

Development and Optimization of Naringin Phytosomes Through a Quality-by-Design Approach for Enhanced Anti-Obesity Activity

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Abstract

Background: Naringin is a citrus-derived flavonoid that has attracted considerable interest because of its reported metabolic, antioxidant, anti-inflammatory and lipid-modulating properties. However, formulation of phytoconstituents with suitable phospholipid carriers may be necessary to improve their pharmaceutical performance. Phytosome technology involves interaction of a phytoconstituent with phospholipids and can provide a suitable delivery system for poorly bioavailable plant-derived compounds.

Objective: The present study was undertaken to develop and optimize naringin phytosomes using a Quality-by-Design approach and Central Composite Design (CCD). Drug:phosphatidylcholine (Drug:PC) ratio and temperature were selected as formulation/process variables, while entrapment efficiency, particle size and zeta potential were considered critical formulation responses.

Methods: Naringin phytosomes were prepared using varying Drug:PC ratios and temperatures. A two-factor Central Composite Design was employed to evaluate the influence of the independent variables on entrapment efficiency (R1), particle size (R2) and zeta potential (R3). Thirteen experimental formulations were evaluated. The optimized formulation was further characterized by microscopic examination, Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), dynamic light scattering (DLS) and scanning electron microscopy (SEM).

Results: The experimental formulations exhibited entrapment efficiencies ranging from **71.12 to 89.65%**, particle sizes from **319.53 to 512.21 nm**, and zeta potentials from **-21.36 to -61.13 mV**. Drug:PC ratio and temperature influenced the measured responses. The ANOVA results indicated significant models for entrapment efficiency, particle size and zeta potential, with model p-values of **0.0055, 0.0001 and 0.0007**, respectively. The optimized formulation showed predicted values of **82.156% entrapment efficiency, 343.25 nm particle size and -40.7 mV zeta potential**, whereas the observed values were **83.23%, 306.4 nm and -38.6 mV**, respectively. The microscopic examination revealed globular particles without visible agglomeration. FTIR, DSC, DLS and SEM analyses were used to characterize the optimized formulation.

Conclusion: The findings demonstrate that CCD can be effectively applied for systematic optimization of naringin phytosomes. The close agreement between predicted and observed values supports the applicability of the selected design for formulation optimization. The optimized naringin phytosomes exhibited satisfactory entrapment and particle characteristics and may serve as a promising formulation platform for further pharmaceutical and pharmacological evaluation of naringin.

Keywords: Naringin; phytosomes; Quality by Design; Central Composite Design; entrapment efficiency; particle size; zeta potential; optimization.

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1. Introduction

Naringin is a naturally occurring flavonoid glycoside predominantly associated with citrus fruits, particularly grapefruit and related Citrus species. It belongs to the flavanone class of polyphenolic compounds and has attracted considerable research interest because of its reported biological activities. Experimental investigations have associated naringin with antioxidant, anti-inflammatory, hepatoprotective, cardioprotective and metabolic effects. Its potential influence on lipid

metabolism and glucose homeostasis has also generated interest in the development of naringin-based interventions for metabolic disorders.

Obesity is a complex metabolic disorder characterized by excessive accumulation of adipose tissue and is frequently associated with dyslipidemia, insulin resistance, hyperglycemia and other metabolic abnormalities. Natural flavonoids such as naringin have been investigated for their ability to modulate several pathways involved in

lipid and glucose metabolism. Consequently, development of suitable pharmaceutical delivery systems for naringin may provide opportunities for improving its therapeutic utilization.

Despite its biological potential, the pharmaceutical development of plant-derived flavonoids may be limited by physicochemical and biopharmaceutical characteristics. Incorporation of such compounds into specialized delivery systems is therefore an important area of formulation research. Phytosomes represent a lipid-based delivery approach in which phytoconstituents are associated with phospholipids, generally phosphatidylcholine, to form a phytophospholipid complex. This approach can alter the physicochemical characteristics of the phytoconstituent and potentially improve its interaction with biological membranes and pharmaceutical performance.

The development of a phytosomal formulation involves several formulation and process variables that may simultaneously influence critical quality attributes. Conventional one-factor-at-a-time optimization is inefficient for identifying interactions between variables and may require a large number of experiments. In contrast, Quality by Design (QbD) provides a systematic framework for formulation development by defining critical quality attributes, identifying critical material attributes and critical process parameters, and applying statistical experimental designs for optimization.

Design of Experiments (DoE), particularly response-surface methodology, permits simultaneous investigation of multiple factors and their influence on formulation responses. Central Composite Design (CCD) is a commonly used response-surface design for developing mathematical models and identifying optimized formulation conditions.

In the present investigation, naringin phytosomes were developed using a QbD-based approach. Drug: phosphatidylcholine (Drug:PC) ratio and temperature were selected as independent variables, while entrapment efficiency, particle size and zeta potential were selected as critical responses. A two-factor CCD was employed to understand the influence of these variables and to establish an optimized formulation.

2. Materials and Methods

2.1 Materials

Naringin was used as the phytoconstituent for phytosome development. Phosphatidylcholine was used as the phospholipid component. Other materials and reagents used during formulation development and characterization were of appropriate pharmaceutical/analytical grade.

2.2 Quality-by-Design Approach

A QbD-based approach was adopted for systematic development of naringin phytosomes. The formulation development strategy involved identification of the major formulation/process

variables and their potential influence on the critical quality attributes of the phytosomes.

The selected independent variables were:

- **Factor A (X1): Drug:PC ratio**
- **Factor B (X2): Temperature**

The responses selected for optimization were:

- **R1: Entrapment efficiency (%)**
- **R2: Particle size (nm)**
- **R3: Zeta potential (mV)**

The experimental ranges used for the naringin phytosome development were:

Variable	Low level	High level
Drug:PC ratio	0.5:1	2.0:1
Temperature	40°C	60°C
Entrapment efficiency	Maximize	—
Particle size	Minimize	—
Zeta potential	Target appropriate magnitude	—

2.3 Experimental Design

A two-factor Central Composite Design was employed for optimization of the naringin phytosomes. The experimental design consisted of 13 experimental runs, covering different combinations of Drug:PC ratio and temperature. The responses measured for each formulation were entrapment efficiency, particle size and zeta potential.

Table 1. Experimental factors and responses selected for CCD optimization

Type	Variable	Sym	Unit	Low level	High level
Independent	Drug:PC ratio	X1	—	0.5:1	2.0:1
Independent	Temperature	X2	°C	40	60
Dependent	Entrapment efficiency	R1	%	Maximize	—
Dependent	Particle size	R2	nm	Minimize	—

Dependent	Zeta potential	R3	mV	Appropriate magnitude	—
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2.4 Preparation of Naringin Phytosomes

Naringin phytosomes were prepared using varying Drug:PC ratios and temperature conditions according to the experimental design. The formulation conditions were varied according to the CCD matrix, and the prepared phytosomal dispersions were subsequently evaluated for entrapment efficiency, particle size and zeta potential. The optimized formulation was selected based on the desirability criteria established for the responses.

2.5 Determination of Entrapment Efficiency

The entrapment efficiency of the prepared naringin phytosomes was determined to assess the proportion of naringin associated with the phytosomal formulation. The entrapment efficiency was expressed as percentage of the total amount of naringin used during formulation.

2.6 Particle Size and Zeta Potential

The particle size and zeta potential of the optimized naringin phytosomes were determined using Dynamic Light Scattering (DLS). Particle size was expressed in nanometers (nm), while zeta potential was expressed in millivolts (mV).

2.7 Microscopic Examination

The optimized naringin phytosomes were examined microscopically to assess their general morphology and the presence or absence of visible aggregation. The microscopic examination demonstrated globular particles without visible agglomeration.

2.8 Fourier-Transform Infrared Spectroscopy

FTIR analysis was performed to investigate the characteristic functional groups of naringin and to assess changes associated with phytosome formation. FTIR spectra of pure naringin and optimized naringin phytosomes were compared to evaluate the compatibility and interaction between naringin and the phospholipid component.

2.9 Differential Scanning Calorimetry

DSC analysis was performed for pure naringin and the optimized naringin phytosomes to investigate the thermal behavior of the phytoconstituent and the formulation.

The thermograms were compared to determine changes in the thermal characteristics following phytosome formation.

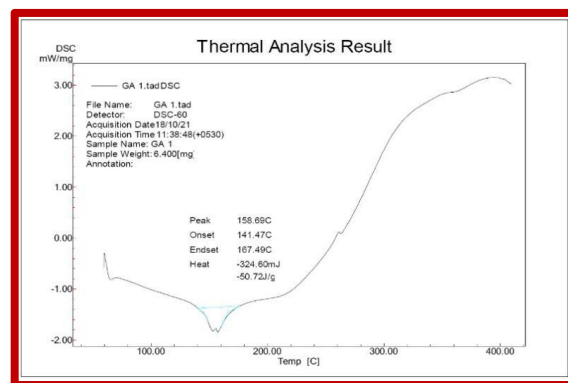


Figure: DSC Thermograph of Naringin

2.10 Scanning Electron Microscopy

The surface morphology of the optimized naringin phytosomes was evaluated using scanning electron microscopy. SEM analysis was performed to obtain detailed information regarding the morphology and surface characteristics of the optimized formulation.

2.11 Statistical Analysis

The experimental data obtained from the CCD runs were subjected to statistical analysis using Design-Expert software. Regression analysis was performed to identify the relationship between the independent variables and the selected responses. Analysis of variance (ANOVA) was used to evaluate the significance of the developed models. Model adequacy was assessed using the model F-value, p-value, lack-of-fit, adjusted R^2 , predicted R^2 and adequate precision.

A probability value of $p < 0.05$ was considered indicative of statistical significance.

3. Results and Discussion

3.1 Central Composite Design and Experimental Results

The CCD was successfully employed to investigate the influence of Drug:PC ratio and temperature on the characteristics of naringin phytosomes. Thirteen experimental runs were performed. The obtained entrapment efficiency ranged from 71.12 to 89.65%, particle size ranged from 319.53 to 512.21 nm, and zeta potential ranged from -21.36 to -61.13 mV.

Table 2. CCD experimental runs and observed responses for naringin phytosomes

S	R	Drug :PC ratio	Temperature (°C)	EE (%)	Particle size (nm)	Zeta potential (mV)
3	1	0.5	60	73.20	366.40	-61.13
9	2	1.25	50	85.08	394.23	-50.27

10	3	1.25	50	83.03	384.78	-46.80
1	4	0.5	40	78.28	415.30	-21.36
8	5	1.25	64.142135	72.34	512.21	-40.25
6	6	2.310660	50	89.65	335.12	+37.21
7	7	1.25	35.857864	80.36	469.42	-29.34
4	8	2.0	60	81.17	465.20	+35.31
2	9	2.0	40	88.39	332.46	-36.50
12	10	1.25	50	81.20	372.56	-46.28
5	11	0.189339	50	71.12	319.53	-42.56
11	12	1.25	50	84.24	375.60	-30.20
13	13	1.25	50	77.23	410.56	-29.95

The results indicate considerable variation in the three responses with changes in Drug:PC ratio and temperature, demonstrating the importance of formulation and process conditions in determining the characteristics of naringin phytosomes. The highest experimentally observed entrapment efficiency was 89.65%, obtained in run 6, while the lowest was 71.12%, observed in run 11. Particle size varied from 319.53 nm to 512.21 nm, demonstrating that the selected variables had a substantial effect on particle characteristics.

3.2 Effect of Drug:PC Ratio and Temperature on Entrapment Efficiency

Entrapment efficiency is an important quality attribute because it indicates the amount of naringin successfully incorporated or associated with the phytosomal system. The experimental data showed that entrapment efficiency increased with increasing Drug:PC ratio within the investigated formulation space. The highest observed entrapment efficiency was 89.65%. The regression analysis indicated that Drug:PC ratio and temperature influenced entrapment efficiency. The regression equation for R1 was:

$$Y1 = 81.44 - 12.26X1$$

– 9.66X2

where:

- Y1 = predicted entrapment efficiency

- X1 = Drug:PC ratio

- X2 = temperature

The ANOVA demonstrated that the selected model was statistically significant ($F = 9.20$; $p = 0.0055$). The model statistics showed an adjusted R^2 of 0.7736 and predicted R^2 of 0.5834, indicating that the model explained a substantial proportion of the variability in the response.

3.3 Effect of Variables on Particle Size

Particle size is a critical quality attribute of phytosomal formulations because it can influence dispersion characteristics, physical stability and biological interaction. The particle size of the experimental formulations ranged from 319.53 to 512.21 nm.

The regression equation for particle size was:

$$Y2 = 198.64 - 21.54X1$$

– 23.66X2

where Y2 represents the predicted particle size.

The model for particle size showed a high degree of statistical significance, with an F-value of 37.68 and p-value of 0.0001. The adjusted R^2 was 0.9386, while the predicted R^2 was 0.8775, indicating good agreement between the predicted and adjusted model performance. The adequate precision value was 20.1791, which was substantially higher than the desirable value of 4. This indicates an adequate signal-to-noise ratio for the particle-size response. The experimental findings demonstrated that changes in Drug:PC ratio and temperature produced measurable changes in particle size.

3.4 Effect of Variables on Zeta Potential

Zeta potential provides information regarding the surface charge characteristics of dispersed phytosomal systems and can be useful in assessing their physical stability. The experimental zeta-potential values ranged from -21.36 to -61.13 mV, with one experimental run showing a positive value of +37.21 mV.

The regression equation for the zeta-potential response was:

$$Y3 = -29.68 - 6.16X1 - 21.68X2 -$$

8.26X1X2

where Y3 represents the predicted zeta potential.

The model was statistically significant with an F-value of 17.88 and p-value of 0.0007.

The adjusted R^2 and predicted R^2 values were 0.8755 and 0.6776, respectively. The adequate precision value was 14.3538, indicating an adequate signal-to-noise ratio. The response-surface and perturbation analyses demonstrated that changes in Drug:PC ratio and temperature influenced the surface charge characteristics of the naringin phytosomes.

3.5 ANOVA and Model Adequacy

Table 3. ANOVA and regression statistics for the developed models

Parameter	Entrapment Efficiency	Particle Size	Zeta Potential
r			1

Model suggested	Linear	Quadratic	Quadratic
F-value	9.20	37.68	17.88
p-value	0.0055	0.0001	0.0007
Lack of fit	0.5332	0.6709	1.44
Predicted R ²	0.5834	0.8775	0.6776
Adjusted R ²	0.7736	0.9386	0.8755
Adequate precision	9.4026	20.1791	14.3538

All three models exhibited p-values below 0.05, demonstrating statistical significance. The lack-of-fit values supplied for the models were not statistically significant, supporting the adequacy of the developed models for describing the observed responses within the investigated experimental domain. The adequate precision values for all three responses were greater than 4, indicating an adequate signal relative to experimental noise.

3.6 Response-Surface and Perturbation Analysis
Perturbation, contour and three-dimensional response-surface plots were used to visualize the influence of Drug:PC ratio and temperature on the responses. The response-surface analysis demonstrated that the formulation responses varied with changes in both independent variables. Increasing the Drug:PC ratio was associated with changes in entrapment efficiency, particle size and zeta potential, whereas variation in temperature also produced measurable changes in the responses. The three-dimensional plots provided a visual representation of the interaction between the independent variables and the three responses. The regression analysis indicated the following overall trends:

Table 4. Effect of independent variables on the selected responses

Factor	Effect	EE	Particle Size	Zeta Potential
Drug:PC ratio (X1)	Increased	Increased	Increased*	Increased
Temperature (X2)	Increased	Decreased	Decreased	Decreased

3.7 Optimization of Naringin Phytosomes

Numerical optimization was performed using the desirability approach in Design-Expert software. The optimized formulation was selected by

simultaneously considering the desired values of entrapment efficiency, particle size and zeta potential.

Table 5. Predicted and observed responses of optimized naringin phytosomes

Response	Predicted value	Observed value
Entrapment efficiency (%)	82.156	83.23
Particle size (nm)	343.25	306.4
Zeta potential (mV)	-40.7	-38.6

The observed values were reasonably close to the predicted values.

The difference between predicted and observed values was:

- **Entrapment efficiency:** 1.074 percentage points
- **Particle size:** 36.85 nm
- **Zeta potential:** 2.1 mV

The observed entrapment efficiency was slightly higher than the predicted value, while the experimentally measured particle size was lower than predicted. The observed zeta potential was also close to the predicted value. These findings demonstrate reasonable predictive performance of the optimization model within the studied formulation space.

3.8 Microscopic Examination of Optimized Naringin Phytosomes

Microscopic examination of the optimized naringin phytosomes revealed globular particles with no apparent agglomeration. The absence of visible agglomeration suggested relatively uniform dispersion of the prepared phytosomal particles under the conditions of microscopic examination.

Figure 1. Microscopic view of optimized naringin phytosomes

3.9 FTIR Studies

FTIR spectroscopy was performed for pure naringin and optimized naringin phytosomes.

The FTIR spectrum of naringin was compared with that of the optimized phytosomal formulation to evaluate possible interactions between naringin and phosphatidylcholine.

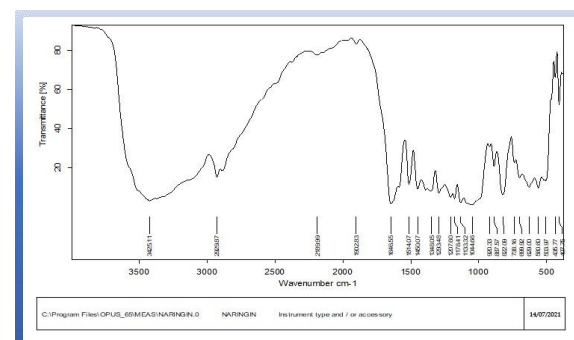


Figure 2. FTIR spectrum of naringin.

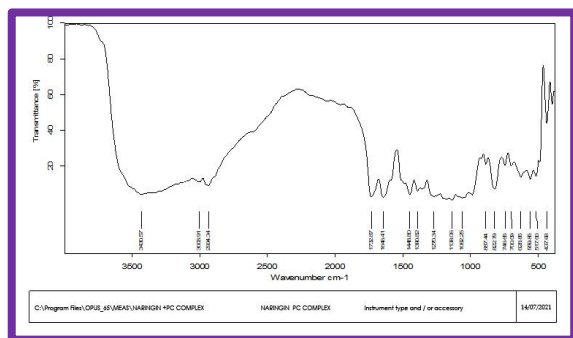


Figure 3. FTIR spectrum of optimized naringin phytosomes.

3.10 DSC Studies

DSC thermograms of pure naringin and optimized naringin phytosomes were obtained to investigate their thermal characteristics.

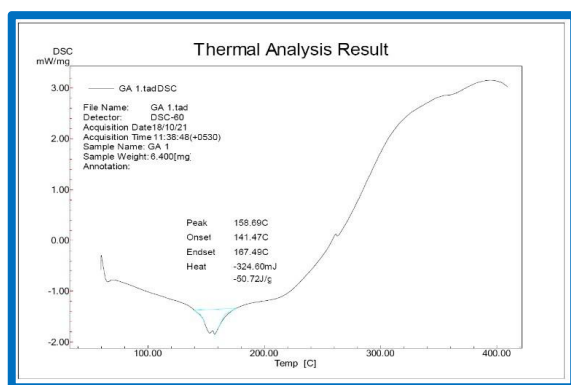


Figure 4. DSC thermogram of naringin.

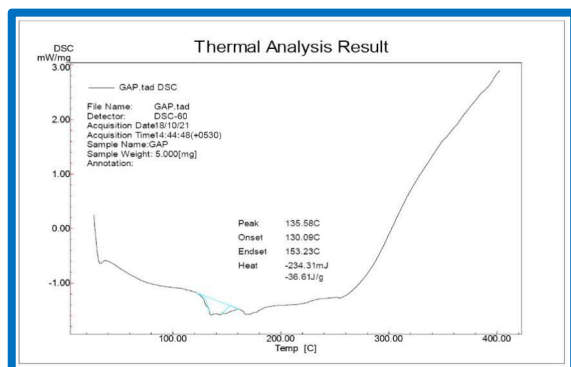


Figure 5. DSC thermogram of optimized naringin phytosomes.

3.11 DLS Characterization

The optimized formulation was subjected to dynamic light scattering analysis for determination of particle size and zeta potential. The optimized formulation exhibited an experimentally observed particle size of 306.4 nm and zeta potential of -38.6 mV.

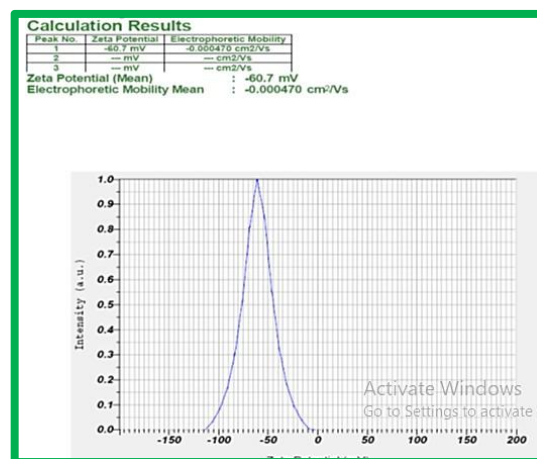


Figure 6. Particle-size distribution of optimized naringin phytosomes.

3.12 SEM Analysis

Scanning electron microscopy was performed to further investigate the morphology of the optimized naringin phytosomes. The SEM image provides morphological information regarding the shape and surface characteristics of the optimized formulation.

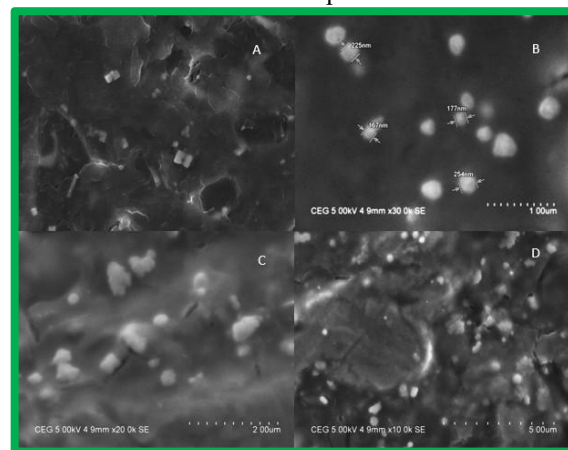


Figure 8. SEM image of optimized naringin phytosomes.

4. Discussion

The present study demonstrates the application of a systematic QbD and CCD-based strategy for development and optimization of naringin phytosomes. The use of Drug:PC ratio and temperature as independent variables enabled assessment of their influence on important formulation characteristics. The experimental results demonstrated considerable variation in entrapment efficiency, particle size and zeta potential across the CCD formulations. Entrapment efficiency ranged from 71.12 to 89.65%, indicating that the Drug:PC ratio and processing temperature influenced the incorporation of naringin into the phytosomal system.

The particle size showed a broader range of 319.53–512.21 nm. Such variation demonstrates the sensitivity of particle characteristics to the selected formulation and processing variables. The statistical model for particle size showed the highest adjusted R^2 among the three responses (0.9386), indicating a

strong relationship between the investigated variables and particle size within the experimental design.

Zeta potential varied from -21.36 to -61.13 mV in most of the experimental formulations, with the experimental matrix also containing positive values in some runs. This variation demonstrates the influence of the formulation conditions on surface charge characteristics. Since zeta potential is associated with electrostatic interactions between dispersed particles, an appropriate magnitude of surface charge is important when considering physical stability.

The ANOVA results supported the statistical significance of all three models. The p-values for entrapment efficiency, particle size and zeta potential were 0.0055, 0.0001 and 0.0007, respectively. The lack-of-fit values were not significant, while adequate precision values exceeded 4 for all responses.

The optimization step provided predicted values of 82.156% for entrapment efficiency, 343.25 nm for particle size and -40.7 mV for zeta potential. Experimental verification yielded values of 83.23%, 306.4 nm and -38.6 mV, respectively. The closeness of the predicted and experimental results provides experimental support for the applicability of the developed design for optimization. Microscopic examination demonstrated globular particles without apparent agglomeration. DLS analysis further characterized the optimized formulation, while FTIR, DSC and SEM analyses provided additional characterization of the optimized phytosomal system. The findings demonstrate that systematic application of QbD and CCD can reduce empirical formulation development and facilitate simultaneous optimization of multiple formulation attributes.

5. Conclusion

The present study successfully demonstrated the development and optimization of naringin phytosomes using a Quality-by-Design approach and Central Composite Design. Drug:PC ratio and temperature were identified as important variables influencing entrapment efficiency, particle size and zeta potential. Across the 13 experimental formulations, entrapment efficiency ranged from 71.12 to 89.65%, particle size ranged from 319.53 to 512.21 nm, and zeta potential ranged from -21.36 to -61.13 mV, with the experimental design also producing positive zeta-potential values in selected formulations. ANOVA confirmed that the developed models were statistically significant, with p-values of 0.0055, 0.0001 and 0.0007 for entrapment efficiency, particle size and zeta potential, respectively. The optimized formulation exhibited predicted values of 82.156% entrapment efficiency, 343.25 nm particle size and -40.7 mV zeta potential, compared with observed values of 83.23%, 306.4 nm and -38.6 mV, respectively.

The microscopic examination showed globular particles without visible agglomeration, while DLS, FTIR, DSC and SEM analyses provided additional characterization of the optimized formulation. Thus, the study establishes a systematic formulation-development strategy for naringin phytosomes and demonstrates the utility of CCD for simultaneous optimization of multiple formulation responses. Further pharmacological evaluation is required to establish the anti-obesity efficacy of the optimized naringin phytosomes.

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