. Compared with conventional herbal extracts, phytosomes show better stability, higher permeability, and improved therapeutic performance. Although several nanocarrier-based systems for hesperidin have been explored, very few studies have focused on phytosomal gel formulations specifically designed to improve skin permeation and provide sustained release[6].

The present research aims to develop a hesperidin–phosphatidylcholine phytosomal system, optimizing its physicochemical characteristics, and incorporating it into a topical gel. The novelty of the study lies in utilizing a phosphatidylcholine-based phytosomal complex to improve hesperidin's solubility, dermal penetration, and prolonged drug release, thereby enhancing its topical therapeutic efficiency.

2. MATERIALS AND METHODS

2.1. COLLECTION OF DRUG AND EXCIPIENTS

Hesperidin was procured from Otto chemie, Mumbai. Phosphotidyl choline and Cholesterol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

2.2 PREFORMULATION STUDIES:

PHYSICOCHEMICAL PROPERTIES OF DRUG

ORGANOLEPTIC PROPERTIES:

Assess the drug's color, odor, and texture by spreading a small amount on a white surface.

SOLUBILITY:

A number of solvents were tested to determine how well Hesperidin dissolved. Put 5 millilitres of water, DMSO, and methanol into a conical flask with extra medication. Before analysing the concentration using UV spectroscopy, shake, filter, and dilute the sample.[7]

MELTING POINT:

The drug's melting point was determined by use of the capillary melting point apparatus.[8]

Calculation of the Calibration Curve and Absorption Maxima (λmax)

Finding the Maximum Absorption (λmax)

A phosphate buffer solution (pH 6.8) containing 100 µg/mL of hesperidin was produced. The next step was to scan this solution using a UV-Visible spectrophotometer, which operates between the 200 to 400 nm wavelength range. The wavelength that produced the greatest absorbance value was determined to be the absorption maximum (λmax).[9]

Hesperidin Calibration Curve

First, we made a main stock solution by dissolving 100 milligrammes of hesperidin in phosphate buffer (pH 6.8) according to the specified weight. Using the same buffer, the solution was filled up to the mark in a 100 mL volumetric flask, resulting in a final concentration of 1000 µg/mL.

A 100 mL volumetric flask was used to create a working standard solution, which was made by pipetting 1 mL of the main stock solution. Using phosphate buffer (pH 6.8), the volume was reduced to the mark in order to create a secondary stock solution with a concentration of 100 µg/mL. Subsequent dilutions for the calibration curve were prepared using this solution as their basis.[9]

Preparation of serial dilutions for standard calibration curve:

Hesperidin standard solutions with concentrations of 10, 20, 30, 40, and 50 µg/mL were made by gradually diluting the secondary stock solution. Using a double-beam UV-Visible spectrophotometer, the absorbance of every concentration was assessed at the pre-established λmax of 285 nm. By aligning the concentration on the X-axis with the observed absorbance on the Y-axis, a calibration curve was generated.[9]

FTIR: DRUG – EXCIPIENT COMPATIBILITY STUDY

Pure drug and physical mixtures with excipients were analyzed at 4000-400 cm^{-1} to detect chemical interactions.[10]

2.3 Method for Preparing Phytosomes

The thin-film hydration approach was used to synthesise phytosomes that contained the medication. In a round-bottom flask, a combination of dichloromethane and methanol (in a 2:1 ratio) was used to dissolve hesperidin, cholesterol, and phospholipid. A thin lipid coating formed on the inner wall of the flask after the organic solvent was evaporated using a rotary evaporator (Aditya Scientific) running at 60 rpm and 40°C under reduced pressure for 15 minutes. After drying, the film was rehydrated for an hour in phosphate-buffered saline (PBS, pH 7.4) while being continuously spun at 60 rpm. For a more even distribution and size reduction, the produced multilamellar vesicles (MLVs) were probe sonicated for 30 minutes using Mangaldeep Tech Solutions. The completed phytosomal mixture was kept at 4°C until further analysis.[11]

FORMULATION OF HESPERIDIN PHYTOSOMES

Table 1: Formulation of phytosomes

Ingredients	F1 (0.5:1)	F2 (0.5:2)	F3 (0.5:3)	F4 (1:1)	F5 (1:2)	F6 (1:3)	F7 (1.5:1)	F8 (1.5:2)	F9 (1.5:3)
Hesperidin(mg)	100	100	100	100	100	100	100	100	100
Phosphatidylcholine (mg)	100	200	300	100	200	300	100	200	300
Cholesterol (mg)	50	50	50	100	100	100	150	150	150
Dichloromethane: Methanol (ml)	20	20	20	20	20	20	20	20	20
7.4 Phosphate buffer	30	30	30	30	30	30	30	30	30

Figure 1: Rotary evaporator used in the preparation of hesperidin phytosomes

Figure 2: Sonication of samples and prepared samples of phytosomes

CHARACTERIZATION OF HESPERIDIN-LOADED PHYTOSOME

1. ENTRAPMENT EFFICIENCY:

Five millilitres of phytosomes containing Hesperidin were combined with pH 7.4 and centrifuged at 4000 rpm for 45 minutes at 4°C to segregate encapsulated medication from the non-encapsulated fraction. This technique created a sediment with the drug that had been trapped in it and a supernatant with the drug that had not been trapped in it. We gently took off the supernatant and dissolved the sediment in methanol so we could look at it. A UV–Visible spectrophotometer was used to measure the solution's absorbance at 285 nm.[12]

EE% = [Amount of Entrapped Hesperidin/Total Amount of Hesperidin] /100

2. Surface Morphology (Vesicle Size) – SEM:

The optimized hesperidin phytosomes were dried, coated, and imaged under SEM to observe vesicle shape and surface morphology[13].

3. Particle Size

Particle size and distribution were determined using a Horiba SZ-100 analyzer after diluting the samples with distilled water[14].

4. Zeta-potential:

Zeta potential was measured using a Malvern Zetasizer Nano ZS, with samples diluted in distilled water prior to analysis[15].

5. In Vitro Diffusion Experiment

A Franz diffusion cell equipment with a dialysis membrane was used to test the formulation's release profile. A day before to the experiment, the membrane was soaked in distilled water to prepare it for use. An upper donor chamber and a lower receptor chamber make up the assembly. To create an effective diffusion area of 2 cm^2, phosphate buffer (pH 7.4) was put into the receptor chamber and 5 mL of the phytosomal formulation was introduced into the donor compartment. For 600 minutes, the whole thing was spun on a magnetic stirrer set at 600 rpm. The receptor compartment was sampled hourly for a duration of 10 hours. To keep the sink conditions constant, an equal amount of new buffer was added after every sample. The drug content of the obtained samples was measured using a UV-Visible spectrophotometer set at 285 nm after the samples were properly diluted.[16]

2.4 FORMULATION OF GELS OF PHYTOSOMES

Preparation of Gel:

To make the gel formulations, Carbopol 934 was dissolved in distilled water and mixed continuously at a moderate speed with a mechanical shaker to make sure it was evenly distributed. A transparent and stable gel was formed after the polymer was hydrated. The pH of the prepared base was carefully adjusted to 5.5-6.5 by slowly adding triethanolamine.[17]

Incorporation of Phytosomes into the Gel:

The hydrated base was treated with the phytosomal dispersion containing hesperidin, and constant stirring was used to distribute the phytosomes evenly. As a preservative, methyl paraben was added. A homogenous gel was then produced by gradually adding triethanolamine to the liquid while stirring constantly. This neutralised the mixture. After the gels were made, they were placed in appropriate containers and left to cool to room temperature until further testing.[17]

Table 2: Formulation table of phytosomal gel

Ingredients	F1	F2	F3	F4	F5	F6
Phytosomes (ml)	5	5	5	5	5	5
Carbopol 934 (mg)	100	150	200	250	300	350
Methyl paraben (ml)	0.01	0.01	0.01	0.01	0.01	0.01
Triethanolamine (ml)	1	1	1	1	1	1
Water	q. s	q. s	q. s	q. s	q. s	q. s

Characterization of Hesperidin-Loaded Phytosomal Gel:

To guarantee the quality and homogeneity of the gels, they were tested for visual clarity, consistency, and the lack of extraneous particle matter.

1. Physical Appearance:

The created preparation was carefully examined visually to determine its appearance, which was categorised as either white, opaque, or clear.

2. Homogeneity:

A little amount was placed between the thumb and index finger for visual examination to determine the gel's uniformity. The homogeneity of the gel was determined by looking at its appearance, detecting any aggregates, and general uniformity.[18]

3. **Spreadability:**

The formulation's spreadability was assessed by means of the parallel plate method. A little amount of the material was placed between two glass slides, and the slides themselves had dimensions of 20 cm x 20 cm. On top of the slide, we placed a 100 g weight to make sure everything spread out evenly into a thin layer. After then, the weight was taken off, and the upper slide could travel downwards unhindered by the tied weight. A stopwatch was used to record the amount of time it took for the top slide to separate from the bottom slide. Because of its ease of use and low cost, this method is commonly chosen to measure and assess the spreadability of semisolid formulations. The spreadability (S) was determined by dividing m by L/t.[18]

4. Determination of viscosity:

A 50 ml beaker containing 30 grammes of gel preparation was left at room temperature with the spindle set to 5, 10, 20, 50, and 100 rpm.[18]

5. Measurement of pH:

A digital pH meter was used to measure the pH after dispersing 1 g of gel in 20 mL of distilled water. The technique was carried out three times in order to determine the mean value and standard deviation.[18]

6. Drug content

1-gram sample was dissolved in 100 mL of water using a phosphate buffer (pH 6.8) to assess the drug content of the prepared phytosomal gel. Following filtering, the drug concentration was determined by means of ultraviolet (UV) spectrophotometry.

7. A profile of in vitro diffusion

A membrane dialysis device and diffusion cells were used to assess the phytosomal gel's in vitro release profile. Here is the procedure: An internal diameter of 24 mm diffusion cell was used, and a 1 mL portion of the formulation was added to the donor compartment. A newly made phosphate buffer with a pH of 7.4 was added to the receptor compartment. The two sections were separated using a dialysis membrane.

To make sure the formulation touched the membrane directly, the donor compartment was placed in such a way. After that, a magnetic stirrer that was controlled by a thermostat was used to put the whole assembly on. The whole experiment was conducted with the diffusion medium maintained at a temperature of 37.0 ± 0.5 °C. Timed withdrawals of 1 mL from the receptor compartment occurred at 1, 2, 3, 4, 5, 6, 7, and 8 hour intervals. To keep the sink conditions constant, an equal amount of new pre-warmed buffer was promptly added after each withdrawal. To find the cumulative drug release, the samples were mixed with 10 mL of distilled water in a volumetric flask and then examined using a UV spectrophotometer.

8. Drug Release Kinetics:

The experimental data were fit into four regularly used kinetic

models—Zero Order, First Order, Higuchi, and Korsmeyer-Peppas equations—in order to analyse the release pattern[18].

Zero-Order Kinetics:

In this model, the drug's concentration has no effect on the release rate, which remains constant throughout the procedure. The formula for it is $Q = k_0t$, where k_0 is the zero-order release rate constant and Q is the total quantity of medication released at time *t*. A zero-order release kinetics plotted against *t* shows a straight line.

First-Order Kinetics:

It seems that many sustained-release formulations follow apparent first-order kinetics for drug release, according to Wagner's model. This means that the rate of release depends on the concentration. The first-order release rate constant, denoted as k_1, and the proportion of medication released at time *t* are described by the equation: $\log(1 - Q) = -k_1t / 2.303$. The first-order kinetics is confirmed by a straight-line plot of the logarithm of the proportion of medication remaining (log(1 - Q)) vs time.

Korsmeyer–Peppas Model:

This model refines the fit for drug release data: $Mt/ M\infty = k\,tn$. A linear plot of logarithm of drug release fraction versus the logarithm of time indicates the Peppas-Korsemeyer.

Higuchi Equation: This model shows a linear relation between drug released per unit surface area (Q) and square root of time: $Q = k2t1/2$. A linear plot of drug release versus square root of time indicates the Higuchi equation.

9. Stability studies

To show how the quality of the medicinal product varies with time in reaction to external variables like humidity and temperature is the primary goal of stability testing. A three-month stability chamber experiment was carried out on the phytosomal gel formulation in accordance with ICH requirements.[19]

RESULTS AND DISCUSSION:

PHYSICO-CHEMICAL PROPERTIES OF HESPERIDIN:

Table 3: Physicochemical Properties of Drug

PROPERTIES	RESULTS
APPEARANCE	Powder
ODOR	Characteristic herbal
PHYSICAL STATE	Solid
COLOUR	yellow

MELTING POINT OF HESPERIDIN:

Table 4: Melting Point of the Drug

PURE DRUG	STANDARD REFERENCE RANGE	OBSERVED RANGE
Hesperidin	250-255°C	254°C

Discussion: Based on the data presented, it seems that the melting point of hesperidin is 254 °C. This agreement proves that the sample is suitable for further formulation investigations as it is of sufficient purity and devoid of significant contaminants.

SOLUBILITY STUDIES

Table 5: Solubility studies of drug

SOLVENTS	SOLUBILITY(mg/ml)
Water	0.5
DMSO	51.04
METHANOL	40.3

Observation: DMSO shows the greatest solubilizing ability for hesperidin, followed by methanol and then water.

Determination of absorption maxima of Hesperidin

UV- SPECTROSCOPIC ANALYSIS OF HESPERIDIN

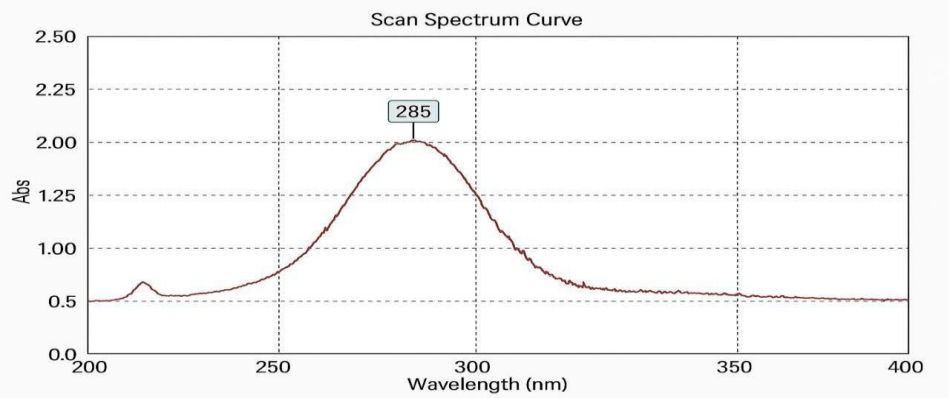


Figure 3: λmax for Hesperidin

Observation: We scanned a hesperidin solution from 200 to 400 nm with a concentration of 10 µg/mL. A sharp peak, with a maximum intensity of 285 nm, was seen in the absorption spectrum. The detection with a pH of 6.8, was thus accomplished at this particular wavelength.

CALIBRATION CURVE OF HESPERIDIN

Table 6: Calibration Curve data

CONCENTRATION (µg/ml)	ABSORBANCE nm
0	0
10	0.122 ±0.010
20	0.223 ±0.012
30	0.334 ±0.014
40	0.457 ±0.015
50	0.564 ±0.019

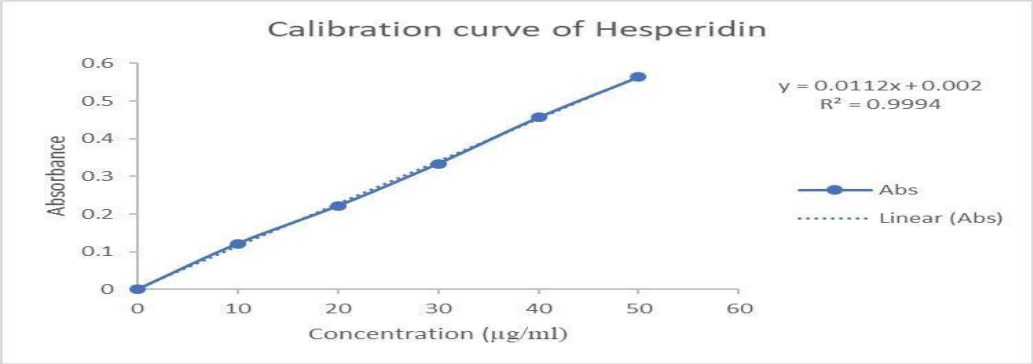


Figure 4: Calibration curve of Hesperidin

Observation: The graph followed the Beer-Lambert law, showing good linearity with an R2=0.9994.

FTIR- SPECTROSCOPIC ANALYSIS:

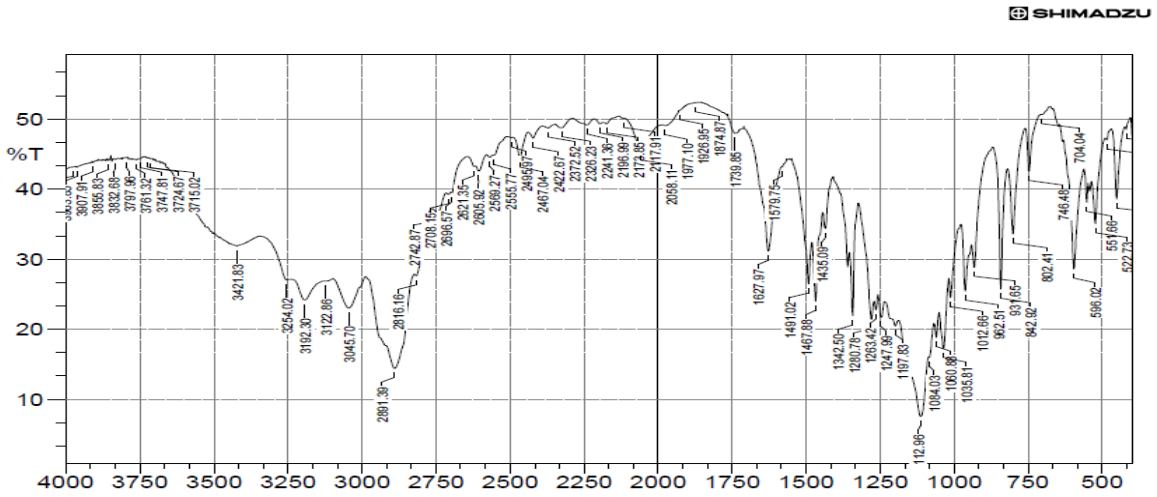


Figure 5: FT-IR Sample for Hesperidin

Table 7: Characteristic Peaks and frequency of Hesperidin

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3500-3000	3254.02
2	OH Bending	3000-2750	2891.39
3	C-H stretching	1750-1250	1342.50
4	C-N stretching	1250-1000	1112.96

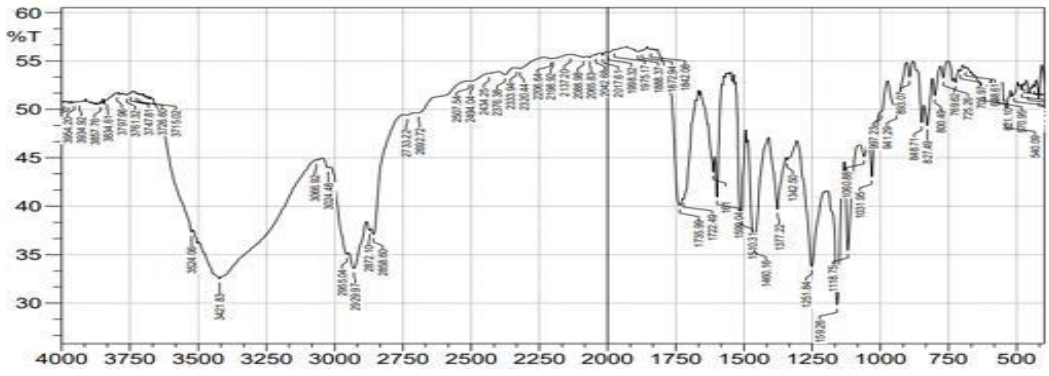


Figure 6: FT-IR Sample for Optimized formulation

Table 8: Characteristic Peaks and frequency of Optimized formulation

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3500-3000	3425.83
2	OH Bending	3000-2750	2929.97
3	C-H stretching	1750-1250	1530.04
4	C-N stretching	1250-1000	1251.36

Observation: This finding provides more evidence that the medication and excipients are compatible, suggesting that there are no major interactions.

CHARACTERIZATION OF HESPERIDIN LOADED PHYTOSOMES:

TABLE 9: CHARACTERIZATION OF HESPERIDIN PHYTOSOMES

FORMULATION	F1	F2	F3	F4	F5	F6	F7	F8	F9
ENTRAPMENT EFFICIENCY(%)	52.50	76.31	80.13	79.86	63.35	85.52	75.63	44.15	83.69
IN VITRO DRUG RELEASE (%)	85.67	87.57	90.37	88.78	88.83	92.69	86.58	88.55	90.25
PARTICLE SIZE	244	485	356	219	403	168	243	382	202

Observation: The phytosomes of hesperidin that were synthesised showed a high encapsulation efficiency (EE%), with values ranging from 44.15% to 85.52, as illustrated in the table.

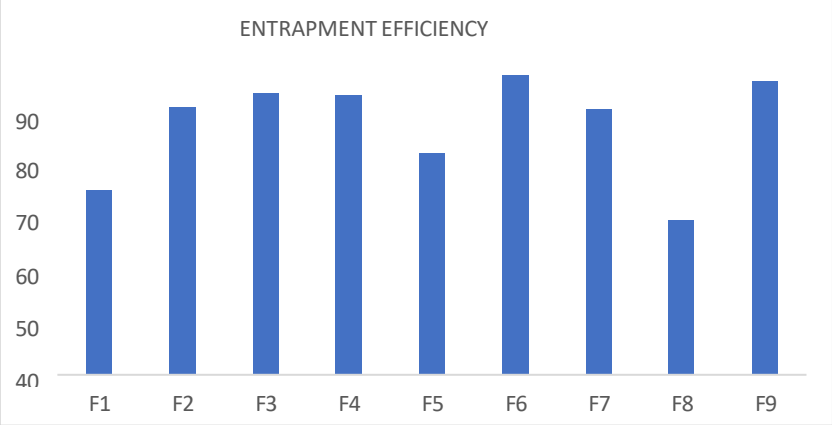


Figure 7: Entrapment efficiency of Hesperidin phytosomes

In vitro release study:

Table 10: *In vitro* drug release profiles of Phytosomes (F1-F9)

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	22.69	18.10	16.32	21.12	27.63	23.56	16.98	15.37	22.91
2	34.69	30.49	26.10	33.92	30.24	32.68	28.52	25.13	24.04
3	56.32	50.47	43.10	54.37	45.93	48.55	41.28	46.49	39.10
4	66.16	52.13	49.37	66.50	51.36	54.55	62.27	56.10	44.52
6	70.85	59.19	53.10	65.22	70.55	68.12	72.33	65.25	53.56
8	77.48	71.82	78.12	74.50	78.82	79.98	80.64	76.34	73.69
10	80.21	81.20	83.20	85.25	83.55	86.88	82.55	84.35	83.10
12	85.67	87.57	90.37	88.78	88.83	92.69	86.58	88.55	90.25

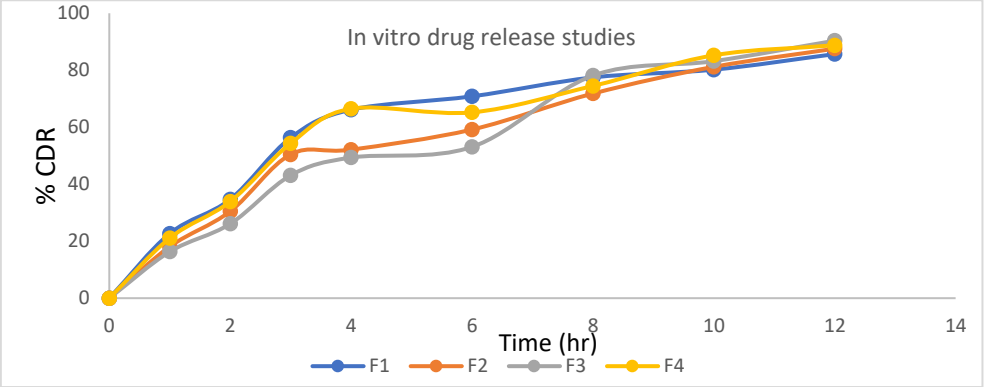


Figure 8: In vitro drug release studies of F1-F4 formulations

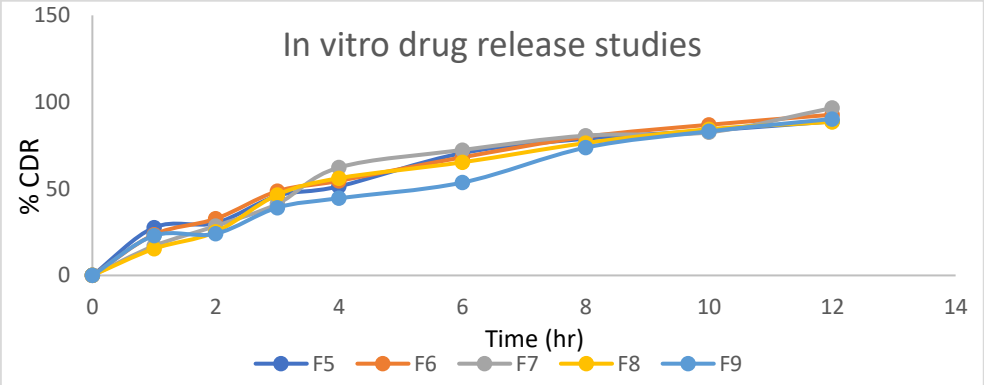
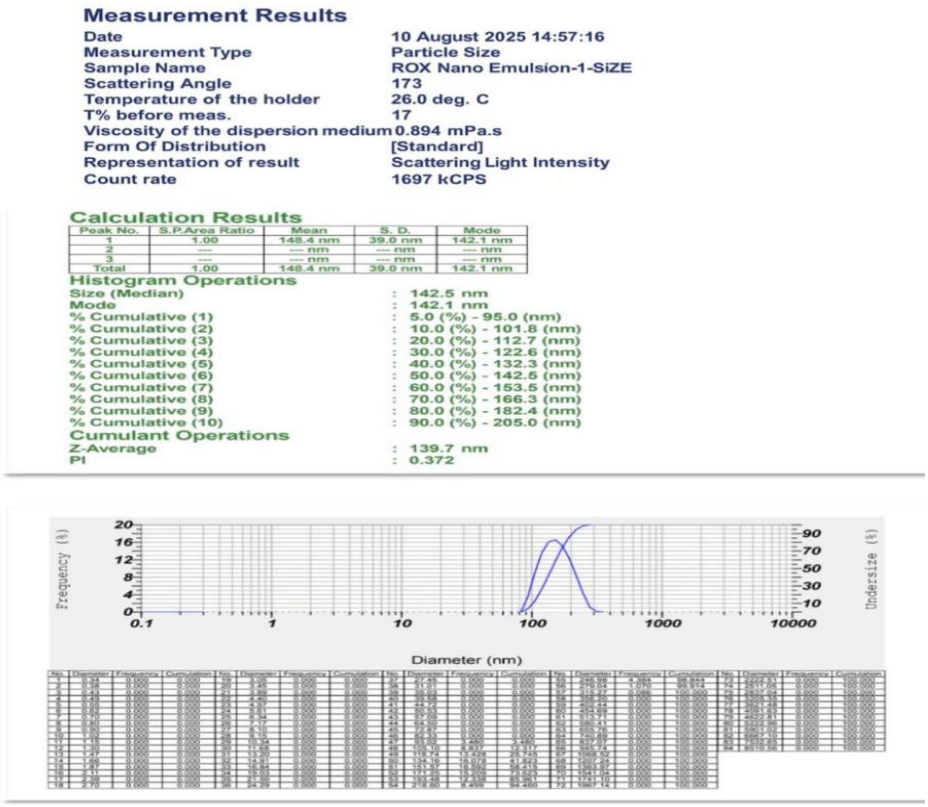


Figure 9: In vitro drug release studies of F5-F9 formulations

Observation: The highest percentage of drug release was observed in formulation Hesperidin F-6, reaching 92.69%.

CHARCTERIZATION OF OPTIMIZED HESPERIDIN PHYTOSOMES

A. PARTICLE SIZE OF HESPERIDIN OPTIMIZED PHYTOSOME(F6)



Measurement Results

Date	10 August 2025 14:57:16
Measurement Type	Particle Size
Sample Name	ROX Nano Emulsion-1-SIZE
Scattering Angle	173
Temperature of the holder	26.0 deg. C
T% before meas.	17
Viscosity of the dispersion medium	0.894 mPa.s
Form Of Distribution	[Standard]
Representation of result	Scattering Light Intensity
Count rate	1697 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	148.4 nm	39.0 nm	142.1 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	148.4 nm	39.0 nm	142.1 nm

Histogram Operations

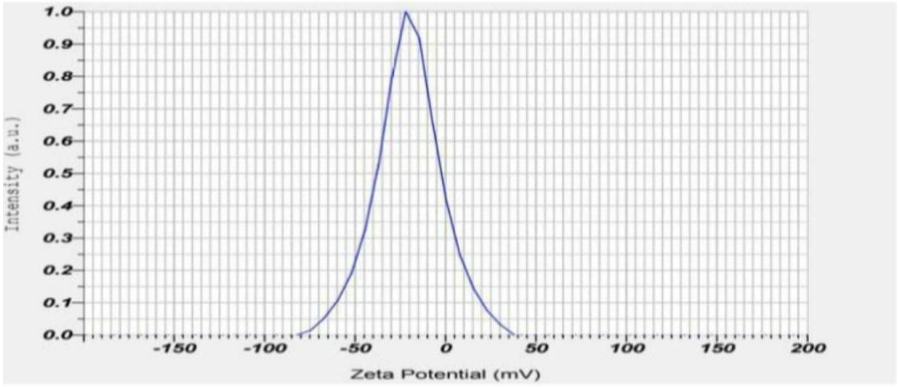
Size (Median)	: 142.5 nm
Mode	: 142.1 nm
% Cumulative (1)	: 5.0 (%) - 95.0 (nm)
% Cumulative (2)	: 10.0 (%) - 101.8 (nm)
% Cumulative (3)	: 20.0 (%) - 112.7 (nm)
% Cumulative (4)	: 30.0 (%) - 122.6 (nm)
% Cumulative (5)	: 40.0 (%) - 132.3 (nm)
% Cumulative (6)	: 50.0 (%) - 142.5 (nm)
% Cumulative (7)	: 60.0 (%) - 153.5 (nm)
% Cumulative (8)	: 70.0 (%) - 166.3 (nm)
% Cumulative (9)	: 80.0 (%) - 182.4 (nm)
% Cumulative (10)	: 90.0 (%) - 205.0 (nm)

Cumulant Operations

Z-Average	: 139.7 nm
PI	: 0.372

Figure 10: Particle size Analysis of Hesperidin Phytosomes [F6]

B. ZETA POTENTIAL OF HESPERIDIN OPTIMIZED PHYTOSOME(F6)



Measurement Results

Date	:	10 August 2025 12:06:08
Measurement Type	:	Zeta Potential
Sample Name	:	ROX Nano Emulsion-1-ZETA
Temperature of the holder	:	25.0 deg. C.
Viscosity of the dispersion medlum	:	0.834 mPa.s
Conductivity	:	0.376 mS/cm
Electrode Voltage	:	3.5 V

Calculation Results

Peak NO	Zeta Retential	Electroghoretic Mobility
1	-20·2 mV	0.000157 cm2/Vs
2	– ·mV	– cm2/Vs
3	– ·mV	– cm2/Vs

Zeta Potential (Mean)	:	-20.2 mV
Electrophoretic Mobility mean	:	-0.000157 cm²/Vs

Figure 11: Zeta potential of optimizrd Hesperidin Phytosomes[F6]

C. SEM OF HESPERIDIN OPTIMIZED PHYTOSOME (F6)

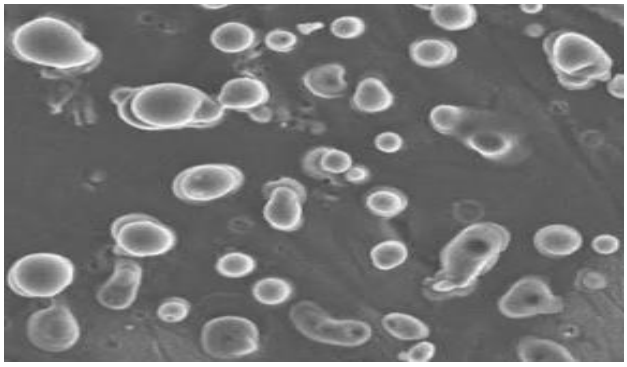


Figure 12: SEM of Optimized Phytosome (F6)

Microscopic analysis of the optimized Hesperidin phytosomes: In order to examine the shape and size of the Hesperidin phytosomes, scanning electron microscopy was used. Thefindings demonstrated the existence of tiny, spherical vesicles that were evenly dispersed.

EVALUATION OF HESPERIDIN PHYTOSOMAL GEL

A. PHYSICO-CHEMICAL PROPERTIES OF OPTIMIZED HESPERIDIN PHYTOSOMAL GEL

Table 11: Physicochemical properties of hesperidin phytosomal gel

Parameters	F-1	F-2	F-3	F-4	F-5	F-6
Appearance	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Homogenicity	Good	Good	Good	Good	Good	Good
pH	7.5	7.2	6.6	7.8	6.4	5.97
Viscosity(cps)	5200	4329	5890	4703	5532	4069
Spread ability(cm)	2.8	3.9	4.8	3.4	2.6	5.5

Appearance Evaluation: All of the hesperidin phytosomal gel formulations were consistent in colour and had a transparent, clear appearance.

pH Evaluation: The phytosomal gels that were created had pH values that fell between 5.97 and 7.8, which is quite close to the skin's physiological pH. This means that they can be applied topically without causing any irritation.

Viscosity Analysis: The formulations showed a low viscosity, which means the medicine can be released more quickly. Formulation F-6 had the most mild viscosity of all of them.

Spreadability:

Distinct variations in their spreading capacity were observed in the measured spreadability values of the phytosomal gel formulations, which ranged from 2.6 g·cm/s to 5.5 g·cm/s.

B. Drug Content:

Table 12: The percentage drug content of the hesperidin formulations

Parameters	F-1	F-2	F-3	F-4	F-5	F-6
Drug Content (%)	87.98	90.6	85.6	92.1	76.4	98.3

The drug concentration of the Hesperidin phytosomal gel varied between 76.4% and 98.3%, indicating that its formulation was consistently excellent.

C. In-vitro diffusion studies

After 8 hours of in-vitro diffusion testing, the samples were examined by ultraviolet (UV) spectroscopy set at 285 nm.

Table 13: *In vitro* diffusion profiles of Phytosomal gel (F1-F9)

Time (hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	5.8	6.2	4.5	7.9	12.1	6.7
2	12.7	13.6	16.2	14.9	26.9	13.6
3	18.2	25.6	23.1	22.37	45.3	27.8
4	21.9	33.9	28.7	48.5	51.36	33.7
5	43.1	49.8	33.1	56.2	62.5	48.6
6	55.3	73.82	64.2	63.7	69.8	73.84
7	66.7	88.4	71.2	79.25	70.5	87.2
8	79.24	92.7	89.3	88.78	74.3	95.6

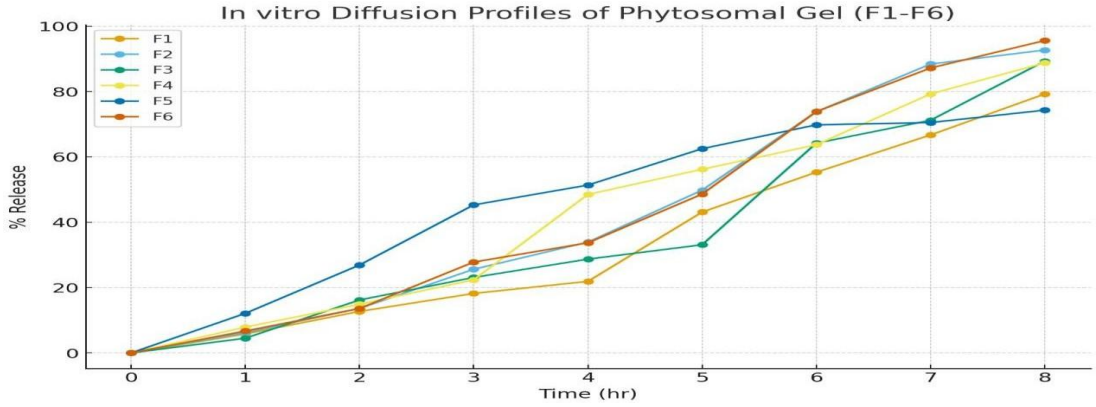


Figure 13: The In-Vitro Diffusion of the hesperidin formulation

In-vitro diffusion: we compared the release patterns of different formulations; the F6 version showed the best drug release, at 95.6%.

D. CHARACTERIZATION OF THE OPTIMIZED HESPERIDIN PHYTOSOMAL GEL

Table 14: Summary Findings of the Optimized Hesperidin Phytosomal Gel F6

EVALUATION	RESULTS
Appearance	Yellow
Homogenecity	Good
pH	5.97
Viscosity(cps)	4069
Spreadability(cm)	5.5
Drug content (%)	98.3
In-vitro drug release	95.6

Observation: Formulation F6 was determined to be the most effective formulation after undergoing evaluations for factors including in-vitro diffusion, homogeneity, viscosity, homogeneity, and pH. A skin-friendly pH of 5.97 and an appropriate viscosity of 4069 cps were its rheological attributes. Patients are more likely to comply with treatment plans when they are easy to apply, and the 5.5 cm spreadability made that possible. The constant consistency of the formulation was further confirmed by its excellent homogeneity. In addition, the formulation attained the maximum drug release profile of 95.6% and the drug content was found to be 98.3%.

E. KINETIC MODELLING OF DRUG RELEASE

Table 15: Release kinetics of optimzed formulate

Time (hrs)	%CDR	SQARE T	LOG T	LOG%CDR	ARA	LOG%ARA
0	0	0	0	0	100	0
1	19.56	1	0	1.29136885	80.44	0
2	29.68	1.41421	0.29103	1.472463897	70.32	0.15051
3	39.55	1.73205	0.45712	1.597146488	60.45	0.23856
4	49.55	2	0.62689	1.695043659	50.45	0.30103
6	66.12	2.44949	0.70815	1.820332845	33.88	0.38908
8	6.98	2.82843	0.91031	1.886377907	23.02	0.45154
10	86.88	3.16228	0.96025	1.938919812	13.12	0.5
12	98.69	3.4641	1.07449	1.994273149	1.31	0.53959

Zero order kinetics

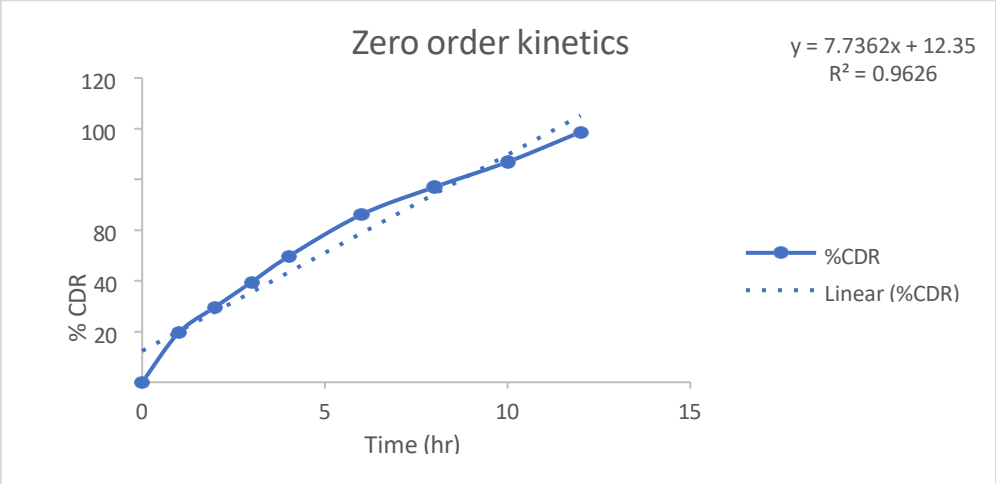


Figure 14: Zero Order Release Kinetics

First order kinetics

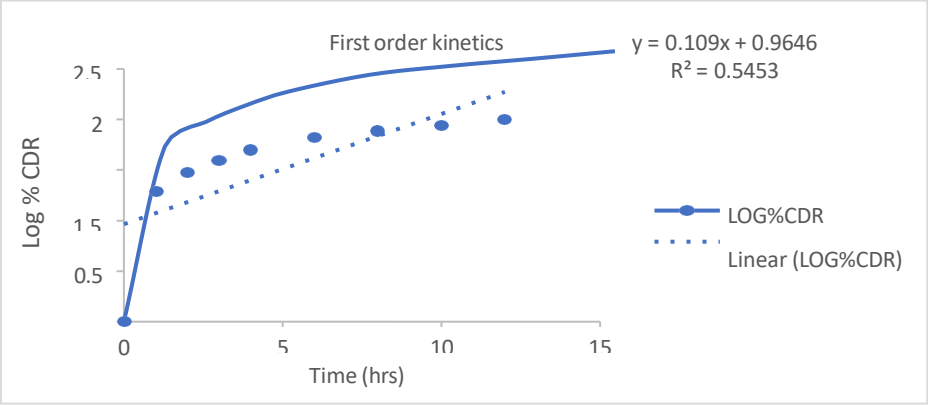


Figure 15: First Order Release Kinetics

Higuchi model

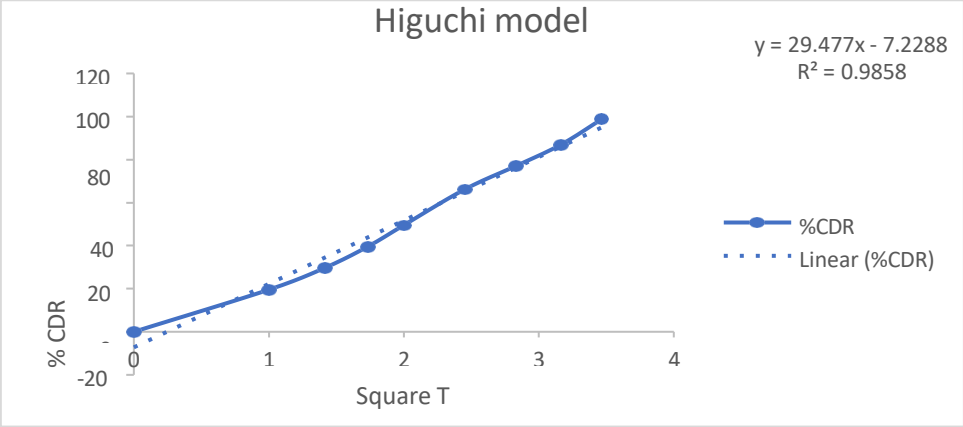


Figure 16: Higuchi Release Kinetics

Korsmeyer peppas

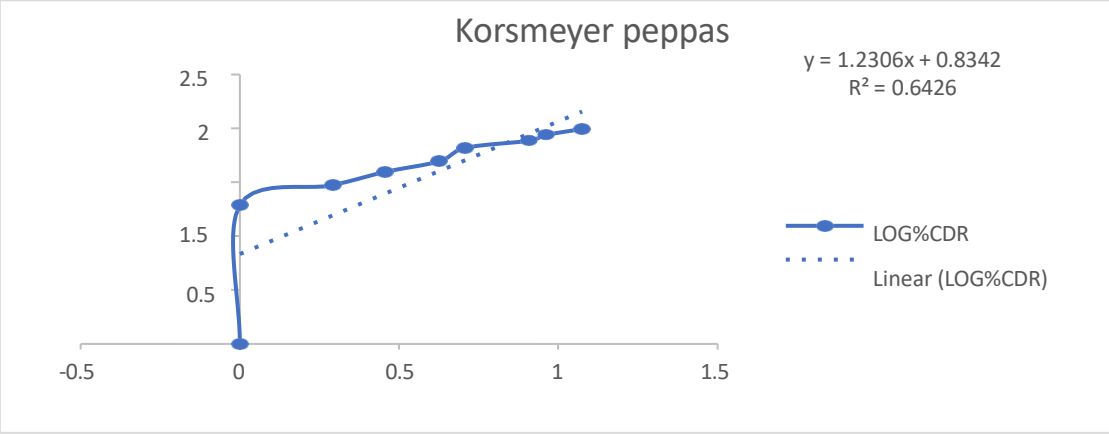


Figure 17: Korsmeyer Peppas Release Kinetics

Based on Fick's law of diffusion and first-order release kinetics, the regression analysis shows that formulation F6 releases the drug through a diffusion mechanism. —Higuchi layout.

STABILITY STUDIES

Table 16: Stability studies of optimized hesperidin formulation (F6)

Parameters	Initial	25.2^{0}C, 60.5 %RH			40.2 ^{0}C,75.5 %RH		
		30	60	90	30	60	90
		Days					
Viscosity (Cps)	4069	4069	4069	4058	4067	4055	4054
Spread ability (cm)	5.5	5.5	5.5	5.4	5.3	5.3	5.2
pH	5.98	5.98	5.98	5.85	5.84	5.84	5.7
Drug Content (%)	98.3	98.3	98.3	98.3	97.9	96.8	96.8

OBSERVATION: The stability study samples exhibited uniform physical characteristics and drug content throughout the testing period. No noticeable changes were detected during storage, confirming that the phytosomal gel maintained excellent stability.

SUMMARY OF FINDINGS

The study demonstrated the successful formulation of a hesperidin-loaded phytosomal gel using the thin-film hydration method. Among the developed formulations, F6 was identified as the optimized system, showing nanosized vesicles with a mean particle size of 168 nm and high entrapment efficiency (85.52%), indicating efficient phytosome formation. Incorporation of the optimized phytosomes into a Carbopol 934 gel produced a stable topical formulation with acceptable pH, viscosity, spreadability, and uniform drug content. In-vitro diffusion studies revealed sustained release behavior, with 95.6% drug release observed over 8 hours. FTIR analysis confirmed the absence of chemical interaction between hesperidin and formulation excipients, supporting formulation compatibility and stability. Release kinetic analysis indicated that the drug release followed the Higuchi model, suggesting diffusion-controlled release. Overall, the findings confirm that phytosomal gel formulation is an effective approach to enhance the solubility, stability, and topical delivery performance of hesperidin.

CONCLUSION:

The study successfully developed an optimized hesperidin phytosomal gel with improved topical delivery performance. Among all formulations, F6 demonstrated superior characteristics, including nanosized vesicles (~168 nm), a stable zeta potential, and high entrapment efficiency (85.52%), confirming efficient phytosome formation. Incorporation of the optimized phytosomes into a Carbopol gel produced a formulation with suitable pH (5.9–6.2), acceptable viscosity, uniform homogeneity, and high drug content (98.3%). In-vitro diffusion studies showed significantly enhanced and sustained release from the phytosomal gel, achieving 95.6% drug release within 8 hours, compared with slower release from other formulations. Release kinetics followed the Higuchi model, indicating diffusion-controlled drug transport. Stability studies confirmed no significant changes in physicochemical properties over the test period. Overall, the hesperidin phytosomal gel demonstrates strong potential as an effective topical delivery system for antioxidant and anti-inflammatory therapy.

Acknowledgement: The authors express their sincere gratitude to the Management and Principal of the Department of Pharmacy, Deccan School of Pharmacy, Darussalam, Hyderabad, for their continuous support and for providing the necessary research facilities and instrumentation to carry out this study.

Author Contributions: Romisha Naila*[1]: Conceptualization, methodology, investigation, writing – original draft.

Abdul Mannan[2]: Supervision, validation, formal analysis, writing –review.

Conflict of Interest: The authors declare that there are no conflicts of interest, financial or otherwise, that may have influenced the design, execution, or interpretation of the study.

Funding Source: This research received no specific financial support from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Statement: Ethical approval was not required for this study, as no human or animal subjects were involved. All experimental procedures were conducted using in-vitro models.

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