Optimization of Resveratrol Phytosomes by Response Surface Methodology: Formulation, Characterization and In Vitro Release Evaluation

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Abstract

Resveratrol, a bioactive polyphenolic compound is known to possess potent antioxidant, anti-inflammatory, cardioprotective and anticancer effects, but its clinical use is restricted due to its low aqueous solubility and oral bioavailability. The objective of the present study was to develop and optimize the resveratrol loaded phytosomes with soya lecithin by using rotary evaporation method. The effects of soya lecithin concentration and processing temperature were examined using a 3" factorial design, with respect to particle size and entrapment efficiency. The optimized formulation was subjected to Fourier-transform infrared (FTIR) Spectroscopy, Differential scanning calorimetry (DSC), Scanning electron microscopy (SEM), Particle size analysis, Determination of zeta potential, Drug loading and In vitro drug release study. The optimized formulation (F8) exhibited a particle size of 120.7 ± 0.4 nm, zeta potential of −25.2 ± 1.2 mV, entrapment efficiency of 98.43 ± 1.05%, and drug loading of 98.43 ± 0.46%. FTIR and DSC studies confirmed the formation of a drug–phospholipid complex and the SEM analyses showed the formation of nanosized particles evenly distributed. The optimized phytosomal formulation had 98.85% cumulative drug release while pure resveratrol had 85.76% cumulative drug release. From these results, it can be concluded that phytosomal complexation is an effective method to enhance the physicochemical properties and release behavior of resveratrol with the potential of becoming a good oral delivery system.

Keywords: Resveratrol; Phytosomes; Soya Lecithin; Response Surface Methodology; Entrapment Efficiency; Oral Drug Delivery.

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1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring polyphenolic stilbene, which is found primarily in grapes, berries, peanuts and Polygonum cuspidatum. Due to its wide range of pharmacological properties, resveratrol is a promising therapeutic agent for the treatment of several chronic diseases. It has been well studied for its powerful antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antidiabetic and anticancer activity [1,2]. The biological effects are mostly mediated by modulation of multiple cell signaling pathways, such as activation of sirtuin-1 (SIRT1) and the AMP-activated protein kinase (AMPK) pathways, involved in the regulation of energy metabolism, oxidative stress, and inflammatory response (Nowacka et al., 2024). Moreover, resveratrol has been found to have a potent effect in the treatment of obesity and metabolic disorders by stimulating lipid oxidation, enhancing insulin sensitivity and inhibiting adipogenesis (Chimento et al., 2019; Nie et al., 2020). Although resveratrol shows great therapeutic potential, its clinical use is hindered by some biopharmaceutical barriers.

One drawback of resveratrol is its poor bioavailability when taken orally. The aqueous solubility of resveratrol is low and it shows only limited gastrointestinal fluid solubility, which leads to limited absorption after oral administration (Gausuzzaman et al., 2022). Furthermore, the compound is extensively metabolised in the intestine and liver by glucuronidation and sulfation processes, resulting in a high degree of first-pass metabolism and low availability of the active parent compound (L. Wang et al., 2021). Moreover, resveratrol is chemically unstable and prone to photoisomerization and

degradation at physiological conditions, which limits its therapeutic value (Chimento et al., 2019).

Thus, a number of formulation approaches such as nanoparticles, liposomes, solid dispersions, self-microemulsifying drug delivery systems and nanosuspensions have been evaluated to enhance its solubility and bioavailability (Kuk et al., 2019; Pandita et al., 2014; Rawat et al., 2021; P. Wang & Sang, 2018). While these methods have been shown to be successful in different situations, there are difficulties associated with formulation stability, manufacturing complexity, and absorption enhancement, which have not yet been solved. One of the new delivery systems that has received a lot of attention is phytosome technology which aims to enhance the delivery of poorly soluble phytoconstituents. Phytosomes are molecular complexes of bioactive plant compounds and phospholipids, usually soya lecithin-based phosphatidylcholine, that are formed by a specific interaction between the bioactive plant components and the phospholipids (Jagwani et al., 2020; Luo et al., 2021). In phytosomal systems, the active constituent is effectively incorporated into the phospholipid as a result of hydrogen bonding and molecular interactions that increases its amphiphilic properties. The structural modification enhances the permeability of the membrane, gastrointestinal absorption and systemic bioavailability of the compound incorporated (Mehta et al., 2024).

Phytosomes exhibit better stability, better drug incorporation and better penetration of biological membranes compared to the conventional liposomes (Kumar et al., 2017). The use of phytosomal technology to improve the oral delivery and therapeutic effect of a variety of phytoconstituents has proved to be successful in previous studies, and thus could prove to be a valuable delivery system for resveratrol. Many formulation and processing parameters are critical, and the performance of phytosomal formulations is strongly affected by the concentration of phospholipids, the composition of solvents and the processing temperature. The following variables have direct effects on the important quality attributes such as particle size, entrapment efficiency, stability, and drug release behavior (Kapse & Mulla, 2024). So, it is crucial to optimize the formulation systematically to build a strong and repeatable formulation. Quality by Design (QbD) approaches that use Design of Experiments (DoE) have gained ground in recent years and have been successfully used to optimize pharmaceutical formulations. Of these methods, factorial experimental designs are a more efficient way of assessing individual and interactive effect of formulation variables and reducing the number of experimental runs needed (Shriram et al., 2022). Using a 3^2 factorial design allows for the study of two independent variables at three different levels and for optimisation of the formulation conditions.

Hence, the present study was aimed at development and optimization of resveratrol loaded phytosomes using soya lecithin by rotary evaporation process. The influence of soya lecithin concentration and processing temperature was studied by using 3^2 factorial design for the particle size and EE. The optimized formulation was then investigated by some additional characterization techniques such as Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), particle size analysis and determination of zeta potential and in vitro drug release. The developed phytosomal delivery system is supposed to overcome the biopharmaceutical problem of resveratrol and would help to find an effective approach for improving the oral delivery and therapeutic activity of resveratrol.

2. Materials and Methods

2.1 Materials

Resveratrol was used as the model bioactive compound. Soya lecithin was employed as the phospholipid for phytosome preparation, while dichloromethane (DCM) was used as the organic solvent. Methanol, ethanol, dimethyl sulfoxide (DMSO), potassium dihydrogen phosphate, sodium hydroxide, and other analytical-grade reagents were used throughout the study without further purification.

2.2 Preformulation Studies

Preformulation studies were performed to assess the physicochemical properties of resveratrol before developing the formulation. Various organoleptic properties such as physical appearance, colour, smell and physical state were studied. Standard pharmacopeial methods were used for solubility studies in methanol, ethanol, dichloromethane, DMSO and phosphate buffer (pH 7.4). The melting point of resveratrol was determined by capillary tube method. The drug-excipient compatibility studies were carried out by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC).

2.3 Determination of λmax and Calibration Curve

The wavelength of maximum absorbance (λmax) of Resveratrol was determined using a UV–Visible spectrophotometer (Jasco V-630, Japan). Standard solutions of Resveratrol were scanned over the wavelength range of 200–400 nm to determine the wavelength of maximum absorbance.

Calibration curve in phosphate buffer (pH 7.4): Accurately weighed 10 mg of Resveratrol was transferred into a 100 mL volumetric flask and dissolved in phosphate buffer (pH 7.4) to obtain a primary stock solution of 100 µg/mL. From this stock solution, suitable aliquots were withdrawn and diluted

with phosphate buffer (pH 7.4) to obtain concentrations of 1, 2, 4, 6, 8 and 10 µg/mL. The absorbance of each solution was measured at 306 nm against phosphate buffer as blank. A calibration curve was constructed by plotting concentration versus absorbance, and the regression equation and coefficient of determination (R^2) were calculated.

Calibration curve in methanol: Similarly, 10 mg of Resveratrol was dissolved in 100 mL methanol to prepare a stock solution of 100 µg/mL. Serial dilutions of 1–10 µg/mL were prepared using methanol and their absorbance was measured at 305 nm. A calibration curve was obtained by plotting concentration against absorbance and the regression equation along with the coefficient of determination (R^2) was calculated.

2.4 Preparation of Resveratrol-Loaded Phytosomes

Resveratrol-loaded phytosomes were prepared by the rotary evaporation technique. Accurately weighed 100 mg of Resveratrol and the required quantity of soya lecithin (100, 200 or 300 mg according to the experimental design) were transferred into a clean round-bottom flask. The components were dissolved in 25 mL of dichloromethane (DCM) with gentle stirring until a clear homogeneous solution was obtained. The flask was attached to a rotary evaporator and the solvent was removed under reduced pressure at the predetermined temperature (40°C, 50°C or 60°C) with a rotation speed of 90 rpm, resulting in the formation of a thin drug–phospholipid film on the inner wall of the flask. The obtained film was washed with n-hexane to remove excess phospholipid and any uncomplexed material, followed by filtration. The collected phytosomal complex was dried under vacuum, stored in a desiccator, and used for further characterization studies.

2.5 Experimental Design and Optimization

The formulation variables were optimized by Design-Expert® software which was a 3^2 factorial design. The soya lecithin concentration (A) and processing temperature (B) were chosen as independent variables, with each having three levels. The dependent responses chosen were the particle size (Y_1) and entrapment efficiency (Y_2) at three different concentrations of soya lecithin (100, 200 and 300 mg) and three different temperatures (40, 50 and 60°C). The factorial design matrix was used to create nine experimental formulations. Response surface methodology (RSM) was used for the statistical analysis of the individual and interactive effects of the formulation variables to determine the optimum conditions of the formulation.

2.6 Particle Size and Zeta Potential Analysis

The average particle size and the zeta potential of the prepared phytosomal formulations were measured by dynamic light scattering (DLS) analyzer (HORIBA SZ-100). Samples were sonicated for an appropriate amount of time before analysis and then appropriately dispersed in distilled water. All measurements were carried out at room temperature (25 °C) and the mean results were taken.

2.7 Determination of Entrapment Efficiency and Drug Loading

Accurately weighed 100 mg of Resveratrol-loaded phytosomes was dispersed in 10 mL methanol and sonicated to release the entrapped drug completely. The dispersion was centrifuged at 20,000 rpm for 60 min at 4°C. The amount of free drug present in the supernatant was determined spectrophotometrically at 306 nm after suitable dilution.

The percentage entrapment efficiency was calculated using the following equation:

EE(%) = (Encapsulated Drug % of Total Drug) x 100

Drug loading was determined using a similar analytical procedure and expressed as percentage drug content within the phytosomal system. Drug loading was calculated using the following equation:

DL (%) = (Amount of Entrapped Drug / Total Weight of Phytosomal Formulation) × 100

2.8 Fourier Transform Infrared Spectroscopy (FTIR)

To study the drug–excipient interaction and to verify the phytosome formation, FTIR analysis was carried out on JASCO FT/IR-4600 Plus spectrophotometer. Pure resveratrol, soya lecithin, physical mixture and Resveratrol-loaded phytosomes were prepared and mixed with potassium bromide to form samples. The spectra were measured in the range of 4000–400 cm^{-1}.

2.9 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed using a DSC 200 (Hitachi, Japan) to evaluate the thermal behavior of pure Resveratrol and Resveratrol-loaded phytosomes. Approximately 5–10 mg of each sample was placed in a sealed aluminum pan and heated from 25°C to 300°C at a heating rate of 10°C/min under a nitrogen purge maintained at 40 mL/min. The thermograms obtained were used to evaluate drug crystallinity, thermal behavior, and possible interactions between Resveratrol and phospholipids.

2.10 Scanning Electron Microscopy (SEM)

The Resveratrol-loaded phytosomes was analyzed by scanning electron microscopy (Nova NanoSEM) in terms of surface morphology. Dried samples were placed on aluminum stubs with double-sided adhesive tape and then coated with a thin layer of gold under vacuum and then imaged at appropriate magnifications.

2.11 In Vitro Drug Release Study

The in vitro drug release study of Resveratrol-loaded phytosomes was performed using the dialysis membrane diffusion method. A quantity of formulation equivalent to 100 mg of Resveratrol was placed inside a dialysis membrane and immersed in 900 mL phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C with continuous stirring at 50 rpm. At predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18 and 24 h), 5 mL of dissolution medium was withdrawn and immediately replaced with an equal volume of fresh phosphate buffer to maintain sink conditions. The withdrawn samples were analyzed spectrophotometrically at 306 nm, and the cumulative percentage drug release was calculated.

2.12 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical evaluation and optimization were performed using Design-Expert® software. Analysis of variance (ANOVA) was used to identify the significance of model terms, and response surface plots to show the influence of the formulation variables on the selected responses. A difference was defined as statistically significant if the p value was < 0.05.

3. Results

3.1 Preformulation Studies

3.1.1 Organoleptic Properties of Resveratrol

The organoleptic properties of resveratrol were evaluated prior to formulation development. The drug was observed as a white crystalline powder with no characteristic odor. The results are presented in Table 1.

Table 1

Organoleptic properties of resveratrol

Sr. No.	Property	Observation
1	Physical state	Solid
2	Appearance	Crystalline powder
3	Odor	Odorless
4	Color	White solid

3.1.2 Melting Point Determination

The melting point of resveratrol was measured by capillary tube method. The melting point observed was 262°C and within the reported range of 261–263°C. The results are reported in the Table 2.

Table 2: Melting point determination of resveratrol

Identification Test	Reported Standard	Observed Result
Melting point	261–263°C	262°C

3.1.3 Solubility Study

Resveratrol solubility profile was tested in various solvents. The drug proved to be highly soluble in methanol, dichloromethane and ethanol, soluble in DMSO and freely soluble in phosphate buffer (pH 7,4). The summary of the results is shown in Table 3.

Table 3: Solubility profile of resveratrol

Sr. No.	Solvent	Observation
1	Methanol	Very soluble
2	Dichloromethane	Very soluble
3	Phosphate buffer pH 7.4	Soluble
4	Ethanol	Very soluble
5	DMSO	Freely soluble

3.1.4 Determination of λmax and Calibration Curve

UV spectrophotometric analysis of Resveratrol was carried out in phosphate buffer (pH 7.4) and methanol. The maximum absorbance (λmax) of Resveratrol was observed at 306 nm in phosphate buffer and 305 nm in methanol. Calibration curves prepared over the concentration range of 1–10 µg/mL exhibited excellent linearity in both media. The regression equations showed a strong linear relationship between concentration and absorbance, with coefficients of determination (R^2) approaching unity, confirming the suitability of the analytical method for quantitative estimation of Resveratrol.

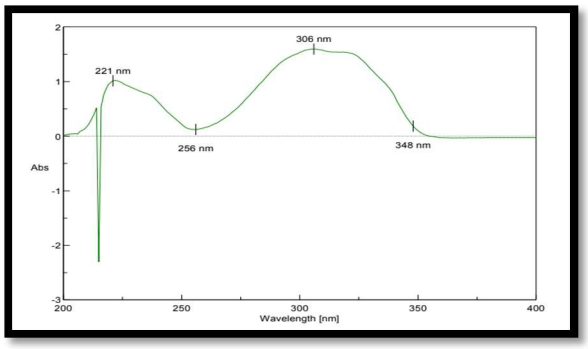


Figure 1: UV absorption spectrum of resveratrol in phosphate buffer pH 7.4 showing λmax at 306 nm.

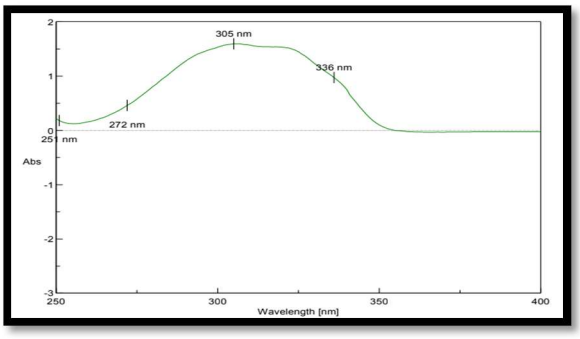
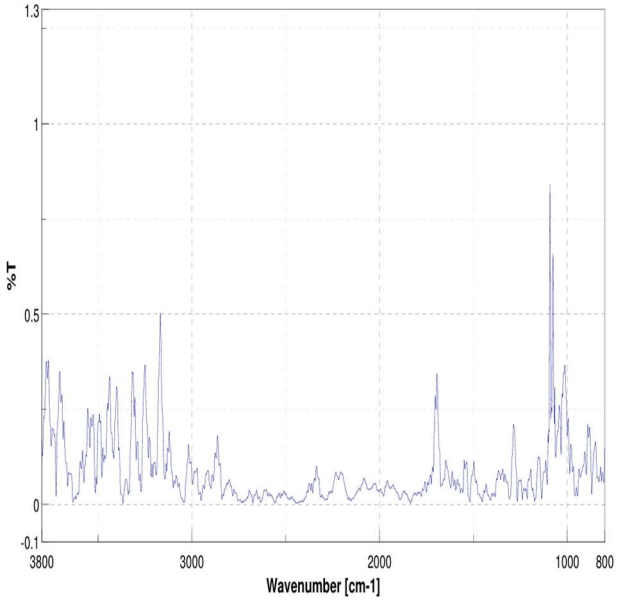


Figure 2: Calibration curve of resveratrol in methanol.



3.1.5 Fourier Transform Infrared Spectroscopy (FTIR)

Optimized formulation of the phytosomal formulation was characterized by FTIR spectroscopy along with pure resveratrol and soya lecithin. The spectra we get are shown in Fig. 3-6. The FTIR spectrum of pure resveratrol showed characteristic absorption bands at 3200–3500 cm^{-1} corresponding to O–H stretching, another band was observed in the region of 3000 cm^{-1} which corresponds to C–H stretching of the aromatic ring, one of the absorption bands around 1600 cm^{-1} is due to the aromatic C=C stretching and another band in the region of 1000–1300 cm^{-1} is due to the stretching of C–O bonds. FTIR spectrum of soya lecithin revealed the presence of characteristic absorption bands for C–H stretching vibration of the aliphatic groups in the vicinity of 2850 cm^{-1} – 2920 cm^{-1}, carbonyl stretching vibration (C=O) in the range of 1730 cm^{-1}, and phosphate group vibrations in the range of 1000 cm^{-1} – 1250 cm^{-1}. FTIR spectrum of a physical mixture showed the presence of the characteristic peaks of both resveratrol and soya lecithin. The FTIR spectrum of the optimized resveratrol loaded phytosomes revealed the presence of peaks corresponding to resveratrol as well as the soya lecithin with minor differences in the intensity and position of the peaks. The spectral pattern was observed and it confirmed the presence of the drug in the phytosomal formulation.

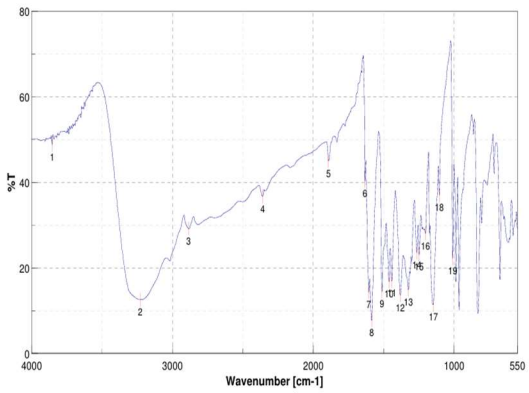


Figure 3: FTIR spectrum of pure resveratrol.

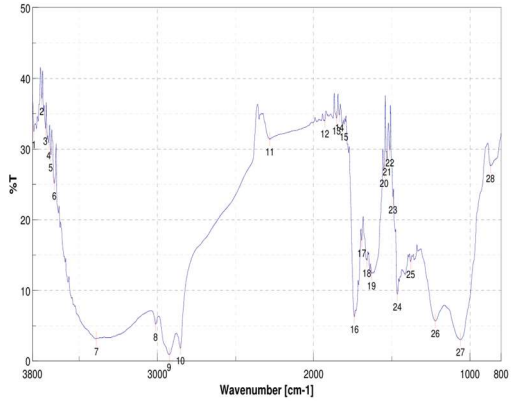


Figure 4: FTIR spectrum of soya lecithin.

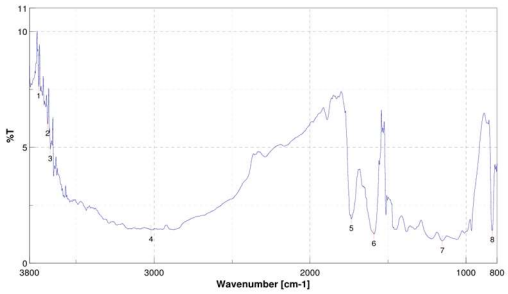


Figure 5: FTIR spectrum of physical mixture of resveratrol and soya lecithin.

Figure 6: FTIR spectrum of optimized resveratrol-loaded phytosomes.

The overlay spectrum showed that the characteristic functional groups of resveratrol were retained in the phytosomal formulation with only minor shifts in peak position and intensity. These slight variations indicate molecular interactions between resveratrol and phospholipids during complex formation without any evidence of chemical incompatibility. The retention of characteristic peaks confirms successful incorporation of resveratrol into the phytosomal system

3.2 Particle Size and Zeta Potential Analysis

Particle size and zeta potential of the prepared resveratrol-loaded phytosomal formulations (F1–F9) were determined using Dynamic Light Scattering (DLS). The obtained results are presented in Table 4.

Table 4: Particle size and zeta potential of resveratrol-loaded phytosomes

Sr. No.	Batch	Particle Size (nm)	Zeta Potential (mV)
1	F1	156.4 ± 1.0	-25.7 ± 0.8
2	F2	185.3 ± 1.1	-20.0 ± 0.9
3	F3	184.6 ± 0.9	-21.5 ± 0.9
4	F4	210.2 ± 1.2	-20.7 ± 1.0
5	F5	160.5 ± 0.8	-24.9 ± 0.8
6	F6	139.9 ± 0.6	-23.2 ± 1.1
7	F7	210.1 ± 1.3	-22.1 ± 1.0
8	F8	120.7 ± 0.4	-25.2 ± 1.2
9	F9	140.8 ± 0.7	-26.5 ± 1.3

Particles sizes of the prepared formulations were between 120.7 ± 0.4 nm and 210.2 ± 1.2 nm. The particle size for formulation F8 was the smallest (120.7 ± 0.4 nm) while formulation F4 was the largest (210.2 ± 1.2 nm). The zeta potential values of the formulations varied between −20.0 ± 0.9 mV to −26.5 ± 1.3 mV. The zeta potential was the highest for formulation F9 (−26.5 ± 1.3 mV) and the lowest for formulation F2 (−20.0 ± 0.9 mV). The formulation F8 was chosen for further characterization studies according to the particle size and zeta potential obtained.

3.3 Entrapment Efficiency and Drug Loading

The entrapment efficiency and drug loading of the prepared resveratrol-loaded phytosomal formulations were determined and the results are presented in Table 5.

Table 5: Entrapment efficiency and drug loading of resveratrol-loaded phytosomes

Sr. No.	Batch	Entrapment Efficiency (%)	Drug Loading (%)
1	F1	98.43 ± 0.35	98.43 ± 0.12
2	F2	89.8 ± 1.1	89.8 ± 0.51
3	F3	95.1 ± 0.8	95.1 ± 0.40
4	F4	96.2 ± 1.0	96.2 ± 0.51
5	F5	92.1 ± 0.7	92.1 ± 0.40
6	F6	94.2 ± 1.0	94.2 ± 0.46
7	F7	90.5 ± 1.0	90.5 ± 0.51
8	F8	98.43 ± 1.05	98.43 ± 0.46
9	F9	93.6 ± 0.8	93.6 ± 0.40

The entrapment efficiency of the formulations prepared was between 89.8 ± 1.1% and 98.43 ± 1.05%. The formulation with maximum entrapment efficiency was found to be formulations F1 (98.43%) and F8 (98.43%) and minimum entrapment efficiency of formulation F2 (89.8%). Drug loading values ranged from 89.8 ± 0.51% to 98.43 ± 0.46%. The drug loading of the formulations was the highest for the formulation F1 and the lowest for the formulation F2. The optimized formulation was F8 with particle size of 120.7 ± 0.4 nm, entrapment efficiency of 98.43 ± 1.05% and drug loading of 98.43 ± 0.46% which was chosen for further characterization studies.

3.4 Morphological Analysis

The surface morphology of the optimized resveratrol loaded phytosomal formulation was analysed by Scanning Electron Microscopy (SEM). Representative SEM images of different magnifications are shown in Figures 9–12. Well-defined morphology of discrete phytosomal particles was observed in the SEM images. The particles seemed to be distributed evenly over the field of view. No foreign particles or crystalline drug deposits were seen in the samples analysed. The optimized formulation had nanosized particles that matched with the particle size analysis results.

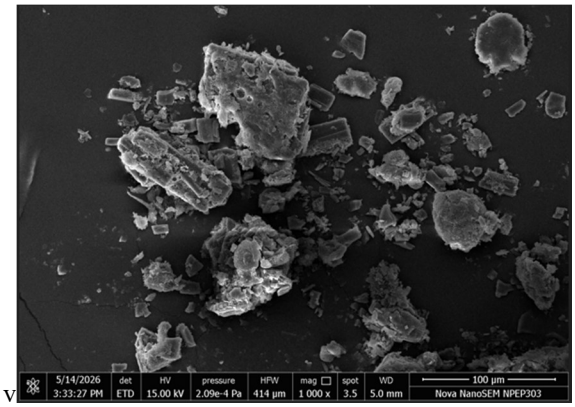


Figure 9: Scanning electron micrograph of optimized resveratrol-loaded phytosomes at low magnification.

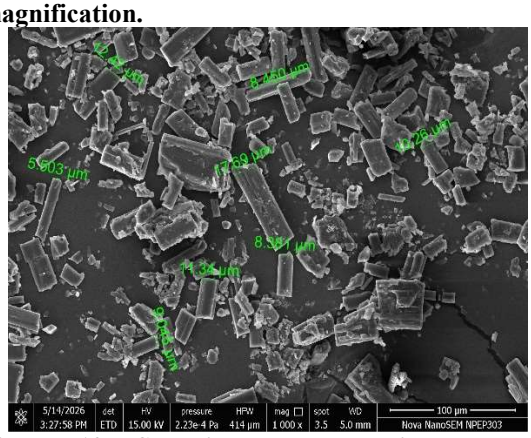


Figure 10: Scanning electron micrograph of optimized resveratrol-loaded phytosomes showing particle distribution.

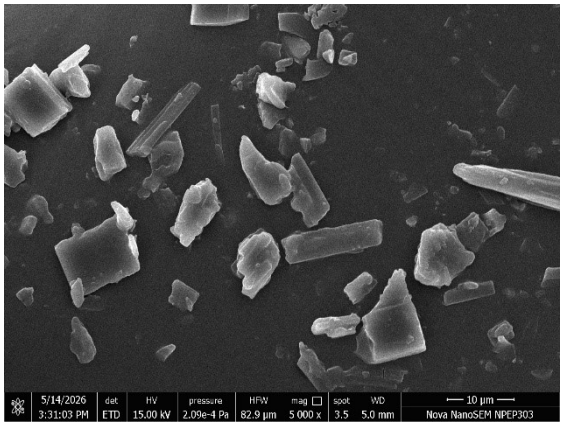
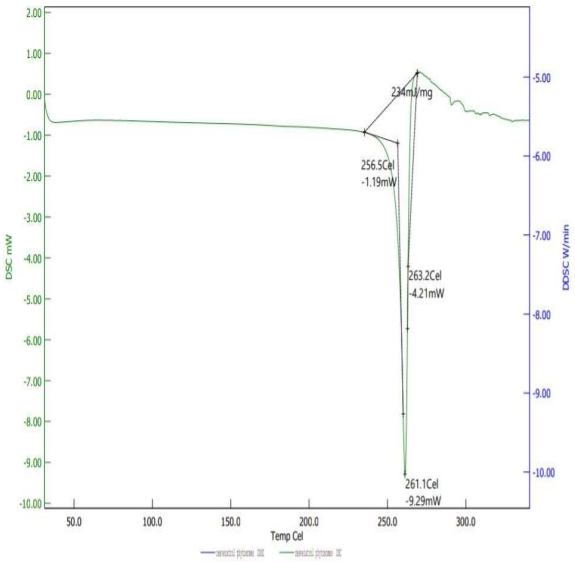


Figure 11: Scanning electron micrograph of optimized resveratrol-loaded phytosomes at higher magnification.



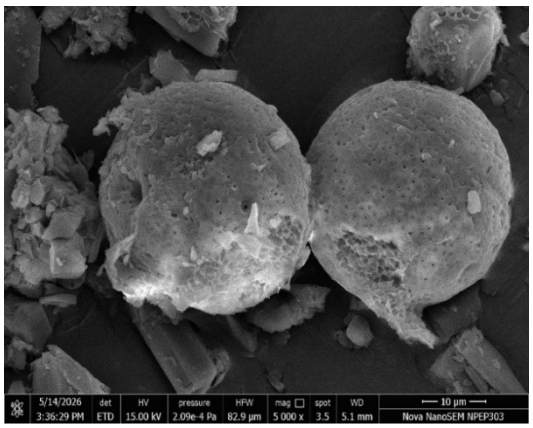


Figure 12: Scanning electron micrograph of optimized resveratrol-loaded phytosomes illustrating surface morphology of phytosomal particles.

3.5 Differential Scanning Calorimetry (DSC)

Optimized resveratrol-loaded phytosomal formulation was analyzed by DSC in comparison to pure resveratrol. Figures 13 and 14 show the thermograms obtained. The DSC thermogram of pure resveratrol showed a characteristic endothermic peak, typical of the melting property of resveratrol. A thermogram of optimized phytosomal formulation demonstrated that the thermogram of the optimized formulation differed from that of pure resveratrol. Pure resveratrol and optimized phytosomal formulation was subjected to thermal behaviour over the selected temperature range and the resulting thermograms were utilized for further characterization of the formulation.

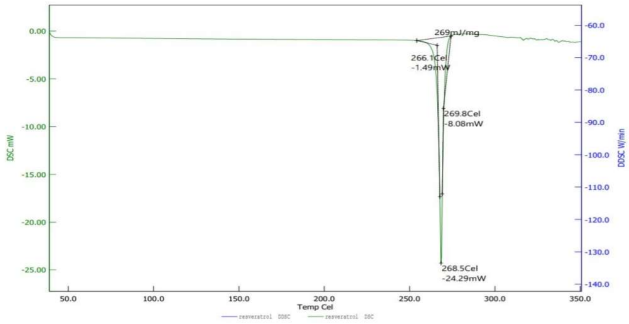


Figure 13: DSC thermogram of pure resveratrol.

Figure 14: DSC thermogram of optimized resveratrol-loaded phytosomes.

The DSC thermogram of pure resveratrol exhibited a characteristic sharp endothermic melting peak corresponding to its crystalline nature. In contrast, the thermogram of the optimized phytosomal formulation showed a reduction in peak intensity along with a slight shift in the melting endotherm, indicating partial loss of crystallinity following phytosome formation. These findings suggest molecular interaction between resveratrol and phospholipid molecules and confirm successful formation of the drug–phospholipid complex while maintaining thermal stability of the formulation.

3.7 Optimization of Resveratrol-Loaded Phytosomes Using 3^2 Factorial Design

A 3^2 factorial design was employed to evaluate the effect of soya lecithin concentration (Factor A) and processing temperature (Factor B) on particle size and entrapment efficiency of resveratrol-loaded phytosomes. The experimental design matrix and observed responses are presented in Table 7.

Table 7

Experimental design matrix and observed responses

Run	Soya Lecithin (mg)	Temperature (°C)	Particle Size (nm)	Entrapment Efficiency (%)
F1	300	50	156.4	98.4
F2	100	60	185.3	89.8
F3	300	40	184.6	95.1
F4	300	60	210.2	96.2
F5	100	50	160.5	92.1
F6	200	40	139.9	94.2
F7	100	40	210.1	90.5
F8	200	50	120.7	98.4
F9	200	60	140.8	93.6

Among the prepared formulations, F8 exhibited the lowest particle size (120.7 nm) and the highest entrapment efficiency (98.4%).

3.7.1 Optimization of Particle Size

The fit summary identified the quadratic model as the most suitable model for particle size.

Table 9
ANOVA for particle size response

Source	F-value	p-value
Model	27.43	0.0104
A	0.0638	0.8169
B	0.0084	0.9329
AB	11.01	0.0451
A^2	89.19	0.0025
B^2	36.89	0.0090

Table 10
Fit statistics for particle size response

Parameter	Value
Standard Deviation	7.59
Mean	167.61
C.V. (%)	4.53
R^2	0.9786
Adjusted R^2	0.9429
Predicted R^2	0.8379
Adeq Precision	15.5515

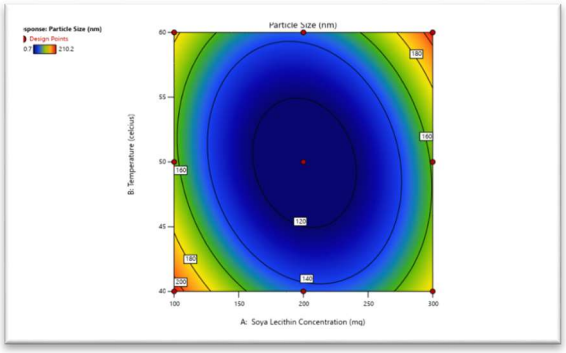


Figure 16: Contour plot showing the effect of soya lecithin concentration and temperature on particle size.

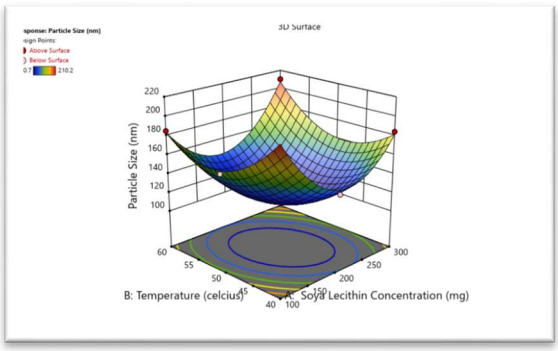


Figure 17: Three-dimensional response surface plot showing the influence of formulation variables on particle size.

3.7.2 Optimization of Entrapment Efficiency

The fit summary indicated that the quadratic model was the most suitable model for the entrapment efficiency response.

Table 12
ANOVA for entrapment efficiency response

Source	F-value	p-value
Model	9.58	0.0461
A	42.47	0.0073
B	0.1149	0.7569
AB	0.6897	0.4672
A^2	0.5151	0.5248
B^2	4.09	0.1363

Table 13
Fit statistics for entrapment efficiency response

Parameter	Value
Standard Deviation	0.9094
Mean	94.26
C.V. (%)	0.9648
R^2	0.9681
Adjusted R^2	0.9150

Predicted R^2	0.7370
Adeq Precision	12.5471

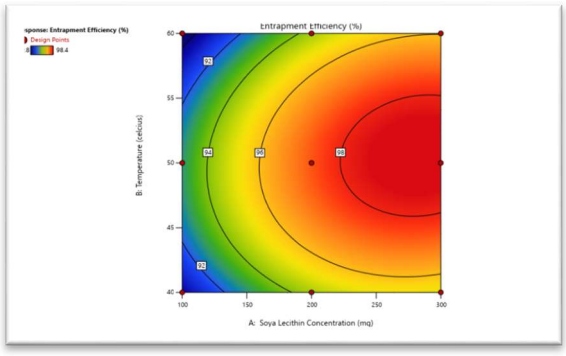
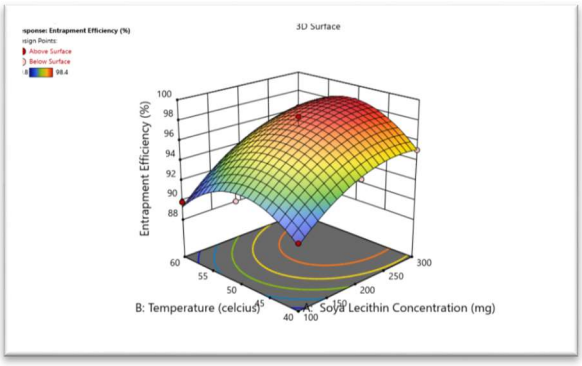


Figure 18: Contour plot showing the effect of soya lecithin concentration and temperature on entrapment efficiency.

Figure 19: Three-dimensional response surface



plot showing the influence of formulation variables on entrapment efficiency.

3.7.3 Overlay Plot and Selection of Optimized Formulation

The overlay plot generated from the optimization study identified the design space satisfying the desired criteria for both particle size and entrapment efficiency. The optimized region corresponded to intermediate levels of soya lecithin concentration and temperature.

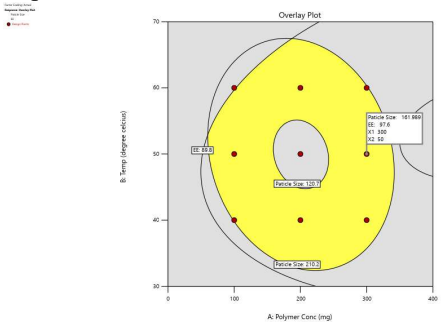


Figure 20: Overlay plot showing the optimized design space for particle size and entrapment efficiency.

Based on the optimization results, formulation F8 containing 200 mg of soya lecithin and processed at 50°C was selected as the optimized formulation. The optimized batch exhibited a particle size of 120.7 nm and an entrapment efficiency of 98.4%.

3.8 In Vitro Drug Release Study

The in vitro drug release behavior of pure resveratrol and the optimized resveratrol-loaded phytosomal formulation (F8) was evaluated using the dialysis membrane diffusion method in phosphate buffer (pH 7.4). Samples were collected at predetermined time intervals and analyzed spectrophotometrically at 306 nm. The cumulative percentage drug release of both formulations was compared to evaluate the effect of phytosomal complexation on the release profile. The optimized phytosomal formulation exhibited a sustained release pattern throughout the study and achieved 98.85% cumulative drug release after 24 h, whereas pure resveratrol showed 85.76% drug release over the same period. The controlled release behavior may be attributed to the incorporation of resveratrol within the phospholipid matrix, which prolonged drug diffusion and improved dissolution characteristics.

Table 6. In vitro drug release profile of pure resveratrol and optimized resveratrol-loaded phytosomes

Time (h)	Pure Resveratrol (%)	Optimized Phytosomes (%)
0.5	3.40	1.50
1	11.70	2.90
2	27.30	9.50
3	51.50	21.60
4	70.90	28.89
5	75.79	42.35
6	77.54	49.07
8	81.90	61.68
10	83.67	71.51
12	84.78	79.93
18	85.12	87.56
24	85.76	98.85

4. Discussion

The present study aims to formulate and optimize a resveratrol loaded phytosomal system that would overcome the biopharmaceutical drawbacks inherent in resveratrol. Despite its remarkable antioxidant, anti-inflammatory, cardioprotective, neuroprotective,

antidiabetic and anticancer properties, the therapeutic use of resveratrol is limited by its low aqueous solubility, high metabolism and low bioavailability, especially when given orally (Kaushal et al., 2022; Shekh & Gupta, 2022). Thus, the rational approach to enhance the release behavior and to modify the physicochemical properties of the phytosomal carrier was considered the development of a phospholipid based phytosomal carrier.

The preformulation studies are proving the suitability of resveratrol for the formulation of phytosomes. The observed melting point (262°C) was found in a good agreement with the reported melting point range (261–263°C) which proved the identity and purity of the drug. The solubility study showed that resveratrol was more soluble in organic solvents (methanol, ethanol, dichloromethane, and DMSO) than aqueous phosphate buffer. This discovery is in line with previous reports that resveratrol is a hydrophobic polyphenolic compound with poor aqueous solubility and dissolution properties (Kaushal et al., 2022; Rawat et al., 2021; Shekh & Gupta, 2022). Its low aqueous solubility is believed to be one of the key reasons for its low oral bioavailability and inconsistent therapeutic effect. Therefore, it was anticipated that the incorporation of resveratrol into a phospholipid-based delivery system would increase the interaction of resveratrol with biological membranes, and increase its release in aqueous environments.

The effects of formulation variables on critical quality attributes were investigated using a systematic optimization procedure in the form of 3^2 factorial design. The use of response surface methodology (RSM) has been widely used in recent years in the pharmaceutical industry to evaluate several factors simultaneously, while reducing the number of experimental runs (Rawat et al., 2021). The particle size model showed good predictive ability, with a coefficient of determination ($R^2 = 0.9786$), adjusted R^2 (0.9429) and predicted R^2 (0.8379) values. Likewise, the entrapment efficiency model had good statistical results with R^2 value of 0.9681. Based on these findings, it can be concluded that the mathematical models selected were able to describe the relationship between the formulation variables and the response parameters.

The interaction term (AB) and the quadratic effects of lecithin concentration and temperature (A^2 and B^2) were significant factors affecting particle size by analysis of variance. The individual effect of the concentration of lecithin and temperature was not statistically significant, however. This behavior indicates that the formation of the particles followed a combined and non-linear effect of the formulation variables than just their independent effects. The concentration of phospholipid and the processing temperature during rotary evaporation both affect the process of removing solvent, phospholipid organization and vesicle formation. Too much phospholipid content may lead to higher viscosity and aggregation of particles, while too little content can lead to an incomplete complex. Likewise, the processing temperature can influence the fluidity of phospholipids and molecular mobility, which in turn can influence the assembly of phytosome (Aundhia et al., 2024; Rawat et al., 2021). The optimized formulation F8 obtained by the use of 200 mg of soya lecithin at 50 °C had the smallest particle size (120.7 nm) showing that there is an optimum processing window for the formation of phytosomes.

The concentration of lecithin had a strong effect on entrapment efficiency while the temperature had no statistically significant effect. This observation points to the importance of the phospholipid level to include the drugs in the phytosomal matrix. The soya lecithin contains phosphatidylcholine molecules, which have both hydrophilic and lipophilic domains that promote the interaction of hydroxyl groups from resveratrol by the hydrogen-bonding forces and molecular association. Increasing the number of phospholipids led to an increased matrix available for complex formation, leading to improved drug entrapment. This resulted in an optimized formulation with an extremely high entrapment efficiency of 98.43%, demonstrating almost complete incorporation of the drug in the phytosomal structure.

One of the most crucial parameters to characterize nanocarrier systems is the particle size. Optimised phytosomal formulation showed mean particle size of 120.7nm in nanoscale range which is optimum for oral drug delivery. Reduction of particle size results in an increase in the surface area for interaction with dissolution media and leads to improved dissolution and release behavior. In addition, nanosized carriers can interact better with the intestinal epithelial cells and have better transport across biological membranes (Isailović et al., 2013; Kaushal et al., 2022). The particle size observed is in the similar range or less than reported for several previously studied phospholipid-based delivery systems, indicating that an efficient formulation design and processing has been achieved.

The information obtained from the zetapotential analysis is helpful in elucidating information about the colloidal stability. The optimized formulation showed a moderately high negative surface charge of −25.2 mV. This surface charge is part of the charge that causes electrostatic repulsion between adjacent particles, and that helps to prevent aggregation during storage and dispersion. The zeta potential value obtained suggests acceptable physical stability of the phytosomal system as per general rule, stable

nanosuspensions have zeta potential values > ±20 mV (Aundhia et al., 2024). The negative surface charge could be a result of the ionization of functional groups of phospholipids inside the phytosomal structure.

The FTIR study offered valuable information on resveratrol-compatibility with soya lecithin. The characteristic bands of resveratrol remained in the phytosomal formulation, suggesting that no changes occurred in the structure of the drug during formulation processing. Likewise, the dominant functional group vibrations of phosphatidylcholine were still observed. Small changes in the position of the peaks and in the intensity of the peaks were noted, indicating intermolecular interactions between the hydroxyl groups of resveratrol and the polar head groups of phospholipids. The spectral changes observed are similar to those reported for phospholipid complexes of other phytoconstituents and are believed to reflect successful formation of a phytosome (Agarwal et al., 2014; Jagwani et al., 2020). Importantly, no new peaks or loss of characteristic peaks were observed, which suggests that there was no chemical incompatibility or degradation.

Furthermore, successful formation of phytosomal particles was confirmed by morphological examination by scanning electron microscopy. SEM images showed individual and evenly dispersed particles of nanometer size. Significant is the lack of crystals in the drug that are visible, as this suggests that the resveratrol was incorporated into the phospholipid matrix, and not simply a physical mixture. Good formulation reproducibility and reproducible release behavior is often correlated with uniform particle morphology.

Differential scanning calorimetry was used to determine the thermal properties of the formulation. The pure resveratrol showed a typical endothermic peak which was associated with its endothermic crystalline melting point. However, the optimized phytosomal formulation showed a different thermal profile with decreased intensity and the characteristic drug peak modified. These changes are often accompanied with partial loss of crystallinity and molecular dispersion of the drug in the phospholipid matrix. This phenomenon has also been noted in phytosomal and phospholipid-complex systems where the drug is transformed from the crystalline state to more amorphous or molecularly dispersed state after complexation (Jagwani et al., 2020). Thermodynamic activity and dissolution is increased and often desirable in the amorphous forms of drug. Reduction in crystallinity is generally considered to be advantageous.

The in vitro drug release study showed that the release of resveratrol improved significantly after the formation of resveratrol-phytosome. The optimized formulation released 98.85% of drug, whereas pure resveratrol released 85.76% of drug. Several complimentary mechanisms may account for the improved release profile. The first is that the nanoscale size of the phytosomes makes more surface area available for dissolution. Secondly, the wettability and dispersion of hydrophobic drug molecules is enhanced in an aqueous medium by the use of phospholipids. Third, molecular dispersion of resveratrol in the phospholipid matrix renders the need to dissolve of large crystals of the drug before release is eliminated. All these factors combine to lead to better release of the phytosomal formulation. One of the most often claimed benefits of phospholipid-based delivery systems has been enhanced release behavior, which has been consistently reported and usually linked to better bioavailability following oral administration (Agarwal et al., 2014; Isailović et al., 2013).

The efficacy of the optimized phytosomal formulation supports the efficacy of phytosome technology as delivery system for poorly water-soluble phytoconstituents. This nanosized particle size along with high entrapment efficiency, favorable zeta potential, successful interaction between drug and phospholipid, and improved drug release indicates that the drug formulation developed was able to overcome the major drawbacks of resveratrol. In addition, the use of QbD and RSM allowed an optimized formulation to be identified, which had reproducible properties and a statistically validated performance.

The results of the present investigation show that the phytosomal complexation is a successful method to enhance the physicochemical and release characteristics of resveratrol. The optimized formulation showed very good nanoscale characteristics with high drug incorporation efficiency, drug stability for 60 days was very good and the release performance was very good. The improvements could lead to better oral absorption and effective treatment of resveratrol. In future, pharmacokinetic study, long term stability evaluation, and in vivo therapeutic studies should be conducted to further strengthen the clinical potential of the developed phytosomal system.

Conclusion

The present study has successfully developed and optimized resveratrol loaded phytosomal delivery system using soya lecithin using rotary evaporation technique. The systematic optimization of formulation variables using 3^2 factorial design showed that Quality by Design (QbD) principles proved to be helpful in the formulation of phytosomal products. This optimized batch F8 gave the best results with particle size 120.7 ± 0.4 nm, zeta potential of −25.2 ± 1.2 mV, entrapment efficiency of 98.43 ± 1.05 % and drug loading of 98.43 ± 0.46 %.

Successful interaction between resveratrol and phospholipid components was confirmed by FTIR analysis with no signs of chemical incompatibility while DSC study showed modification of the crystalline nature of the drug after the formation of phytosomal complex. The results of particle size analysis were supported by the SEM micrographs which showed uniformly distributed nanosized particles. The optimized phytosomal formulation exhibited better release profile in vitro with 98.85% of cumulative drug release in comparison to the pure resveratrol with 85.76% release.

In general, the developed phytosomal system proved to be an effective solution of major drawbacks of resveratrol, including its low aqueous solubility and release behaviour. The use of nanoscale particle size, high drug incorporation, good colloidal stability and favorable release characteristics, indicates that phytosome technology could be a beneficial approach for the oral delivery of resveratrol. The optimized formulation is promising for further investigation of its therapeutic and translational potential through pharmacokinetic, stability, and in vivo efficacy studies in the future.

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