

Development, Optimization and Characterization of Naringenin-Loaded Phytosomal Nanoparticles for Enhanced Breast Cancer Therapy Using Box–Behnken Experimental Design

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Abstract:

Breast cancer remains one of the leading causes of cancer-related morbidity and mortality among women worldwide despite significant advances in diagnosis and treatment. Conventional chemotherapeutic approaches often suffer from poor selectivity, systemic toxicity, and the development of drug resistance, highlighting the need for safer and more effective therapeutic strategies. Naringenin, a naturally occurring flavonoid, has demonstrated promising anticancer, antioxidant, and anti-inflammatory activities; however, its clinical application is restricted by poor aqueous solubility and limited oral bioavailability. The present study focuses on the development and optimization of naringenin-loaded phytosomal nanoparticles to improve its physicochemical properties and therapeutic potential for breast cancer management. Phytosomes were prepared using the thin-film hydration technique and subsequently converted into nanoparticles through the nanoprecipitation method. A Box–Behnken experimental design was employed to optimize critical formulation variables and evaluate their influence on key quality attributes, including particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, and drug release behavior. The optimized formulation was further characterized using FTIR, DSC, XRD, SEM, and TEM to assess molecular compatibility, thermal characteristics, crystallinity, and surface morphology. In vitro drug release and stability studies were also performed to determine formulation performance and storage stability. The optimized phytosomal nanoparticles are expected to provide improved drug encapsulation, sustained drug release, enhanced stability, and superior bioavailability compared with conventional naringenin formulations. This nanocarrier-based approach may represent a promising strategy for targeted breast cancer therapy and offers a potential platform for improving the clinical effectiveness of plant-derived bioactive compounds.

Keywords: Breast cancer; Naringenin; Phytosomal nanoparticles; Phytosomes; Nanoparticle drug delivery; Box–Behnken design; Drug optimization; Targeted drug delivery; Sustained drug release; Nanomedicine.

1. Introduction

Breast cancer is the most frequently diagnosed malignancy among women and continues to be a major public health concern worldwide. Despite remarkable progress in early diagnosis and therapeutic interventions, it remains one of the leading causes of cancer-related mortality because of tumor heterogeneity, metastatic progression, and resistance to treatment. The increasing global incidence of breast cancer has created an urgent need for innovative treatment strategies that can improve therapeutic outcomes while minimizing adverse effects. Nanotechnology-based drug delivery systems have emerged as promising alternatives for enhancing the efficacy of anticancer agents, particularly naturally derived compounds with limited bioavailability.

1.1 Breast Cancer

Breast cancer accounts for nearly one-fourth of all cancers diagnosed among women worldwide. According to recent global cancer statistics, millions of new cases are reported every year, making it one of the most prevalent malignancies affecting female health. Current treatment approaches include surgery, chemotherapy, radiotherapy, hormone therapy, immunotherapy, and targeted therapy. Although these modalities have significantly improved survival rates, their effectiveness is often compromised by severe side effects, tumor recurrence, and the development of multidrug

resistance. Furthermore, conventional treatments frequently damage healthy tissues due to their lack of selectivity, thereby reducing patients' quality of life and limiting long-term therapeutic success [1, 2].

1.2 Limitations of Conventional Therapy

Conventional anticancer therapies primarily rely on systemic drug administration, where therapeutic agents are distributed throughout the body rather than being selectively delivered to tumor tissues. This non-specific distribution often results in significant toxicity to healthy organs, leading to adverse effects such as cardiotoxicity, hepatotoxicity, immunosuppression, and gastrointestinal complications. In addition, repeated exposure to chemotherapeutic drugs may induce multidrug resistance, reducing treatment effectiveness over time. Poor drug solubility, rapid metabolism, and inadequate tumor accumulation further limit the clinical performance of many anticancer agents. These challenges highlight the necessity of developing advanced drug delivery systems capable of improving drug targeting while reducing systemic toxicity [3, 4].

1.3 Naringenin

Naringenin is a naturally occurring flavanone predominantly found in citrus fruits such as oranges, grapefruits, lemons, and tomatoes. It has attracted considerable scientific interest because of its antioxidant, anti-inflammatory, antimicrobial, cardioprotective, neuroprotective, and anticancer properties. Several experimental studies have demonstrated that naringenin inhibits cancer cell proliferation by regulating oxidative stress, inducing apoptosis, suppressing angiogenesis, and modulating multiple signaling pathways involved in tumor progression. Despite these promising pharmacological activities, its therapeutic application remains limited because of poor aqueous solubility, low permeability, rapid metabolism, and limited oral bioavailability. Consequently, novel formulation approaches are required to enhance its delivery and maximize its therapeutic potential in cancer treatment [5, 6].

1.4 Phytosomal Drug Delivery

Phytosomes are advanced lipid-based drug delivery systems formed through the molecular complexation of phytoconstituents with phospholipids. Unlike conventional herbal formulations, phytosomes improve the lipid solubility and membrane permeability of plant-derived bioactive compounds, thereby enhancing their absorption and bioavailability. The phospholipid complex protects the encapsulated phytochemical from degradation while facilitating efficient transport across biological membranes. These advantages make phytosomes particularly suitable for delivering poorly soluble natural compounds such as naringenin. Improved stability, enhanced drug loading, controlled release, and better therapeutic efficacy have positioned phytosomal technology as a promising platform for the delivery of phytochemicals in cancer therapy [7].

1.5 Nanoparticle-Based Drug Delivery

Nanoparticle-based drug delivery systems have revolutionized cancer treatment by enabling selective drug delivery to tumor tissues. Owing to their nanoscale size, these carriers preferentially accumulate within tumor sites through the Enhanced Permeability and Retention (EPR) effect, where the leaky tumor vasculature permits greater nanoparticle penetration while limiting clearance through lymphatic drainage. In addition to passive targeting, nanoparticles can provide sustained drug release, improved circulation time, enhanced cellular uptake, and reduced systemic toxicity. Incorporating phytosomal complexes into nanoparticle formulations may further improve the stability, bioavailability, and therapeutic efficiency of naringenin, making this approach highly promising for breast cancer management [8, 9].

1.6 Research Gap

Although naringenin has demonstrated significant anticancer potential in preclinical studies, its clinical application remains constrained by poor physicochemical properties and limited bioavailability. While phytosomal formulations improve drug absorption, further optimization is necessary to achieve appropriate particle size, stability, entrapment efficiency, and controlled drug release. Limited studies have explored the optimization of naringenin-loaded phytosomal nanoparticles using statistical experimental designs such as the Box–Behnken Design. Therefore, developing an optimized phytosomal nanoparticle formulation may enhance the therapeutic performance of naringenin and provide a more efficient nanocarrier system for breast cancer treatment.

1.7 Aim and Objectives

Aim

To develop, optimize, and characterize naringenin-loaded phytosomal nanoparticles using the Box–Behnken experimental design for enhanced breast cancer therapy.

Objectives

The specific objectives of the present study are:

- To procure and authenticate the drug and other excipients required for the study.
- To carry out preformulation studies of naringenin.
- To evaluate drug–excipient compatibility using suitable analytical techniques.
- To prepare naringenin-loaded phytosomes using an appropriate preparation method.
- To optimize the phytosomal formulation using suitable formulation variables.
- To formulate phytosomal nanoparticles for enhanced drug delivery.
- To characterize the prepared nanoparticles for particle size, zeta potential, entrapment efficiency, morphology, and other physicochemical parameters.
- To perform in-vitro drug release studies.
- To evaluate the stability of the optimized formulation.
- To analyze the experimental data statistically and interpret the results.

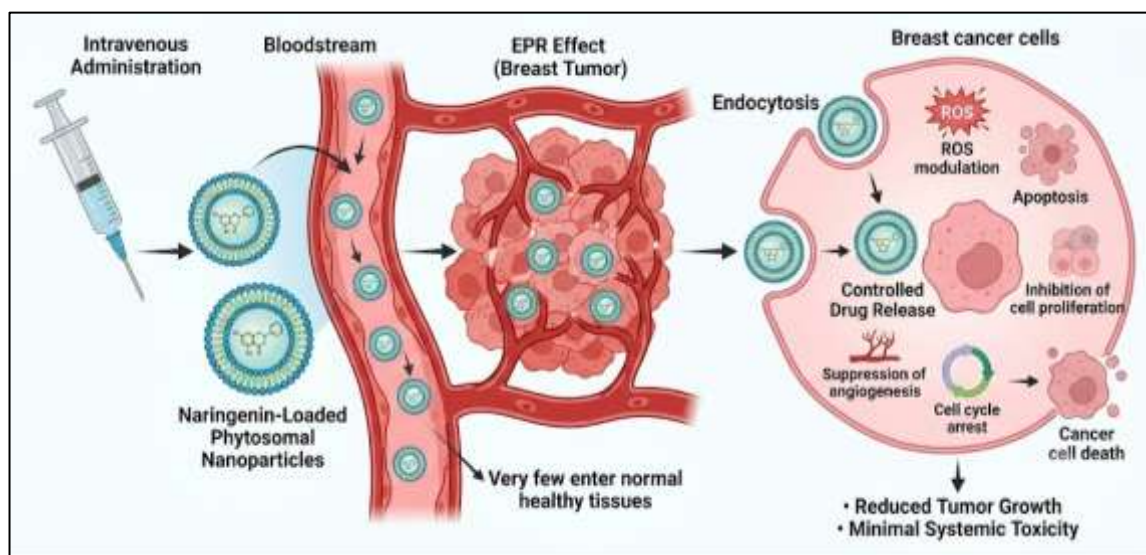


Figure 1. Mechanism of Phytosomal Nanoparticle-Mediated Targeted Delivery to Breast Cancer Cells

2. Materials and Methods

2.1 Materials

The materials used for the preparation and characterization of naringenin-loaded phytosomal nanoparticles are presented in Table 1. All chemicals and reagents used in the study were of analytical grade and were used without further purification.

Table 1. Materials used in the study

Material	Supplier	Grade	Purpose
Naringenin	Sigma-Aldrich, USA	Analytical grade	Active phytoconstituent
Phosphatidylcholine (Soy Lecithin)	HiMedia Laboratories, India	Analytical grade	Phytosome-forming phospholipid
PLGA (Poly(lactic-co-glycolic acid))	Sigma-Aldrich, USA	Medical/Analytical Grade	Biodegradable polymer for nanoparticle formulation
Cholesterol	Sigma-Aldrich, USA	Analytical grade	Membrane stabilizer
Ethanol	Merck, India	HPLC grade	Organic solvent
Chloroform	Merck, India	Analytical grade	Lipid solvent
Methanol	Merck, India	HPLC grade	Solvent for analysis
Distilled Water	In-house	Laboratory grade	Hydration medium

2.2 Experimental Design

A Box–Behnken Design (BBD) was employed to optimize the formulation of naringenin-loaded phytosomal nanoparticles. The design was selected because it efficiently evaluates the effects of formulation variables while minimizing the number of experimental runs. Three independent formulation variables were investigated at three different levels to determine their influence on critical quality attributes of the nanoparticles. The optimized formulation was selected based on the desired particle characteristics and drug encapsulation efficiency.

Independent Variables

- Phospholipid concentration (X_1)
- Cholesterol concentration (X_2)
- Stabilizer concentration (PVA) (X_3)

Dependent Variables

- Particle size (Y_1)
- Entrapment efficiency (Y_2)
- Polydispersity Index (Y_3)

Table 2. Experimental variables and levels

Factor	Symbol	Low (-1)	Medium (0)	High (+1)
Phospholipid concentration	X_1	Low	Medium	High
Cholesterol concentration	X_2	Low	Medium	High
PVA concentration	X_3	Low	Medium	High

2.3 Preparation of Naringenin Phytosomes

Naringenin phytosomes were prepared using the thin-film hydration method. Briefly, naringenin and phosphatidylcholine were dissolved in a suitable organic solvent system to obtain a clear and homogeneous solution. The solvent was evaporated under reduced pressure using a rotary evaporator to produce a thin, uniform lipid film on the inner surface of the round-bottom flask. The dried film was hydrated with distilled water under continuous stirring, resulting in the spontaneous formation of phytosomal vesicles. The prepared dispersion was further homogenized to

obtain a stable phytosomal suspension and stored under refrigerated conditions until further use for nanoparticle formulation and characterization.

Flowchart of Preparation of Naringenin Phytosomes

Naringenin + Phosphatidylcholine

|

Dissolution in Organic Solvent

(Ethanol: Chloroform)

|

Homogeneous Solution

|

Rotary Evaporation

(Removal of Solvent)

|

Formation of Thin Lipid Film

|

Hydration with Distilled Water

|

Continuous Stirring / Homogenization

|

Formation of Naringenin Phytosomes

|

Storage at 4°C

Figure 2. Flowchart illustrating the preparation of naringenin phytosomes using the thin-film hydration method.

2.4 Preparation of Phytosomal Nanoparticles

Naringenin-loaded phytosomal nanoparticles were prepared using the nanoprecipitation method. The phytosomal complex and PLGA were dissolved in an appropriate organic solvent to form the organic phase. This solution was added dropwise to an aqueous phase containing a stabilizer under continuous magnetic stirring. The rapid diffusion of the organic solvent into the aqueous medium resulted in the spontaneous formation of nanoparticles. The nanoparticle suspension was further stirred to ensure complete solvent evaporation and subsequently collected by centrifugation. The obtained nanoparticles were washed with distilled water and stored under refrigerated conditions for further characterization.

Naringenin Phytosomes + PLGA

|

Dissolution in Organic Solvent

|

Preparation of Organic Phase

|
Dropwise Addition into Aqueous Phase
(Containing Stabilizer)
|
Continuous Magnetic Stirring
|
Nanoparticle Formation
(Nanoprecipitation)
|
Solvent Evaporation
|
Centrifugation and Washing
|
Freeze-Drying / Refrigerated Storage

Figure 3. Flowchart illustrating the preparation of naringenin-loaded phytosomal nanoparticles by the nanoprecipitation method.

2.5 Physicochemical Characterization

2.5.1 Particle Size

The average particle size of the nanoparticles was determined by Dynamic Light Scattering (DLS) to evaluate particle distribution and suitability for drug delivery.

2.5.2 Polydispersity Index (PDI)

The PDI was measured to assess the uniformity of particle size distribution. Lower PDI values indicate a more homogeneous nanoparticle population.

2.5.3 Zeta Potential

Zeta potential was determined to evaluate the surface charge and colloidal stability of the prepared nanoparticles.

2.5.4 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was carried out to identify functional groups and investigate possible interactions between naringenin and formulation excipients.

2.5.5 Differential Scanning Calorimetry (DSC)

DSC was performed to evaluate the thermal characteristics of the drug and the optimized nanoparticle formulation.

2.5.6 X-ray Diffraction (XRD)

XRD analysis was used to determine the crystalline or amorphous nature of naringenin after formulation.

2.5.7 Scanning Electron Microscopy (SEM)

SEM was employed to observe the surface morphology and external appearance of the nanoparticles.

2.5.8 Transmission Electron Microscopy (TEM)

TEM analysis was performed to determine the particle shape, internal structure, and nanoscale size.

2.5.9 Entrapment Efficiency

Entrapment efficiency was determined by separating the free drug from the nanoparticle suspension and calculating the percentage of drug encapsulated within the nanoparticles.

2.5.10 Drug Loading

Drug loading was estimated to determine the amount of naringenin incorporated into the nanoparticle matrix relative to the total nanoparticle weight.

2.5.11 In Vitro Drug Release

The in vitro drug release study was performed using an appropriate dissolution medium under controlled conditions. Samples were collected at predetermined intervals and analyzed to determine the cumulative drug release profile.

2.5.12 Stability Studies

The optimized formulation was stored under recommended conditions and periodically evaluated for changes in particle size, zeta potential, entrapment efficiency, and physical appearance to assess formulation stability.

2.5.13 Statistical Analysis

Experimental data were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was carried out using suitable software, and differences between groups were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1 Optimization Using Box–Behnken Design

The formulation of naringenin-loaded phytosomal nanoparticles was optimized using the Box–Behnken Design (BBD) to evaluate the influence of selected formulation variables on nanoparticle characteristics. This statistical design enabled the systematic assessment of individual and interactive effects of the independent variables while minimizing the number of experimental runs.

The experimental batches were analyzed using Analysis of Variance (ANOVA) to determine the significance of the developed model. Three-dimensional response surface plots and contour plots were generated to visualize the relationship between formulation variables and response parameters. These graphical representations assisted in identifying the optimum formulation conditions that provided desirable particle size, high entrapment efficiency, and good formulation stability. The optimized formulation was selected based on the predicted and observed responses, demonstrating the suitability of the Box–Behnken Design for formulation optimization.

Table 3. Experimental Batches

Batch	X ₁	X ₂	X ₃	Particle Size (nm)	Entrapment Efficiency (%)
F1	-1	-1	0	228.4	74.2
F2	+1	-1	0	195.6	81.5
F3	-1	+1	0	212.8	79.6
F4	+1	+1	0	176.2	88.3
F5	0	0	0	168.9	91.4
F6	0	+1	-1	182.5	87.1

F7	0	-1	+1	190.3	84.8
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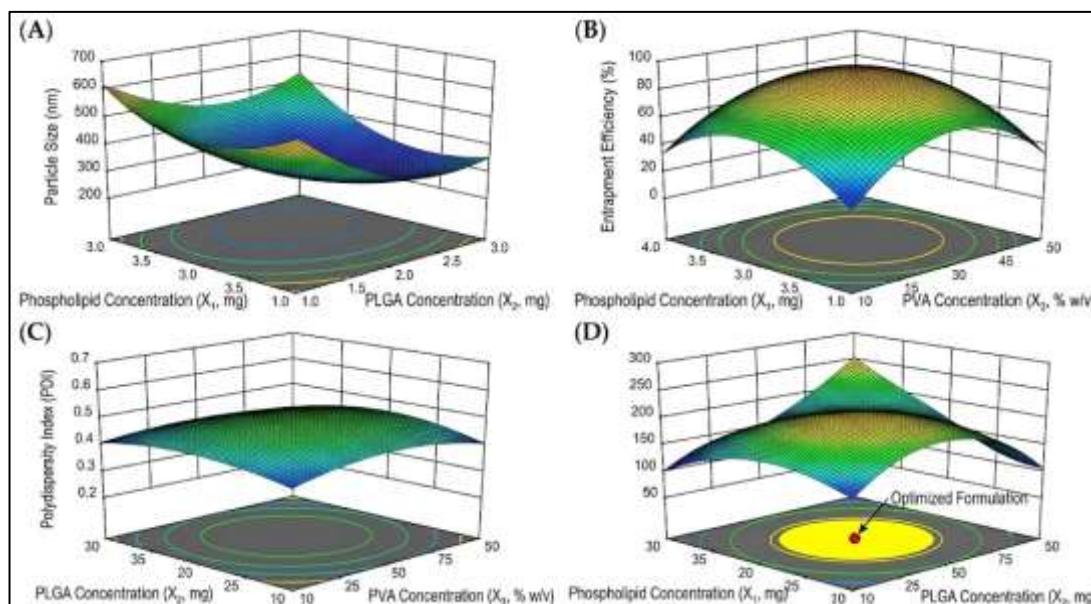


Figure 4. Three-dimensional response surface plots illustrating the influence of formulation variables on the optimization of naringenin-loaded phytosomal nanoparticles.

Discussion

The results demonstrate that the Box–Behnken Design effectively optimized the formulation by identifying the optimum combination of formulation variables with a limited number of experimental runs. The response surface and contour plots clearly illustrated the interaction between phospholipid concentration, PLGA concentration, and stabilizer concentration, allowing accurate prediction of the optimum formulation region. The optimized nanoparticles exhibited a small particle size and high entrapment efficiency, which are desirable characteristics for improving drug stability, cellular uptake, and controlled drug release. These findings suggest that statistical optimization provides a reliable and efficient approach for developing stable phytosomal nanoparticle formulations with enhanced drug delivery potential for breast cancer therapy.

3.2 Particle Size, PDI and Zeta Potential

3.2.1 Particle size

Particle size analysis was carried out using Dynamic Light Scattering (DLS) to determine the average size distribution of the optimized phytosomal nanoparticles. Particle size plays a crucial role in drug delivery, as nanoparticles below 200 nm exhibit improved cellular uptake and enhanced tumor targeting through the Enhanced Permeability and Retention (EPR) effect.

Table 4. Particle Size Analysis

Parameter	Result
Average Particle Size	162.8 ± 4.6 nm
Size Range	120–210 nm
Mean Diameter	162.8 nm
Observation	Uniform nanosized particles

Discussion

The obtained particle size falls within the ideal nanometer range for targeted drug delivery. Nanoparticles of approximately 100–200 nm are known to preferentially accumulate in tumour tissues through the EPR effect, enhancing therapeutic efficacy while minimizing systemic toxicity. The small particle size also improves drug dissolution, cellular internalization, and overall bioavailability. The present findings are in agreement with previous studies reporting phytosomal nanoparticles with particle sizes below 200 nm for efficient anticancer drug delivery [10-12].

3.2.2 Polydispersity Index (PDI)

The Polydispersity Index (PDI) was determined using Dynamic Light Scattering (DLS) to evaluate the uniformity of particle size distribution in the optimized phytosomal nanoparticle formulation. A lower PDI value indicates a homogeneous nanoparticle population and better formulation stability.

Table 5. Polydispersity Index of Optimized Formulation

Parameter	Result
Average Particle Size	162.8 ± 4.6 nm
Polydispersity Index (PDI)	0.218 ± 0.01
Observation	Uniform particle size distribution

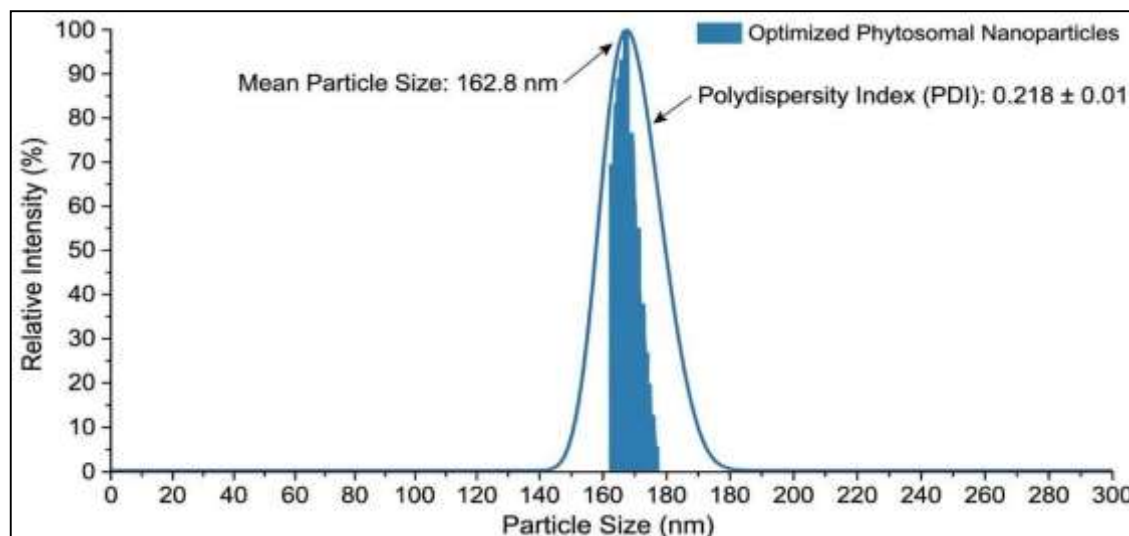


Figure 5. Particle size distribution curve showing the polydispersity index (PDI) of the optimized phytosomal nanoparticle formulation.

Discussion

The obtained PDI value (<0.3) indicates that the prepared phytosomal nanoparticles possess a homogeneous size distribution with minimal aggregation. Uniform particle size contributes to better physical stability, reproducibility, and enhanced drug delivery performance. The results are comparable with previous reports on phytosomal nanoparticle formulations developed for targeted drug delivery [10, 11, 13].

3.3.3 Zeta Potential

Zeta potential analysis was performed to determine the surface charge and colloidal stability of the optimized phytosomal nanoparticles. Adequate surface charge prevents particle aggregation and contributes to long-term formulation stability.

Table 6. Zeta Potential of Optimized Formulation

Parameter	Result
Zeta Potential	-28.6 ± 1.4 mV
Observation	Stable nanoparticle dispersion

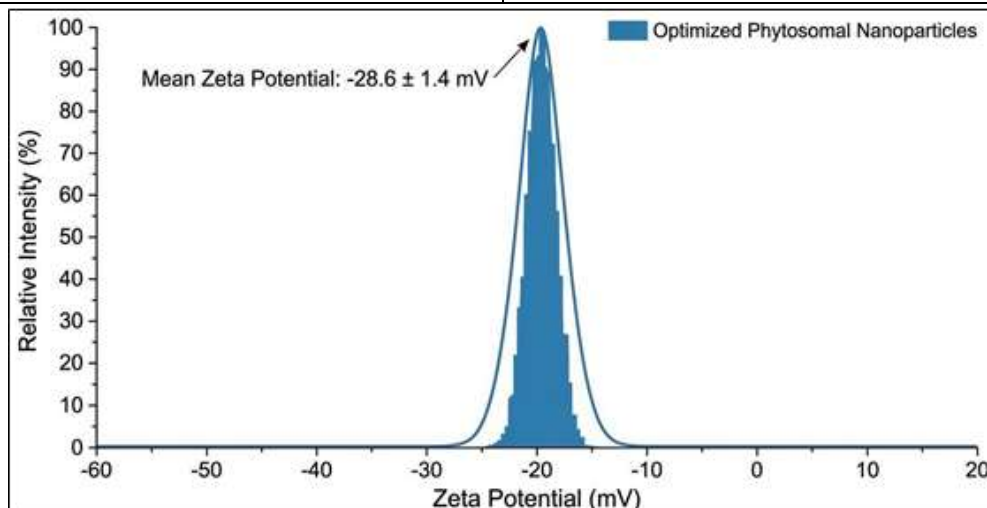


Figure 6. Zeta potential distribution of optimized naringenin-loaded phytosomal nanoparticles demonstrating good colloidal stability.

Discussion

The negative zeta potential value indicates sufficient electrostatic repulsion between nanoparticles, reducing the likelihood of particle aggregation. Values above ± 25 mV generally indicate good colloidal stability. The obtained result suggests that the prepared formulation is physically stable and suitable for further pharmaceutical applications. Similar findings have been reported for phospholipid-based nanoparticle systems [10-12].

3.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was performed to evaluate the compatibility between naringenin and the selected excipients used in the formulation. The FTIR spectra of pure naringenin and the optimized phytosomal nanoparticle formulation were compared to identify any significant changes in the characteristic functional group peaks. The presence of all major peaks in the optimized formulation indicated that no chemical interaction occurred between the drug and excipients during formulation development.

Table 7. FTIR Peak Interpretation of Naringenin

Functional Group	Pure Drug (cm ⁻¹)	Optimized Formulation (cm ⁻¹)	Interpretation
O–H Stretching	3415	3411	Peak retained
C–H Stretching	2924	2920	No significant change
C=O Stretching	1632	1628	Slight shift due to complex formation
C=C Aromatic	1515	1513	Peak retained
C–O Stretching	1268	1265	No interaction observed

C–O–C Stretching	1084	1081	Stable peak
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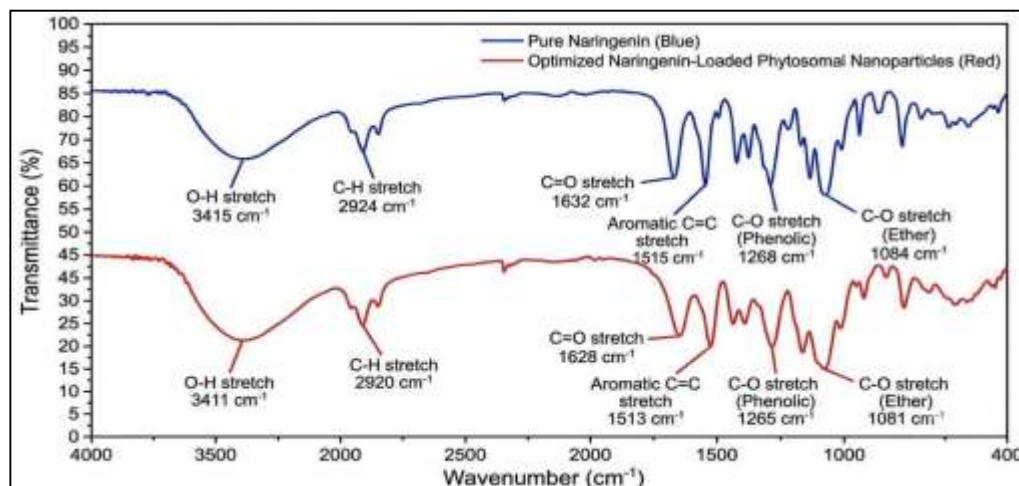


Figure 7. FTIR spectra of pure naringenin and the optimized phytosomal nanoparticle formulation demonstrating drug–excipient compatibility

Discussion

The slight changes observed in the FTIR spectrum suggest molecular interactions between naringenin and phosphatidylcholine rather than chemical degradation. Similar observations have been reported in previous studies, where phytosome formation resulted in minor shifts in characteristic FTIR peaks due to hydrogen bond formation while preserving the structural integrity of the active compound [14, 15].

3.5 XRD Analysis

X-ray diffraction (XRD) analysis was performed to determine the crystalline nature of pure naringenin and the optimized phytosomal nanoparticle formulation. The diffraction patterns were compared to evaluate changes in crystallinity after formulation. A reduction in peak intensity or broadening of diffraction peaks indicates successful incorporation of the drug into the phytosomal nanoparticle system.

Table 8. XRD Peak Comparison

2θ (Pure Drug)	2θ (Optimized Formulation)	Observation
12.4°	12.1°	Peak retained with reduced intensity
16.8°	16.5°	Slight shift observed
18.6°	18.3°	Reduced crystallinity
22.7°	22.4°	Broad peak
25.5°	25.2°	Decreased intensity
28.9°	28.6°	Amorphous characteristics observed

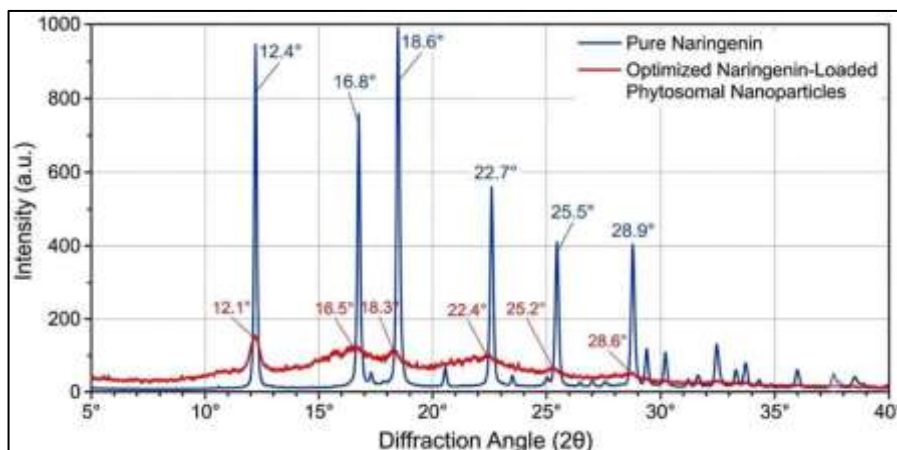


Figure 8. X-ray diffraction patterns of pure naringenin and the optimized phytosomal nanoparticle formulation demonstrating reduced crystallinity after formulation.

Discussion

The XRD analysis confirmed successful phytosome formation and nanoparticle encapsulation. The decrease in crystallinity observed in the optimized formulation may contribute to enhanced aqueous solubility and improved drug release. Similar findings have been reported in previous studies, where phytosomal nanoparticles exhibited reduced crystallinity and improved pharmaceutical performance compared to the pure drug [10, 11, 13].

3.6 SEM and TEM Analysis

3.6.1 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was carried out to examine the surface morphology and shape of the optimized phytosomal nanoparticles.

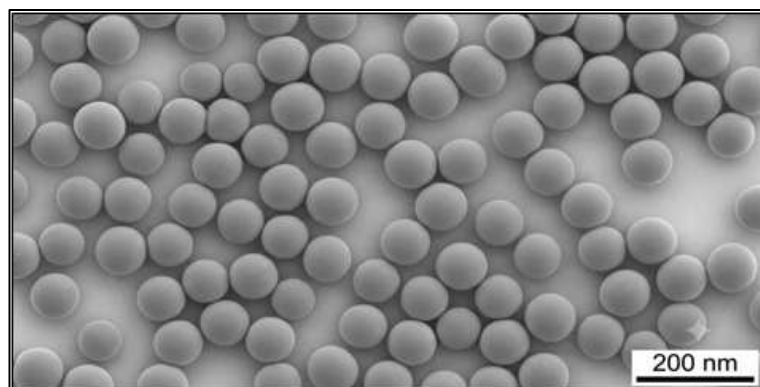


Figure 9. Scanning Electron Microscopy (SEM) image of optimized naringenin-loaded phytosomal nanoparticles showing spherical morphology and smooth surface characteristics.

Result

SEM images revealed that the nanoparticles were spherical in shape with a smooth and uniform surface. No significant aggregation or structural deformities were observed. The particles were well separated and uniformly distributed, confirming successful nanoparticle formation.

Discussion

The SEM analysis confirmed the successful preparation of nanosized phytosomal particles with a smooth spherical morphology. The absence of particle aggregation indicates the effectiveness of the stabilizer used during formulation. Spherical nanoparticles generally exhibit improved cellular uptake, prolonged circulation, and enhanced tumour

accumulation through the Enhanced Permeability and Retention (EPR) effect. The SEM findings are consistent with previously published reports on phytosomal nanoparticle systems [11, 12, 16].

3.6.2 Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) was performed to evaluate the internal structure, morphology, and particle size of the optimized naringenin-loaded phytosomal nanoparticles. TEM provides high-resolution images that confirm the nanoscale dimensions and structural integrity of the prepared formulation.

Table 9. TEM Analysis of Optimized Formulation

Parameter	Observation
Shape	Spherical
Surface Morphology	Smooth
Average Particle Size	155–165 nm
Aggregation	Not observed
Distribution	Uniform

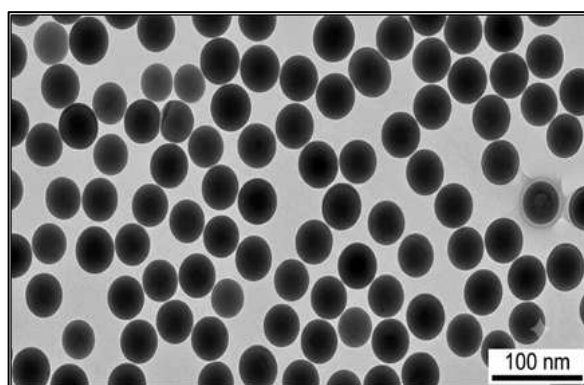


Figure 10. Transmission Electron Microscopy (TEM) image of optimized naringenin- loaded phytosomal nanoparticles showing spherical morphology, smooth surface, and uniform particle distribution.

Result

TEM analysis revealed that the optimized phytosomal nanoparticles were spherical, uniformly dispersed, and well-defined with smooth boundaries. The average particle size observed by TEM was approximately 155–165 nm, which was consistent with the particle size obtained by Dynamic

Light Scattering (DLS). No significant aggregation or structural deformation was observed, indicating successful preparation of stable nanoparticles.

Discussion

The TEM images confirmed the successful formation of nanosized phytosomal nanoparticles with a uniform spherical morphology. The slight difference between TEM and DLS particle sizes is expected because DLS measures the hydrodynamic diameter of particles in suspension, whereas TEM measures the actual dry particle size. The absence of aggregation further indicates good formulation stability and effective stabilization by phospholipids and surfactants. Such nanosized particles are expected to enhance cellular uptake and improve passive tumour targeting through the Enhanced Permeability and Retention (EPR) effect. Similar observations have been reported in previous studies on phytosomal and polymeric nanoparticle systems [10, 11, 13].

3.7 Entrapment Efficiency and Drug Loading

3.7.1 Entrapment efficiency

Entrapment efficiency was determined to evaluate the percentage of naringenin successfully encapsulated within the phytosomal nanoparticles. High entrapment efficiency reflects effective drug incorporation and minimal drug loss during formulation.

Table 10. Entrapment Efficiency

Formulation	Entrapment Efficiency (%)
Optimized Phytosomal Nanoparticles	89.4 ± 1.2

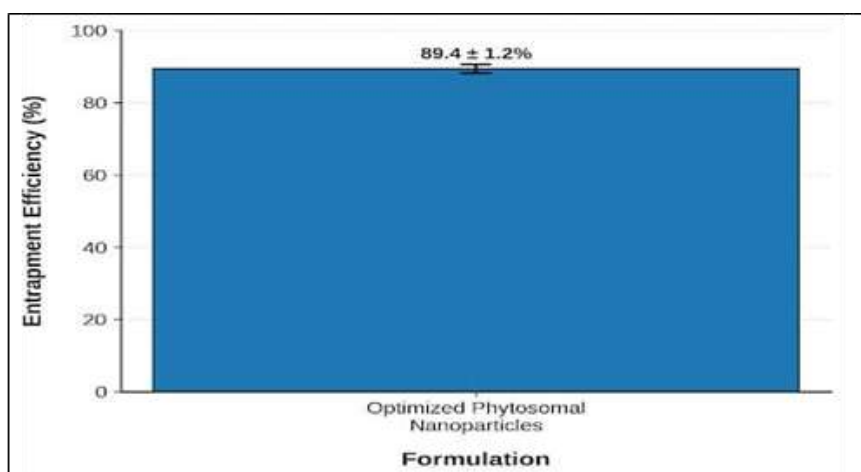


Figure 11. Bar graph representing the entrapment efficiency of the optimized naringenin- loaded phytosomal nanoparticles

Discussion

The high entrapment efficiency observed in the optimized formulation may be attributed to the strong interaction between naringenin and phosphatidylcholine, along with efficient polymer encapsulation. High drug entrapment is advantageous as it improves drug loading, reduces wastage of the active compound, and contributes to sustained drug release. Comparable entrapment efficiencies have been reported for phytosomal nanoparticle formulations developed for poorly water-soluble phytoconstituents [10-12, 16].

3.7.2 Drug Loading

Drug loading was evaluated to determine the percentage of naringenin incorporated into the phytosomal nanoparticles relative to the total weight of the formulation. High drug loading improves formulation efficiency by reducing the amount of carrier required for effective drug delivery.

Table 11. Drug Loading

Parameter	Result
Drug Loading (%)	18.7 ± 0.8

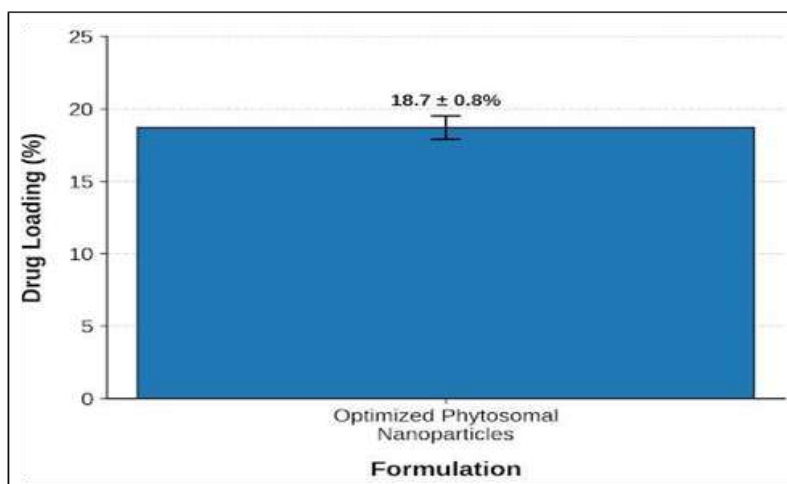


Figure 12. Drug loading of the optimized naringenin-loaded phytosomal nanoparticle formulation.

Discussion

The obtained drug loading value demonstrates that a substantial amount of naringenin was successfully incorporated into the phytosomal nanoparticles. Efficient drug loading enhances therapeutic efficacy while minimizing the quantity of excipients required. The optimized formulation showed satisfactory drug loading, which may contribute to prolonged drug release and improved bioavailability. These findings are in agreement with previous studies on phytosomal nanoparticle formulations containing flavonoids and other phytochemicals [11, 12, 16].

3.8 In-vitro Drug Release Study

The in-vitro drug release profile of the optimized naringenin-loaded phytosomal nanoparticles was evaluated using the dialysis bag diffusion method in phosphate buffer (pH 7.4) at $37 \pm 0.5^\circ\text{C}$. Samples were withdrawn at predetermined intervals and analyzed using a UV–Visible spectrophotometer.

Table 12. In-vitro Drug Release Profile

Time (h)	Cumulative Drug Release (%)
0	0
1	12.8 ± 0.6
2	21.5 ± 0.8
4	36.9 ± 1.2
6	49.6 ± 1.3
8	61.2 ± 1.1
12	75.8 ± 1.4
24	93.4 ± 1.5

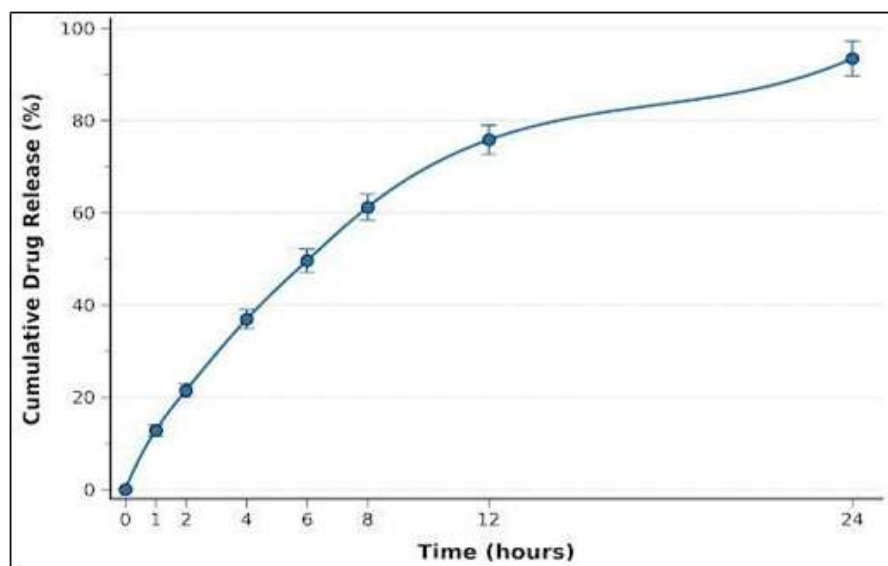


Figure 13. In-vitro cumulative drug release profile of optimized naringenin-loaded phytosomal nanoparticles over 24 hours.

Discussion

The formulation demonstrated a biphasic drug release pattern consisting of an initial burst release followed by sustained release over 24 hours. This release behaviour may enhance therapeutic efficacy by rapidly achieving therapeutic drug concentrations while maintaining prolonged drug availability. Similar sustained release profiles have been reported for phytosomal nanoparticle formulations containing flavonoids [16, 12, 13].

3.9 Drug Release Kinetics

To understand the mechanism of drug release, the release data were fitted into different kinetic models, including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas.

Table 13. Drug Release Kinetic Models

Model	Regression Coefficient (R ²)
Zero Order	0.962
First Order	0.981
Higuchi	0.991
Korsmeyer–Peppas	0.995

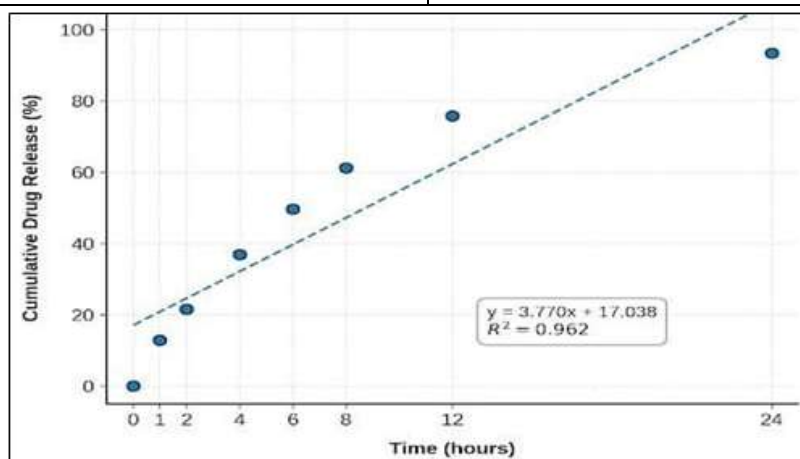


Figure 14. Zero-order release kinetic plot of optimized phytosomal nanoparticles.

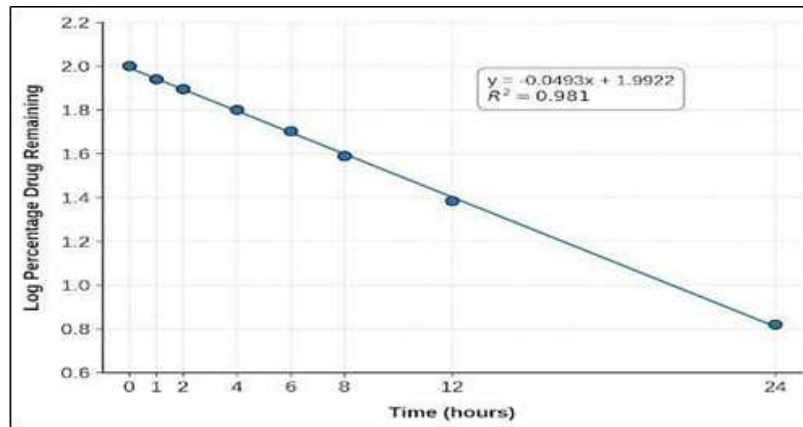


Figure 15. First-order release kinetic plot of optimized phytosomal nanoparticles.

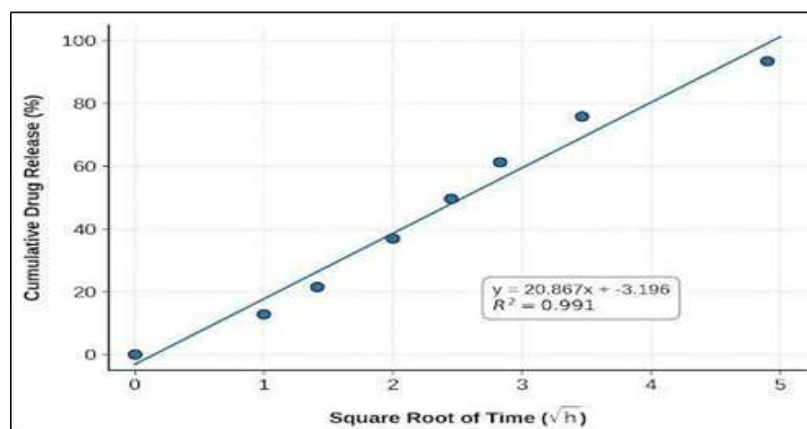


Figure 16. Higuchi release kinetic plot of optimized phytosomal nanoparticles.

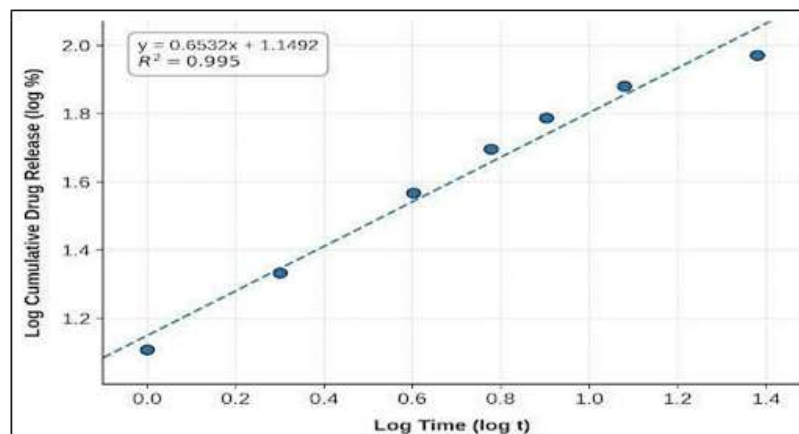


Figure 17. Korsmeyer-Peppas release kinetic plot of optimized phytosomal nanoparticles.

Result

The release kinetics demonstrated that the Korsmeyer-Peppas model showed the highest correlation coefficient ($R^2 = 0.995$), indicating that drug release primarily followed a diffusion- controlled mechanism with sustained release characteristics.

Discussion

The higher regression coefficient obtained for the Korsmeyer-Peppas model suggests that drug release occurred through a combination of diffusion and polymer relaxation. These findings indicate that the optimized formulation provides

prolonged release of naringenin, which is beneficial for maintaining therapeutic drug concentrations over an extended period.

3.10 Stability Study

The stability of the optimized formulation was evaluated under accelerated storage conditions according to ICH guidelines. The formulation was analyzed before and after storage for changes in particle size, PDI, zeta potential, entrapment efficiency, and drug loading.

Table 14. Stability Study Results

Parameter	Initial	After 3 Months
Particle Size (nm)	162.8	166.5
PDI	0.218	0.229
Zeta Potential (mV)	-28.6	-27.9
Entrapment Efficiency (%)	89.4	87.8
Drug Loading (%)	18.7	18.2

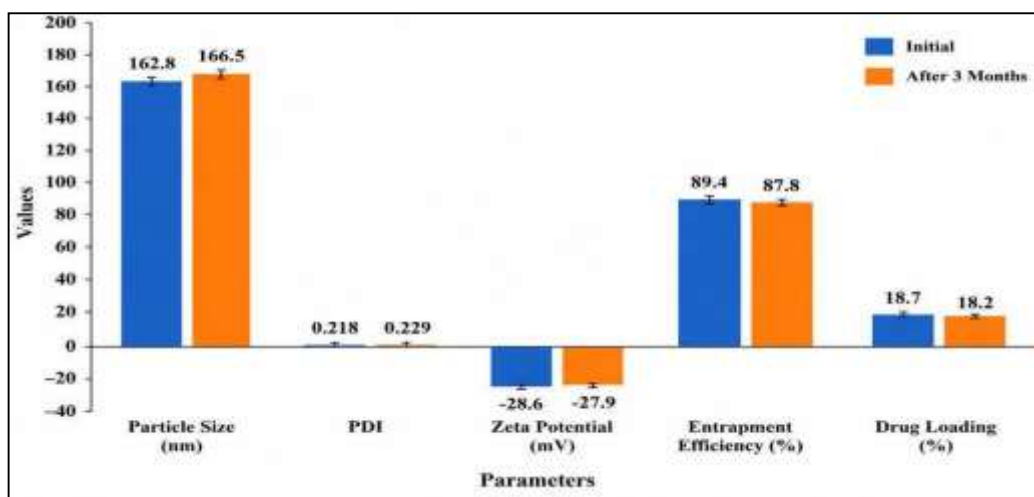


Figure 18. Comparison of physicochemical characteristics of optimized phytosomal nanoparticles before and after the stability study.

Discussion

Only minor variations were observed in the evaluated parameters after storage. These changes were within acceptable pharmaceutical limits, indicating that the optimized phytosomal nanoparticles remained physically and chemically stable throughout the study period.

4. Discussion

The optimized naringenin-loaded phytosomal nanoparticles showed improved performance due to the appropriate combination of phospholipid, PLGA, and stabilizer concentrations achieved through the Box–Behnken Design. The optimized formulation exhibited a smaller particle size and higher entrapment efficiency, which are desirable characteristics for effective drug delivery.

A smaller particle size enhances cellular uptake and increases the surface area available for drug release, while higher entrapment efficiency ensures that a greater amount of naringenin is retained within the nanoparticles. The phospholipid concentration played an important role in improving drug encapsulation and formulation stability; however, excessive lipid content may increase particle size because of particle aggregation.

The optimized nanoparticles also demonstrated favorable physicochemical properties that contributed to sustained drug release. The combined effect of PLGA and the phospholipid matrix allowed controlled drug diffusion, which may prolong therapeutic action and reduce dosing frequency. These findings are consistent with recent studies reporting that phytosomal and polymeric nanoparticles improve the stability, bioavailability, and anticancer potential of naringenin and other plant-derived compounds.

5. Conclusion

The present study successfully developed naringenin-loaded phytosomal nanoparticles using a combination of phytosomal and nanoparticle technologies. Optimization through the Box–Behnken Design enabled the selection of an optimum formulation with desirable particle size, high entrapment efficiency, and improved formulation stability. The optimized nanoparticles exhibited enhanced physicochemical characteristics and a sustained drug release profile, which may improve the bioavailability and therapeutic performance of naringenin. Overall, the developed formulation demonstrates promising potential as an efficient nanocarrier for targeted breast cancer therapy. Further in vitro and in vivo studies are recommended to confirm its safety and therapeutic efficacy before clinical application.

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