

Development, Optimization and In-Vitro Evaluation of Hesperidin-Loaded Phytosomes for Enhanced Solubility and Drug Release

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ABSTRACT

Objective: The present study aimed to develop and optimize a phospholipid-based phytosomal formulation of hesperidin to overcome its poor aqueous solubility and to evaluate the optimized formulation using in-vitro physicochemical and drug-release studies.

Design: Hesperidin phytosomes were prepared by solvent evaporation, salting-out and lyophilization methods. Lyophilization was selected for further development. A three-level factorial randomized quadratic design was employed using hesperidin:soybean phosphatidylcholine (SPC) ratio and mannitol concentration as independent variables, while particle size and drug content were used as responses.

Interventions: The optimized formulation was characterized for particle size, polydispersity index, zeta potential, drug content, DSC, FTIR, SEM, TEM, AFM, XRD, saturation solubility, partition coefficient, in-vitro drug release and release kinetics. DPPH radical-scavenging activity was also assessed as an in-vitro functional evaluation.

Main outcome measures: The primary formulation outcomes were particle size and drug content; secondary outcomes included colloidal characteristics, solid-state properties, solubility enhancement, cumulative drug release and release mechanism.

Results: The quadratic model was significant for particle size ($F=548.36$, $p=0.0001$). The optimized hesperidin phytosomes showed a particle size of 278.42 ± 2.65 nm, PDI of 0.389 ± 0.03 , zeta potential of -30.4 ± 0.12 mV and drug content of $92.16 \pm 0.28\%$ w/w. Aqueous solubility increased from 2.14 ± 0.18 $\mu\text{g/mL}$ for pure hesperidin to 48.92 ± 0.24 $\mu\text{g/mL}$ after phytosome formation. Cumulative drug release at 24 h increased from $22.48 \pm 1.04\%$ for pure hesperidin to $78.60 \pm 1.08\%$ for optimized phytosomes. Release data showed the highest fit to the Higuchi model ($R^2=0.9943$), while the Korsmeyer–Peppas exponent ($n=0.8268$) indicated anomalous/non-Fickian transport. At 50 $\mu\text{g/mL}$, DPPH inhibition increased from $31.42 \pm 0.84\%$ for pure hesperidin to $33.18 \pm 1.16\%$ for the optimized phytosomes.

Conclusion: The optimized lyophilized hesperidin phytosomes demonstrated improved aqueous solubility, favorable nanoscale characteristics and markedly enhanced sustained in-vitro drug release compared with pure hesperidin. The formulation strategy therefore provides a rational platform for improving the pharmaceutical performance of poorly water-soluble hesperidin.

Keywords: Hesperidin; phytosomes; phospholipid complex; soybean phosphatidylcholine; lyophilization; design of experiments; solubility; drug release.

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INTRODUCTION

Hesperidin is a citrus-derived flavanone glycoside belonging to the flavonoid class and is composed of hesperetin linked to a rutinoside moiety. It has been investigated for antioxidant, anti-inflammatory, cardioprotective and other biological activities.^{1–3} However, the pharmaceutical development of hesperidin is constrained by its poor aqueous solubility and limited biopharmaceutical performance. The low aqueous solubility of flavonoids can restrict dissolution in gastrointestinal fluids and consequently reduce the amount available for absorption.^{4,5}

Phospholipid complexation, commonly referred to as phytosome technology, has been developed as a strategy for improving the delivery of plant-derived polyphenols. In a phytosomal system, the active phytoconstituent interacts with phospholipids, particularly phosphatidylcholine, producing a lipid-compatible

complex. This approach can alter the physicochemical environment of the active compound and improve its dispersion, membrane compatibility and delivery characteristics.^{6,7}

Previous studies have demonstrated the feasibility of hesperidin–phospholipid systems. Kalita and Patwary reported improved solubility, partition coefficient and drug release for a hesperidin–phospholipid complex, together with concentration-dependent antioxidant activity.⁸ Studies on related citrus flavonoid–phospholipid complexes have also demonstrated sustained release and improved functional performance.⁹ These findings support the use of phospholipid complexation as a formulation strategy for poorly soluble flavonoids.

The present work was designed to develop hesperidin phytosomes using soybean phosphatidylcholine (SPC), select a suitable manufacturing process, and optimize

critical formulation variables by design of experiments (DoE). The optimized formulation was subsequently subjected to comprehensive in-vitro evaluation, including nanoscale characterization, solid-state characterization, solubility, in-vitro release, release kinetics and antioxidant screening. The study specifically focuses on formulation development and in-vitro performance rather than in-vivo pharmacological or pharmacokinetic evaluation.

OBJECTIVES

1. To develop hesperidin-loaded phytosomes using soybean phosphatidylcholine.
2. To select a suitable preparation method and optimize the formulation using a three-level factorial randomized quadratic design.
3. To characterize the optimized formulation using physicochemical, morphological and solid-state techniques.
4. To determine the effect of phytosome formation on hesperidin solubility, in-vitro drug release and release kinetics.
5. To compare the in-vitro antioxidant activity of pure hesperidin and optimized hesperidin phytosomes.

MATERIALS AND METHODS

Materials

Hesperidin was obtained from Otto Chemie, Mumbai, India. Soybean phosphatidylcholine (Lipoid® S 100) was obtained from Lipoid GmbH, Ludwigshafen, Germany. Methanol, ethanol, chloroform, dimethyl sulfoxide (DMSO), t-butyl alcohol, n-hexane and other analytical-grade reagents were procured from commercial suppliers. DPPH and ascorbic acid were obtained from HiMedia Laboratories, Mumbai, India. All solvents and reagents used were of analytical or HPLC grade.

Preformulation studies

The organoleptic properties, melting point, UV absorption maximum and qualitative solubility of hesperidin were evaluated. The melting point was determined using a digital melting point apparatus. For UV analysis, hesperidin solutions were scanned over 200–400 nm in acidic buffer pH 1.2 and phosphate buffer pH 7.4. Calibration standards were prepared at 5–30 µg/mL and analyzed at the respective absorption maxima.

Preliminary preparation of hesperidin phytosomes

Three preparation approaches were investigated at a hesperidin:SPC ratio of 1:2: solvent evaporation, salting-out and lyophilization. For solvent evaporation, hesperidin and SPC were refluxed in dioxane:methanol (7:3) for 5 h at 45 ± 5 °C and the solvent was removed under reduced pressure. For salting-out, hesperidin and SPC were dissolved in DMSO:dehydrated ethanol:chloroform (2:2:3), stirred overnight and precipitated by gradual addition of n-hexane. For lyophilization, hesperidin was dissolved in DMSO (2.5% w/v) and SPC in t-butyl alcohol (1.5% w/v). The solutions were combined and stirred for 3 h, frozen at –80 °C for 4 h, and lyophilized at a condenser temperature of –70 °C.

Primary drying was performed at 40 mbar and –40 °C for 24 h followed by secondary drying at 25 °C for 24 h. The lyophilized product was selected based on physical appearance, dryness, ease of handling and overall suitability.

Formulation development and DoE optimization

Lyophilized hesperidin phytosomes were optimized using Design-Expert® software (version 11.0.3.0). A three-level factorial randomized quadratic design comprising nine experimental runs was used. Factor A was hesperidin:SPC ratio (1:1, 1:2 and 1:3 w:w), and factor B was mannitol concentration (0.5, 1.0 and 1.5% w/v). Particle size (R1) and drug content (R2) were selected as dependent variables. Mannitol was incorporated as a cryoprotectant, and the resulting dispersion was probe-sonicated for 3 min before freezing and lyophilization. The quadratic model was assessed using analysis of variance (ANOVA), model summary statistics and regression coefficients. Numerical optimization was used to identify the formulation with desirable particle size and high drug content.

Particle size, PDI and zeta potential

Particle size and PDI were determined by dynamic light scattering after dilution of the formulation with distilled water. Zeta potential was measured by electrophoretic light scattering using a Malvern Zeta Nano Sizer. Measurements were performed at 25 ± 0.5 °C and expressed as mean $\pm$ SD.

Drug content

Drug incorporation was determined by a chloroform-based separation method. An accurately weighed quantity of formulation equivalent to approximately 10 mg hesperidin was dispersed in chloroform. The free/unincorporated drug was separated by filtration, dissolved in methanol and quantified spectrophotometrically at 285 nm. Drug content was calculated as the percentage of drug incorporated relative to the amount initially added.

Differential scanning calorimetry

Thermal behavior was evaluated using DSC from 25 to 500 °C at a heating rate of 10 °C/min under nitrogen. Pure hesperidin, SPC, the physical mixture and optimized phytosomes were compared for melting endotherms, peak broadening, shifting and intensity changes.

FTIR spectroscopy

FTIR spectra were recorded by ATR over 4000–500 cm^{–1} for pure hesperidin, SPC, the physical mixture and optimized phytosomes. Changes in characteristic functional-group bands were evaluated to assess compatibility and possible intermolecular interactions.

Morphological characterization

SEM, TEM and AFM were used to assess morphology. SEM was used to observe surface characteristics; TEM was used to visualize nanoscale morphology and vesicular structures; and AFM was used to evaluate surface topography.

X-ray powder diffraction

XRD patterns of pure hesperidin, SPC and optimized phytosomes were recorded using Cu K α radiation over 0–50° (2 θ). Changes in peak sharpness and intensity were evaluated to assess changes in crystallinity.

Saturation solubility and partition coefficient

Saturation solubility was evaluated in water and n-octanol at 37 °C. Excess sample was equilibrated with 10 mL of solvent for 24 h, followed by centrifugation and filtration. Hesperidin concentration was quantified spectrophotometrically at 285 nm. The partition coefficient was determined using n-octanol/phosphate buffer pH 7.4 and calculated as $K = C_1/C_2$, where C_1 and C_2 represent the equilibrium concentrations in the organic and aqueous phases, respectively.

In-vitro drug release

In-vitro release was evaluated using a dialysis-bag diffusion method. Samples equivalent to approximately 10 mg hesperidin were placed in pretreated dialysis bags and suspended in 500 mL release medium maintained at 37 ± 0.5 °C with stirring at 100 rpm. Acidic buffer pH 1.2 was used for the first 2 h, followed by phosphate buffer pH 7.4 from 2 to 24 h. At predetermined intervals, 5 mL samples were withdrawn and replaced with fresh medium. Hesperidin was quantified at 285 nm and cumulative percentage drug release (%CDR) was calculated.

Drug release kinetics

Release data were fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models. The model with the highest coefficient of determination (R^2) was considered the best fit. The Korsmeyer–Peppas release exponent (n) was used to infer the predominant release mechanism.

In-vitro antioxidant activity

DPPH radical-scavenging activity was evaluated for pure hesperidin, SPC and optimized hesperidin phytosomes using ascorbic acid as the reference standard. Samples were evaluated at 10–50 μ g/mL. Absorbance was measured at 517 nm and percentage inhibition was calculated using: % inhibition = [(Absorbance of control – Absorbance of sample)/Absorbance of control] $\times$ 100.

Statistical analysis

DoE responses were analyzed by quadratic regression and ANOVA. Experimental results are presented as mean $\pm$ SD where applicable. Model significance was interpreted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Preformulation characteristics

Hesperidin was obtained as a pale, crystalline powder. The observed melting point was 252 ± 1 °C. The absorption maximum was 285 nm in acidic buffer pH 1.2 and 286 nm in phosphate buffer pH 7.4. Qualitative solubility testing showed poor solubility in water and both buffer media, while clearer solutions were obtained in DMSO and DMF. These findings confirmed the poor aqueous solubility that justified formulation development.

Parameter	Observation
Appearance	Pale crystalline powder
Melting point	252 ± 1 °C
λ_{max} , pH 1.2	285 nm
λ_{max} , pH 7.4	286 nm
Water	Poorly soluble
Acidic buffer pH 1.2	Poorly soluble
Phosphate buffer pH 7.4	Poorly soluble
Methanol / ethanol	Slightly soluble
DMSO / DMF	Soluble; clear solution formed

Table 1. Preformulation characteristics of hesperidin.

Selection of preparation method

All three preliminary methods produced hesperidin–SPC complexes, but the lyophilization process produced a comparatively dry and uniform product, whereas solvent-evaporated and salting-out products showed greater stickiness and aggregation. Lyophilization was therefore selected for subsequent formulation development. The selection is consistent with the use of freeze-drying for phospholipid-based phytosomal systems, where solvent removal under controlled low-temperature conditions can facilitate formation of a dry, readily dispersible product.^{6,7}

DoE optimization of hesperidin phytosomes

The nine experimental batches showed particle sizes from 281.8 to 495.6 nm and drug content from 78.86 to 94.12% w/w. Batch 9, prepared at the central coded levels, showed the smallest measured particle size (281.8 nm) and the highest observed drug content (94.12% w/w) among the nine experimental runs. The model-based numerical optimization subsequently selected the formulation whose experimentally observed particle size and drug content were 278.42 ± 2.65 nm and $92.16 \pm 0.28\%$ w/w, respectively.

Ru n	Hesperidin:SP C	Mannito l (% w/v)	Particl e size (nm)	Drug conten t (% w/w)
1	Low (–1)	1.0 (0)	462.8	81.74
2	High (+1)	1.5 (+1)	348.3	83.25
3	Low (–1)	1.5 (+1)	495.6	78.86
4	High (+1)	0.5 (–1)	329.4	86.18
5	Low (–1)	0.5 (–1)	478.1	80.42

6	High (+1)	1.0 (0)	307.5	88.74
7	Medium (0)	0.5 (-1)	292.6	91.35
8	Medium (0)	1.5 (+1)	304.2	89.41
9	Medium (0)	1.0 (0)	281.8	94.12

Table 2. Experimental design batches and observed responses for hesperidin phytosomes.

Model adequacy and factor effects

For particle size, the quadratic model was suggested by the sequential model comparison and was highly significant ($F = 584.12$, $p = 0.0001$). The model summary showed $R^2 = 0.9991$, adjusted $R^2 = 0.9973$ and predicted $R^2 = 0.9912$, indicating strong agreement between the model and experimental data. In the ANOVA, the hesperidin:SPC ratio was the dominant factor ($F = 1518.42$, $p < 0.0001$), followed by the quadratic terms A^2 ($F = 1126.57$, $p < 0.0001$) and B^2 ($F = 58.82$, $p = 0.0043$). Mannitol also showed a significant linear effect ($F = 17.26$, $p = 0.0248$), whereas the AB interaction was not significant ($p = 0.7442$).

The coded-factor regression equation for particle size was: Particle size = $276.34 - 77.12A + 8.24B + 0.95AB + 114.26A^2 + 27.42B^2$. The negative coefficient for factor A indicates that increasing the hesperidin:SPC ratio from the low level initially reduced particle size, whereas the positive quadratic term indicates curvature in the response. Mannitol had a comparatively smaller positive effect on particle size.

For drug content, the coded-factor model was represented by: Drug content = $93.4821 + 2.3567A - 0.9542B - 0.5480AB - 7.8233A^2 - 3.46B^2$. The positive linear coefficient for factor A indicates an increase in drug content with increasing hesperidin:SPC ratio within the studied region, followed by a decline reflected by the negative quadratic term. Mannitol exerted a smaller negative linear effect. The absence of strong interaction between the two factors indicates that their individual effects were more influential than the combined AB term. Such response-surface optimization is consistent with the broader application of DoE for pharmaceutical formulation development.^{10,11}

Response	Predicted mean	Observed value	R^2 / key statistic	Interpretation
Particle size	266.214 nm	278.42 ± 2.65 nm	$R^2=0.9991$; adj. $R^2=0.9973$; pred. $R^2=0.9912$	Excellent model fit
Drug content	93.4821 %	$92.16 \pm 0.28\%$	Observed value within prediction	Good model agreement

			n interval	
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Table 3. Model prediction and experimental confirmation for the optimized hesperidin phytosomes.

In-vitro characterization of optimized phytosomes

The optimized formulation exhibited a mean particle size of 278.42 ± 2.65 nm, PDI of 0.389 ± 0.03 and zeta potential of -30.4 ± 0.12 mV. The relatively low PDI indicates a comparatively uniform particle-size distribution, while the negative surface charge is consistent with the phospholipid-containing formulation. The drug content was $92.16 \pm 0.28\%$ w/w, indicating efficient incorporation of hesperidin into the phytosomal system.

Parameter	Optimized hesperidin phytosomes
Particle size	278.42 ± 2.65 nm
PDI	0.389 ± 0.03
Zeta potential	-30.4 ± 0.12 mV
Drug content	$92.16 \pm 0.28\%$ w/w

Table 4. In-vitro physicochemical characteristics of optimized hesperidin phytosomes.

DSC and FTIR characterization

Pure hesperidin displayed a sharp endothermic peak around 253°C , characteristic of its crystalline nature. In the optimized phytosomal formulation, the hesperidin endotherm was shifted, broadened and reduced in intensity. These changes suggest a reduction in crystallinity and interaction of hesperidin with the phospholipid matrix. Similar thermal changes have been reported for phospholipid complexes of flavonoids.^{6,8} FTIR analysis showed characteristic hesperidin bands corresponding to hydroxyl, carbonyl, aromatic C=C and ether groups. In the optimized phytosomes, broadening and slight shifts of the hydroxyl band and changes in the phosphate/alkyl regions of SPC were observed. The retention of the major characteristic bands together with modest spectral shifts suggests intermolecular association between hesperidin and SPC rather than formation of a new covalent chemical entity.

Morphological and solid-state characterization

SEM showed crystalline particles for pure hesperidin and an irregular, porous and aggregated morphology for the optimized phytosomes. TEM showed discrete, nearly spherical nanoscale structures without obvious decomposition or extensive aggregation. AFM demonstrated rough and porous surface features with relatively uniform hydrated structures. XRD showed sharp crystalline peaks for pure hesperidin, whereas the optimized phytosomes displayed broad and diffuse diffraction features, indicating a reduction in drug crystallinity. The combined DSC, FTIR, microscopy and

XRD findings support successful formation of the hesperidin–SPC phytosomal complex.

Solubility and partition coefficient

Phytosome formation produced a marked improvement in hesperidin aqueous solubility. Pure hesperidin showed an aqueous solubility of 2.14 ± 0.18 $\mu\text{g/mL}$, whereas the optimized phytosomes showed 48.92 ± 0.24 $\mu\text{g/mL}$, corresponding to an approximately 22.9-fold increase. Solubility in n-octanol also increased from 132.42 ± 1.62 to 224.36 ± 1.14 $\mu\text{g/mL}$. The increase in aqueous solubility is consistent with improved dispersion of hesperidin within the phospholipid matrix. Similar improvements in solubility and dissolution have been reported for hesperidin-phospholipid complexes.⁸

Medium	Pure hesperidin ($\mu\text{g/mL}$)	Optimized phytosomes ($\mu\text{g/mL}$)	Approx. fold change
Water	2.14 ± 0.18	48.92 ± 0.24	22.9-fold
n-Octanol	132.42 ± 1.62	224.36 ± 1.14	1.7-fold

Table 5. Saturation solubility of pure hesperidin and optimized phytosomes at 37 °C.

The partition coefficient in the n-octanol/phosphate-buffer system was 2.82 ± 0.462 for pure hesperidin and 0.74 ± 0.068 for the optimized phytosomes. The lower numerical partition coefficient of the optimized formulation should not be interpreted simply as loss of lipophilicity; rather, it reflects redistribution of the drug within a phospholipid-containing system and the altered equilibrium behavior of the complex. The markedly increased aqueous solubility was the more relevant formulation outcome for the present study.

In-vitro drug release

The release profile demonstrated a clear difference between pure hesperidin and the optimized phytosomes. Pure hesperidin released $11.20 \pm 0.14\%$ in the first 2 h in acidic medium and reached $22.48 \pm 1.04\%$ at 24 h, with a maximum of $37.92 \pm 0.92\%$ at 12 h. In contrast, the optimized phytosomes released $10.10 \pm 0.26\%$ at 2 h and progressively increased to $78.60 \pm 1.08\%$ at 24 h. Thus, the optimized phytosomes provided approximately 3.5-fold greater cumulative release at 24 h than pure hesperidin.

Time (h)	Pure hesperidin (%CDR)	Optimized hesperidin phytosomes (%CDR)
0	0.00	0.00
2	11.20 ± 0.14	10.10 ± 0.26
4	19.40 ± 0.82	25.80 ± 1.34

6	25.73 ± 0.61	29.60 ± 1.48
8	31.18 ± 0.76	33.20 ± 1.27
10	35.84 ± 0.38	39.60 ± 1.42
12	37.92 ± 0.92	46.80 ± 1.94
14	36.28 ± 0.64	54.20 ± 1.83
16	33.74 ± 0.88	60.30 ± 1.75
18	31.16 ± 0.74	64.10 ± 1.39
20	28.82 ± 1.11	68.70 ± 2.06
22	25.94 ± 0.86	73.40 ± 1.22
24	22.48 ± 1.04	78.60 ± 1.08

Table 6. In-vitro cumulative drug release of pure hesperidin and optimized phytosomes in sequential pH conditions.

The lower initial release from the phytosomal formulation during the acidic phase followed by progressively higher release in phosphate buffer suggests that the formulation can limit premature release in acidic conditions while providing enhanced release under intestinal pH conditions. Phospholipid complexation has previously been associated with improved dissolution and sustained release of flavonoid systems.^{8,9}

Drug release kinetics

Model	Slope	R ²
Zero order	2.9642	0.9854
First order	0.0258	0.9817
Higuchi	17.4	0.9943
Korsmeyer–Peppas	0.7924	n = 0.8268

Table 7. Release-kinetic parameters for optimized hesperidin phytosomes.

The Higuchi model provided the highest R² (0.9943), indicating that diffusion was the predominant mathematical description of release from the optimized system. The Korsmeyer–Peppas exponent of 0.8268 indicates anomalous/non-Fickian transport within the studied release range, suggesting that drug diffusion and matrix/structural relaxation or erosion-related effects may contribute to release. These findings are consistent with the prolonged release behavior of phospholipid complexes reported in earlier flavonoid delivery studies.^{8,9}

In-vitro antioxidant activity

DPPH radical-scavenging activity increased with concentration for both pure hesperidin and the optimized phytosomes. At 50 $\mu\text{g/mL}$, pure hesperidin showed $31.42 \pm 0.84\%$ inhibition, while optimized phytosomes showed $33.18 \pm 1.16\%$ inhibition. The modest improvement may be related to improved dispersion of hesperidin within the

phospholipid matrix. The observation is in agreement with previous work demonstrating antioxidant potential of hesperidin-phospholipid complexes.⁸

Concentration (µg/mL)	Pure hesperidin (% inhibition)	Optimized phytosomes (% inhibition)
10	25.68 ± 0.91	27.12 ± 1.46
20	26.17 ± 1.18	27.85 ± 1.21
30	27.84 ± 1.06	28.96 ± 1.33
40	29.63 ± 0.94	31.28 ± 1.08
50	31.42 ± 0.84	33.18 ± 1.16

Table 8. DPPH radical-scavenging activity of pure hesperidin and optimized phytosomes.

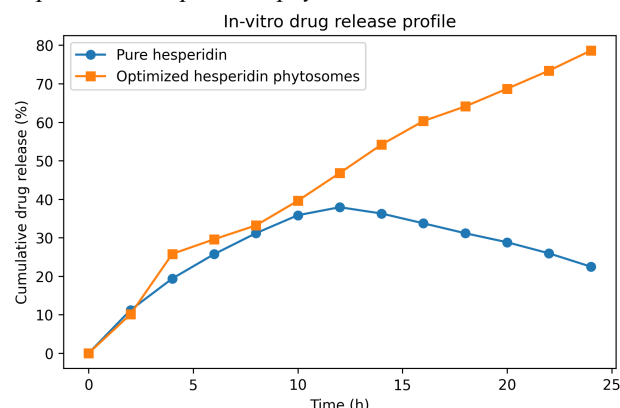


Figure 1. In-vitro cumulative drug release profile of pure hesperidin and optimized hesperidin phytosomes.

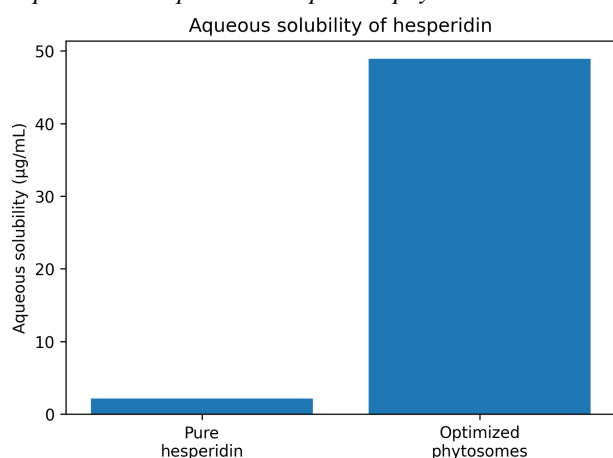


Figure 2. Comparative aqueous solubility of pure hesperidin and optimized hesperidin phytosomes.

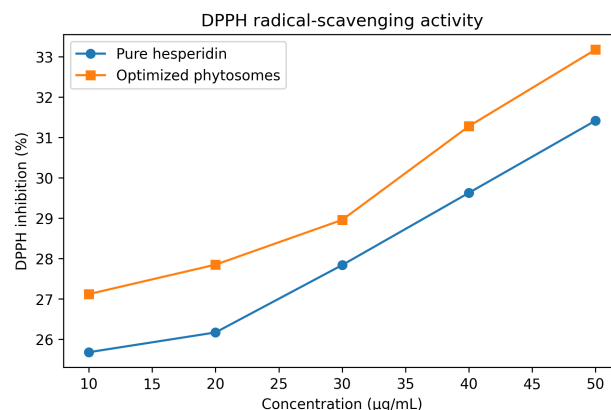


Figure 3. DPPH radical-scavenging activity of pure hesperidin and optimized hesperidin phytosomes.

DISCUSSION

The present study demonstrates a coherent formulation-development pathway for improving the in-vitro pharmaceutical performance of hesperidin. The initial preformulation findings established poor aqueous solubility, providing a clear rationale for phospholipid complexation. The preliminary screening indicated that lyophilization generated a more suitable dry product than solvent evaporation or salting-out, supporting its selection for optimization.

The DoE results showed that the hesperidin:SPC ratio was the principal formulation factor influencing particle size, while both the linear and quadratic effects of mannitol were smaller. The high R^2 and predicted R^2 values of the quadratic model indicate that the response surface adequately described the experimental region. The optimized formulation was not selected solely from the single best experimental batch; rather, the final formulation was obtained through model-based numerical optimization and subsequently confirmed experimentally. This distinction is important because the highest observed response in an individual trial does not necessarily correspond to the model-derived optimum.

The optimized phytosomes combined nanoscale particle size with a PDI below 0.4 and a moderately negative zeta potential. These characteristics are compatible with a relatively uniform dispersed system. The solid-state characterization further demonstrated changes in the crystalline state of hesperidin. The reduced intensity and broadening of the hesperidin DSC and XRD signals, together with FTIR band shifts, are consistent with interaction between the flavonoid and phospholipid. Such molecular association is a defining feature of phyto-phospholipid complex systems.^{6,7}

The most important pharmaceutical improvement was the increase in aqueous solubility from 2.14 to 48.92 µg/mL. This approximately 22.9-fold enhancement provides a direct explanation for the improved release performance. At 24 h, the optimized phytosomes released 78.60% of the drug compared with only 22.48% from pure hesperidin.

The release profile therefore demonstrates that the formulation did not merely increase early dissolution; it also maintained drug release over an extended 24-h period. The high Higuchi R^2 and non-Fickian Korsmeyer–Peppas exponent further indicate that release was controlled by diffusion together with other structural relaxation/transport processes.

The findings are comparable in direction with the study of Kalita and Patwary, who reported improved solubility and release from hesperidin-phospholipid complexes.⁸ The present work extends that concept by incorporating a three-level factorial optimization strategy, lyophilization with a cryoprotectant, nanoscale characterization and a sequential gastrointestinal-pH release design. Related work with hesperetin–HSPC complexes has also demonstrated sustained release from phospholipid-based systems, supporting the broader applicability of phospholipid complexation to citrus flavonoids.⁹

The modest increase in DPPH inhibition suggests that phytosome formation preserved, and slightly enhanced, the in-vitro antioxidant behavior of hesperidin. However, the primary significance of the present study lies in formulation and pharmaceutical performance; the DPPH findings should therefore be considered supportive rather than a substitute for mechanistic or in-vivo antioxidant evaluation.

A limitation of the present formulation-focused manuscript is that it does not establish intestinal permeability, oral bioavailability or in-vivo therapeutic efficacy. These endpoints require separate validated pharmacokinetic, permeability and pharmacodynamic studies. Nevertheless, the combination of improved solubility, favorable particle characteristics, altered solid-state properties and enhanced in-vitro release provides a strong basis for further biopharmaceutical investigation.

CONCLUSION

Hesperidin-loaded phytosomes were successfully developed using soybean phosphatidylcholine and optimized by a three-level factorial quadratic design. Lyophilization was selected as the most suitable preparation method. The optimized formulation exhibited a particle size of 278.42 ± 2.65 nm, PDI of 0.389 ± 0.03 , zeta potential of -30.4 ± 0.12 mV and drug content of $92.16 \pm 0.28\%$ w/w. Phytosome formation increased aqueous solubility approximately 22.9-fold and enhanced cumulative drug release to $78.60 \pm 1.08\%$ at 24 h compared with $22.48 \pm 1.04\%$ for pure hesperidin. Release kinetics were best described by the Higuchi model, with anomalous/non-Fickian transport suggested by the Korsmeyer–Peppas exponent. Overall, the optimized hesperidin phytosomes demonstrated substantially improved in-vitro pharmaceutical performance and represent a promising formulation platform for further biopharmaceutical evaluation.

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