RESEARCH PAPER

Formulation, Optimization, Statistical Evaluation and Stability Assessment of Quercetin-Loaded Solid Lipid Nanoparticles Using Box–Behnken Design

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ABSTRACT

Background: Quercetin is a naturally occurring flavonoid with significant therapeutic potential; however, its clinical application is limited by poor aqueous solubility and low bioavailability. Solid lipid nanoparticles (SLNs) offer a promising approach to enhance solubility and drug delivery performance.

Aim: This study aimed to formulate, optimize, statistically evaluate, and assess the stability of quercetin-loaded solid lipid nanoparticles using Box–Behnken design.

Methods: Quercetin-loaded SLNs were prepared using Glyceryl Monostearate as lipid and Poloxamer 188 as surfactant. Optimization was performed using Box–Behnken design. The optimized formulation was evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, in-vitro drug release, diffusion studies, SEM analysis, release kinetics, and stability.

Results: The optimized formulation showed a particle size of 143.97 ± 1.62 nm, PDI below 0.3, and zeta potential of −28.23 ± 1.05 mV. Entrapment efficiency was 85.30 ± 1.07%. Sustained drug release of 85.92 ± 1.51% at 12 h was observed. Diffusion was significantly enhanced (88.9 ± 2.6%) compared to pure drug (43.8 ± 2.1%). Release followed Higuchi kinetics with non-Fickian diffusion. SEM confirmed spherical morphology, and stability studies indicated minimal changes over 3 months.

Conclusion: Quercetin-loaded SLNs improved solubility, stability, and drug diffusion, demonstrating potential for enhanced bioavailability and pharmaceutical applications.

Keywords: Quercetin, Solid Lipid Nanoparticles, Drug Delivery, Entrapment Efficiency, Diffusion, Box–Behnken Design

How to cite this article: Arshiya S, Chetty CM, Badarinath AV, Reddy DM, Begum SH. Formulation, Optimization, Statistical Evaluation and Stability Assessment of Quercetin-Loaded Solid Lipid Nanoparticles Using Box–Behnken Design. Int J Drug Deliv Technol. 2026;16(18s): 540-549. DOI: 10.25258/ijddt.16.18s.58

1. Introduction:

Quercetin is a naturally occurring flavonoid widely distributed in fruits, vegetables, and medicinal plants such as onions, apples, berries, and tea. It has attracted significant attention due to its broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, anticancer, antiviral, and cardioprotective effects. These biological activities are mainly attributed to its ability to scavenge free radicals, inhibit inflammatory mediators, and regulate multiple cellular signaling pathways. Consequently, quercetin has been investigated for the management of chronic diseases such as cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions. However, despite its promising therapeutic potential, the clinical application of quercetin remains limited because of its poor aqueous solubility, rapid metabolism, and low systemic bioavailability. [1-3]

The poor bioavailability of quercetin is primarily associated with its hydrophobic nature and low aqueous solubility, which significantly restricts its absorption in the gastrointestinal tract. Studies have reported that quercetin exhibits extremely low oral bioavailability due to poor dissolution and extensive first-pass metabolism. Additionally, quercetin undergoes rapid degradation and clearance, which further limits its therapeutic effectiveness. These limitations necessitate the development of novel drug delivery systems capable of improving solubility, stability, and bioavailability of quercetin. Nanotechnology-based delivery systems, including polymeric nanoparticles, liposomes, and solid lipid nanoparticles, have been extensively explored to overcome these challenges and enhance therapeutic efficacy. [4-6]

Among the various nanocarrier systems, solid lipid nanoparticles (SLNs) have emerged as promising drug delivery platforms for poorly soluble drugs. SLNs offer several advantages, including controlled drug release, improved stability, enhanced bioavailability, biocompatibility, and reduced toxicity. These lipid-based nanoparticles can encapsulate both hydrophilic and lipophilic drugs while protecting them from degradation and improving absorption. Additionally, SLNs are relatively easy to scale up and suitable for multiple routes of administration, making them attractive candidates for pharmaceutical applications. The incorporation of quercetin into SLNs has been shown to enhance drug solubility, stability, and therapeutic performance. [7-10]

Optimization of formulation variables is essential to obtain stable and efficient nanoparticle systems. Statistical design of experiments, particularly Box–Behnken design (BBD), provides a systematic approach for evaluating multiple formulation variables with fewer experimental runs. This design helps to optimize critical parameters such as lipid concentration, surfactant concentration, and sonication time, while assessing their influence on particle size, entrapment efficiency, and drug release. Therefore, the present study aimed to develop and optimize quercetin-loaded solid lipid nanoparticles using Box–Behnken design, followed by characterization, *in-vitro* drug release, diffusion, statistical analysis, and stability assessment to develop a robust and reproducible formulation with enhanced drug delivery performance.

Materials and methods

Materials

Quercetin was obtained as a gift sample from Yarrow Chem Products, Mumbai, India. Glyceryl monostearate (GMS) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Poloxamer 188 was procured from Yarrow Chem Products, Mumbai, India. Tween 80 were obtained from SD Fine Chemicals Ltd., Mumbai, India. All chemicals and reagents used in the study were of analytical grade and used without further purification.

2.2 Preparation of Quercetin-Loaded Solid Lipid Nanoparticles

Quercetin-loaded solid lipid nanoparticles (SLNs) were prepared using the hot homogenization followed by ultrasonication method. Briefly, glyceryl monostearate was used as the solid lipid and melted at 5–10 °C above its melting point. Quercetin was accurately weighed and dispersed in the molten lipid phase under continuous stirring to obtain a uniform drug–lipid mixture.[11-13]

Separately, an aqueous phase containing Poloxamer 188 was prepared by dissolving the surfactant in distilled water and heated to the same temperature as the lipid phase. The hot aqueous phase was then slowly added to the molten lipid phase under continuous stirring to form a coarse oil-in-water emulsion. The obtained emulsion was subjected to high-speed homogenization for 10–15 minutes to reduce droplet size.

The coarse emulsion was further processed using probe ultrasonication for a predetermined time to obtain a fine nanoemulsion. The resulting nanoemulsion was allowed to cool gradually to room temperature under continuous stirring. Upon cooling, the lipid recrystallized, resulting in the formation of quercetin-loaded solid lipid nanoparticles. The prepared SLNs were stored at refrigerated conditions (4 ± 2 °C) until further characterization and evaluation.

Table 1: The levels of variables used in the BBD for the preparation of Quercetin–loaded SLN

Inputs	Levels		
	Low	Medium	High
X1 - Glyceryl Monostearate, (% w/v)	1.0	1.5	2.0
X2 – Poloxamer 188, (% w/v)	0.5	1.0	1.5
X3 - Sonication Time (minutes)	5	10	15
Responses	**Goals**		
Y1 - Particle size (nm)	Minimize		
Y2 - Entrapment efficiency (%)	Maximize		
Y3 - % Drug release at 12 h	Maximize		

2.3 Drug–Excipient Compatibility Studies (FTIR)

Drug–excipient compatibility studies were carried out using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra of pure quercetin, glyceryl monostearate, Poloxamer 188, and their physical mixture were recorded using the KBr pellet method. Samples were scanned in the range of 4000–400 cm^{-1}. The characteristic peaks of quercetin were compared with those of the physical mixture to identify any possible interactions. The absence of significant changes in

characteristic peaks indicated compatibility between the drug and excipients.[14-16]

2.4 Experimental Design and Optimization

Optimization of quercetin-loaded solid lipid nanoparticles was performed using Box–Behnken design (BBD). Three independent variables were selected to study their effect on formulation performance: lipid concentration (X_1, Glyceryl monostearate), surfactant concentration (X_2, Poloxamer 188), and sonication time (X_3). The dependent variables (responses) evaluated were particle size (Y_1), entrapment efficiency (Y_2), and percentage drug release at 12 h (Y_3).[17-19]

A total of 17 experimental runs were generated using Design-Expert® software (Version 13, Stat-Ease Inc., USA). The experimental data were fitted to a quadratic polynomial model, and response surface plots were generated to understand the effect of independent variables on responses. The optimized formulation was selected based on minimum particle size and maximum entrapment efficiency and drug release.

2.5. Characterization of Solid Lipid Nanoparticles[20-35]

2.5.1 Particle Size and Polydispersity Index (PDI)

The particle size and polydispersity index (PDI) of quercetin-loaded solid lipid nanoparticles were determined using dynamic light scattering (DLS) technique with a Zetasizer (Malvern Instruments Ltd., UK). Samples were diluted appropriately with distilled water prior to measurement to avoid multiple scattering. The analysis was carried out at 25 ± 0.5 °C. The mean particle size and PDI were recorded, and all measurements were performed in triplicate.

2.5.2 Zeta Potential

Zeta potential of the prepared SLNs was measured using the same instrument based on electrophoretic mobility. Samples were diluted with distilled water and placed in a zeta cell. The measurements were conducted at 25 ± 0.5 °C. Zeta potential values were recorded to determine the stability of the nanoparticles.

2.5.3 Entrapment Efficiency and Drug Loading

Entrapment efficiency and drug loading were determined using ultracentrifugation method. The SLN dispersion was centrifuged at 13,000 rpm for 60 minutes. The supernatant containing free drug was collected and analyzed using UV–Visible spectrophotometer at λmax of quercetin. Entrapment efficiency and drug loading were calculated using standard equations. All measurements were performed in triplicate.

2.5.4 Scanning Electron Microscopy (SEM)

Surface morphology of the optimized quercetin-loaded SLNs was examined using scanning electron microscopy (SEM). A drop of diluted SLN dispersion was placed on an aluminum stub and dried at room temperature. The samples were coated with gold under vacuum and examined under SEM to determine particle shape and surface morphology.

2.5.5 In-vitro Drug Release Study

The in-vitro drug release study was performed using dialysis bag diffusion method. The dialysis membrane was soaked overnight prior to use. SLN dispersion was placed in the dialysis bag and immersed in phosphate buffer (pH 7.4) containing 0.5% Tween 80 at 37 ± 0.5 °C under constant stirring. Samples were withdrawn at predetermined intervals and replaced with fresh medium. The samples were analyzed using UV–Visible spectrophotometer, and cumulative percentage drug release was calculated.

2.5.6 In-vitro Diffusion Study of Optimized Quercetin-Loaded SLN and Pure Drug Suspension

In-vitro diffusion study was performed using Franz diffusion cell. The dialysis membrane was mounted between donor and receptor compartments. The receptor compartment was filled with phosphate buffer (pH 7.4) containing 0.5% Tween 80 maintained at 37 ± 0.5 °C under continuous stirring. The optimized SLN formulation and pure drug suspension were placed in the donor compartment. Samples were withdrawn at predetermined intervals and analyzed using UV spectrophotometer.

2.5.7 Steady-State Flux and Permeability Coefficient

The cumulative amount of drug permeated per unit area was plotted against time. The slope of the linear portion of the curve was calculated as steady-state flux (Jss). The permeability coefficient was calculated by dividing steady-state flux by initial drug concentration.

2.5.8 Kinetic Modeling of Diffusion Data

The diffusion data were fitted to various kinetic models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The model with highest correlation coefficient (R^2) was considered as best fit model.

2.5.9 Statistical Analysis of In-vitro Diffusion Data

Statistical analysis was performed using Student's t-test to compare optimized SLNs and pure drug suspension. All experiments were conducted in triplicate and results were expressed as mean ± standard deviation. A p-value less than 0.05 was considered statistically significant.

2.5.10 Stability Study of Optimized Quercetin-Loaded SLNs

Stability studies were conducted by storing optimized SLNs at 4 ± 2 °C and 25 ± 2 °C for three months. Samples were analyzed at predetermined intervals for particle size, entrapment efficiency, and drug release. Results were expressed as mean ± standard deviation.

3. Results and Discussion

3.1 Compatibility studies

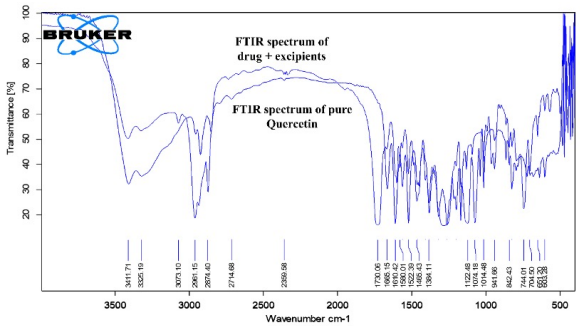

Figure 1: FTIR spectra of pure quercetin and quercetin with excipients indicating drug–excipient compatibility

The FTIR spectrum of pure quercetin showed characteristic peaks at 3411 cm^{-1} (O–H stretching), 3073 cm^{-1} (aromatic C–H), 1730 cm^{-1} (C=O stretching), and 1600–1500 cm^{-1} (aromatic C=C stretching). These peaks confirm the structural integrity of quercetin.

The FTIR spectrum of drug with excipients showed similar characteristic peaks without significant shifts or disappearance. This indicates the absence of chemical interaction between quercetin and excipients. Therefore, glyceryl monostearate and Poloxamer 188 were found to be compatible for formulation of quercetin-loaded solid lipid nanoparticles.

3.2 Optimization Using Box–Behnken Design

Box–Behnken design was employed to optimize the formulation variables for quercetin-loaded solid lipid nanoparticles. The effects of independent variables, namely glyceryl monostearate (X_1), Poloxamer 188 (X_2), and sonication time (X_3), on particle size (Y_1), entrapment efficiency (Y_2), and percentage drug release at 12 h (Y_3) were evaluated using Design-Expert software. The statistical analysis indicated that the selected quadratic model was significant for all responses.

Table 2: ANOVA Results for Quadratic Model Showing Effect of Independent Variables on Particle Size, Entrapment Efficiency and % Drug Release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
ANOVA for particle size						
Model	26754.22	9	2972.69	157.34	< 0.0001	significant
A-GMS	612.50	1	612.50	32.42	0.0007	
B-Poloxamer 188	17578.13	1	17578.13	930.41	< 0.0001	
C-Sonication time	5253.13	1	5253.13	278.05	< 0.0001	
AB	12.25	1	12.25	0.6484	0.4472	
AC	12.25	1	12.25	0.6484	0.4472	
BC	196.00	1	196.00	10.37	0.0146	
A^2	796.05	1	796.05	42.14	0.0003	
B^2	1146.32	1	1146.32	60.67	0.0001	
C^2	825.26	1	825.26	43.68	0.0003	
ANOVA for Entrapment efficiency						
Model	794.68	9	88.30	61.29	< 0.0001	significant
A-GMS	684.50	1	684.50	475.11	< 0.0001	
B-Poloxamer 188	5.45	1	5.45	3.78	0.0930	
C-Sonication time	0.9800	1	0.9800	0.6802	0.4367	
AB	1.21	1	1.21	0.8399	0.3899	
AC	0.6400	1	0.6400	0.4442	0.5264	
BC	3.61	1	3.61	2.51	0.1575	
A^2	12.53	1	12.53	8.70	0.0214	

B^2	73.39	1	73.39	50.94	0.0002	
C^2	5.33	1	5.33	3.70	0.0959	
ANOVA for % Drug release						
Model	1520.43	9	168.94	19.32	0.0004	significant
A-GMS	23.12	1	23.12	2.64	0.1480	
B-Poloxamer 188	920.21	1	920.21	105.24	< 0.0001	
C-Sonication time	403.28	1	403.28	46.12	0.0003	
AB	3.24	1	3.24	0.3706	0.5619	
AC	2.89	1	2.89	0.3305	0.5833	
BC	0.1600	1	0.1600	0.0183	0.8962	
A^2	31.84	1	31.84	3.64	0.0980	
B^2	44.47	1	44.47	5.09	0.0587	
C^2	74.27	1	74.27	8.49	0.0225	

3.2.1 Effect of Independent Variables on Particle Size

The particle size of quercetin-loaded SLNs ranged from 138 ± 1.6 nm to 275 ± 4.0 nm. The smallest particle size (138 ± 1.6 nm) was obtained at moderate lipid concentration and higher surfactant concentration, while the largest particle size (275 ± 4.0 nm) was observed at lower surfactant concentration and shorter sonication time.

ANOVA results showed that the model was significant with an F-value of 157.34 and p-value < 0.0001. Among the formulation variables, Poloxamer 188 (X_2) showed the highest influence with sum of squares 17578.13, followed by sonication time (X_3) with 5253.13, and lipid concentration (X_1) with 612.50. The quadratic terms A^2, B^2, and C^2 were also significant, indicating nonlinear relationships. Increasing surfactant concentration and sonication time significantly decreased particle size due to improved stabilization and reduction in interfacial tension.

3.2.2 Effect of Independent Variables on Entrapment Efficiency

Entrapment efficiency ranged from 65.8 ± 1.4% to 90.2 ± 1.1%. The highest entrapment efficiency (90.2 ± 1.1%) was observed at higher lipid concentration, whereas the lowest value (65.8 ± 1.4%**)** was obtained at lower lipid concentration.

The ANOVA results indicated that the model was significant with an F-value of 61.29 and p-value < 0.0001. Lipid concentration (X_1) showed significant influence with sum of squares 684.50 and p-value < 0.0001, indicating that increasing lipid concentration enhanced drug incorporation within the lipid matrix. Surfactant concentration and sonication time showed comparatively lower influence on entrapment efficiency.

3.2.3. Effect of Independent Variables on Percentage Drug Release

The percentage drug release at 12 h ranged from 55.6 ± 1.6% to 88.3 ± 1.5%**.** The highest drug release (88.3 ± 1.5%) was observed at higher surfactant concentration and moderate lipid concentration, while the lowest drug release (55.6 ± 1.6%) was observed at low surfactant concentration.

ANOVA results indicated that the model was significant with an F-value of 19.32 and p-value of 0.0004. Poloxamer 188 (X_2) showed the highest influence with sum of squares 920.21, followed by sonication time (X_3) with 403.28. Increased surfactant concentration improved drug release due to enhanced solubilization of quercetin, while increased sonication time reduced particle size and improved drug dissolution.

3.2.4. Regression Analysis

The regression analysis demonstrated good agreement between predicted and experimental values. The coefficient of determination (R^2) values for particle size, entrapment efficiency, and percentage drug release were 0.9951, 0.9875, and 0.9613, respectively. The adjusted R^2 values were 0.9888, 0.9714 and 0.9115, confirming good model fitting. The predicted R^2 values were 0.9267, 0.8121 and 0.3940, respectively. The adequate precision values for particle size, entrapment efficiency, and drug release were 43.4954, 24.9569 and 15.7195, respectively, indicating adequate model discrimination.

Table 3: Regression analysis and polynomial equations of responses

Responses	r^2	Adjusted r^2	Predicted r^2	SD	% CV	Adequate precision

Y1	0.9951	0.9888	0.9267	4.35	2.29	43.4954
Y2	0.9875	0.9714	0.8121	1.20	1.50	24.9569
Y3	0.9613	0.9115	0.3940	2.96	3.97	15.7195
Equation						
$Y1 = 169.00+8.75A-46.88B-25.63C-1.75AB+1.75AC+7.00BC+13.75A^2+16.50B^2+14.00C^2$						
$Y2 = 83.10+9.25A+0.8250B-0.3500C-0.5500AB+0.4000AC+0.9500BC-1.73A^2-4.18B^2-1.12C^2$						
$Y3 = 79.20-1.70A+10.73B+7.10C+0.9000AB-0.8500AC+0.2000BC-2.75A^2-3.25B^2-4.20C^2$						

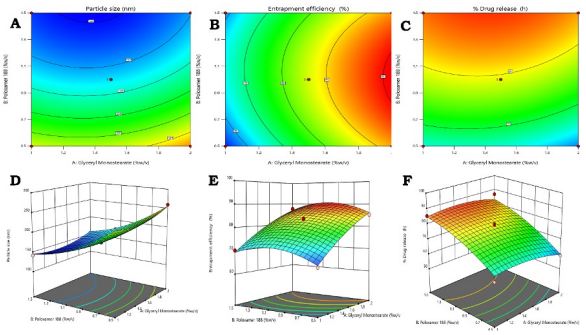

Figure 2: Contour plots of the responses A) particle size B) entrapment efficiency C) % Drug release and 3D plots of the responses D) particle size E) entrapment efficiency F) % Drug release

3.2.5 Statistical Validation of Optimized Formulation

The optimized formulation of quercetin-loaded solid lipid nanoparticles was obtained using Box–Behnken design with glyceryl monostearate (1.80834 % w/v), Poloxamer 188 (1.42576 % w/v), and sonication time (13.2986 min). The optimized formulation was prepared and evaluated experimentally to validate the predicted responses. The observed particle size values were 142.15 nm, 144.5 nm, and 145.25 nm, while entrapment efficiency values were 86.25%, 84.15%, and 85.5%, respectively. The percentage drug release values obtained were 84.55%, 85.65%, and 87.55%, indicating good reproducibility of the optimized formulation.

The predicted particle size, entrapment efficiency, and percentage drug release were 144.587 nm, 85.5106%, and 86.977%, respectively, whereas the experimentally observed values were 143.97 ± 1.62 nm, 85.30 ± 1.07%, and 85.92 ± 1.51%. The observed values were found to be very close to the predicted values and were within the 95% confidence intervals, indicating good agreement and model validity. The small deviation between predicted and observed values confirms the reliability and predictive ability of the developed quadratic model.

The desirability function analysis showed an overall desirability value of 0.90362, indicating that the optimized formulation satisfied all the optimization criteria, including minimum particle size, maximum entrapment efficiency, and controlled drug release. The high desirability value further confirms the robustness and reproducibility of the optimized formulation. Therefore, the statistical validation confirmed that the developed model was significant and suitable for optimization of quercetin-loaded solid lipid nanoparticles for enhanced drug delivery.

3.3. Polydispersity Index (PDI)

The polydispersity index (PDI) values of quercetin-loaded solid lipid nanoparticles ranged from 0.228 ± 0.013 to 0.368 ± 0.024. Most formulations showed PDI values below 0.3, indicating uniform particle size distribution. Lower PDI values were observed with higher surfactant concentration and sonication time, suggesting improved homogeneity. The optimized formulation exhibited PDI below 0.3, confirming uniform distribution and stability of nanoparticles.

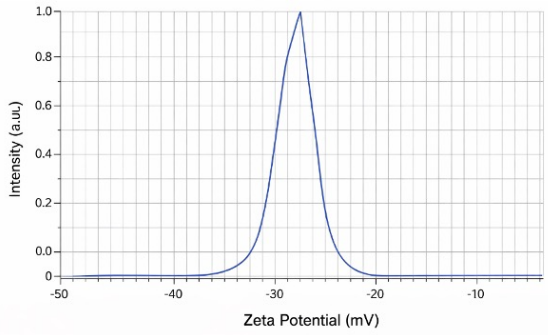

Figure 3: Variation of Polydispersity Index (PDI) Across Different Formulation Runs of Quercetin SLNs

3.4. Drug Loading

The drug loading of quercetin-loaded solid lipid nanoparticles ranged from 13.5 ± 0.7% to 18.9 ± 0.6%. The highest drug loading (18.9 ± 0.6%) was observed in formulation 11, while the lowest (13.5 ± 0.7%) was observed in formulation 7. The variation in drug loading may be attributed to differences in lipid concentration and formulation conditions. Higher lipid

concentration improved drug incorporation within the lipid matrix, resulting in increased drug loading. Overall, the results indicate efficient incorporation of quercetin into solid lipid nanoparticles.

3.5. Zeta Potential

The zeta potential of the optimized quercetin-loaded solid lipid nanoparticles was found to be −28.23 ± 1.05 mV, indicating good stability of the formulation. The negative surface charge helps prevent particle aggregation and ensures uniform dispersion. The narrow peak observed in the zeta potential distribution graph further confirms the stability and homogeneity of the nanoparticles.

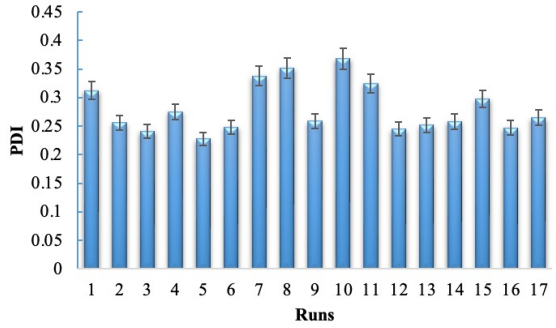

Figure 4: Zeta potential distribution of optimized quercetin-loaded solid lipid nanoparticles

3.6. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) analysis was performed to evaluate the surface morphology of optimized quercetin-loaded solid lipid nanoparticles. The SEM images revealed that the nanoparticles were spherical in shape with smooth surface morphology **and showed** uniform distribution with minimal aggregation. The observed morphology supports the particle size results, confirming the formation of nanosized particles.

The absence of surface irregularities or drug crystals indicated efficient encapsulation of quercetin within the lipid matrix. These results confirm the uniformity and stability of the developed solid lipid nanoparticles.

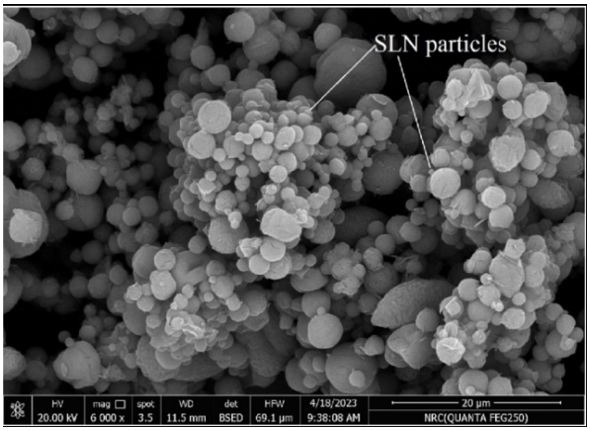

Figure 5: SEM images of optimized quercetin-loaded solid lipid nanoparticles

3.7. *In-vitro* Diffusion Study

The *in-vitro* diffusion study of optimized quercetin-loaded solid lipid nanoparticles (SLNs) and pure quercetin suspension was carried out using Franz diffusion cell. The results showed a significant increase in drug diffusion from SLNs compared to pure drug suspension. The optimized SLNs exhibited 12.6 ± 1.1% drug diffusion at 0.5 h, whereas pure quercetin showed only 5.2 ± 0.6% diffusion, indicating faster initial release from SLNs.

The cumulative drug diffusion from SLNs gradually increased to 45.9 ± 1.8% at 3 h and 68.4 ± 2.2% at 6 h, while pure drug suspension showed 22.4 ± 1.3% and 33.6 ± 1.7% diffusion at the same time points. At 12 h, SLNs exhibited 88.9 ± 2.6% drug diffusion compared to 43.8 ± 2.1% for pure quercetin suspension.

The enhanced diffusion from SLNs may be attributed to nanosized particles, increased surface area, and improved solubility of quercetin. These results confirm that SLNs significantly improved drug diffusion and may enhance bioavailability compared to pure drug suspension.

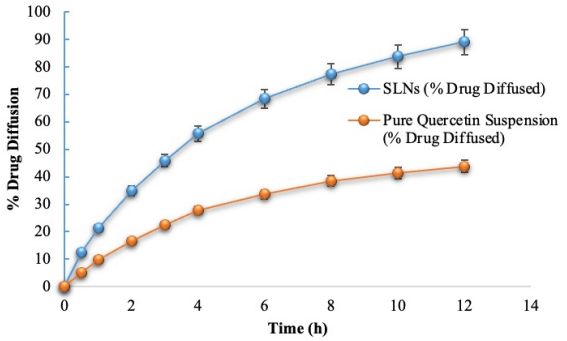

Figure 6: *In-vitro* diffusion profile of optimized quercetin-loaded SLNs and pure quercetin suspension

3.8. Steady-State Flux and Permeability Coefficient

The permeation parameters of optimized quercetin-loaded solid lipid nanoparticles were calculated to evaluate drug diffusion performance. The steady-state flux (Jss) was found to be 2.48 %/cm²/h, indicating efficient diffusion of quercetin across the membrane. The permeability coefficient (Kp) was calculated as 0.0248 h^{-1}, suggesting enhanced permeation of the drug from the SLN formulation.

The improved permeation may be attributed to nanosized particles, increased surface area, and enhanced solubility of quercetin in the lipid matrix. These results indicate that the optimized SLNs improved drug diffusion and permeability compared to

conventional formulations, suggesting their potential for improved drug delivery and bioavailability.

3.9 Release Kinetics

The release kinetics of optimized quercetin-loaded solid lipid nanoparticles were analyzed using different kinetic models. The Higuchi model showed the highest correlation coefficient (R^2 = 0.991), indicating diffusion-controlled drug release. The Korsmeyer–Peppas model also showed good fit (R^2 = 0.987) with release exponent (n = 0.48), suggesting non-Fickian diffusion.

The First-order (R^2 = 0.978) and Zero-order (R^2 = 0.963) models indicated concentration-dependent and non-constant release behavior, respectively. The Hixson–Crowell model (R^2 = 0.972) suggested slight changes in surface area during release. Overall, the optimized SLNs showed diffusion-controlled sustained drug release.

3.10. Statistical Analysis of In-vitro Diffusion Data

The cumulative percentage drug diffusion of optimized quercetin-loaded solid lipid nanoparticles (SLNs) and pure quercetin suspension was statistically analyzed using an unpaired Student's t-test. All experiments were performed in triplicate and results were expressed as mean ± standard deviation.

The cumulative drug diffusion at 12 hours for optimized SLNs was 88.9 ± 2.6%**,** whereas pure quercetin suspension showed 43.8 ± 2.1% diffusion. The calculated t-value was 23.37 with a p-value of 1.98×10^{-5} ($p < 0.0001$), indicating a highly significant difference between the two formulations.

The enhanced diffusion from SLNs may be attributed to nanosized particles, improved solubility, and surfactant-mediated drug release. These results confirm that the optimized SLNs significantly improved drug diffusion compared to pure quercetin suspension.

3.11. Stability Study of Optimized Quercetin-Loaded SLNs

Stability studies were conducted for the optimized quercetin-loaded SLNs at 4 ± 2°C and 25 ± 2°C for 3 months. Samples were evaluated for particle size, entrapment efficiency, and percentage drug release at predetermined intervals.

At 4 ± 2°C, a slight increase in particle size (158.6 ± 4.2 nm to 162.8 ± 5.4 nm) and minimal reduction in entrapment efficiency (88.4 ± 1.3% to 87.1 ± 1.9%) and drug release (89.1 ± 2.1% to 87.6 ± 2.5%) were observed. At 25 ± 2°C, comparatively higher changes were observed, with particle size increasing to 172.6 ± 7.3 nm and slight reduction in entrapment efficiency and drug release.

Overall, the formulation remained stable under both storage conditions with acceptable variations.

Table 4: Stability study of optimized quercetin-loaded SLNs under different storage conditions (Mean ± SD, n = 3)

Time	Particle Size (nm) (4 ± 2°C)	EE (%) (4 ± 2°C)	% Drug Release (12 h) (4 ± 2°C)	Particle Size (nm) (25 ± 2°C)	EE (%) (25 ± 2°C)	% Drug Release (12 h) (25 ± 2°C)
0 month	158.6 ± 4.2	88.4 ± 1.3	89.1 ± 2.1	158.6 ± 4.2	88.4 ± 1.3	89.1 ± 2.1
1 month	160.2 ± 5.0	87.9 ± 1.6	88.5 ± 2.3	165.4 ± 6.1	86.8 ± 2.0	86.9 ± 2.8
3 months	162.8 ± 5.4	87.1 ± 1.9	87.6 ± 2.5	172.6 ± 7.3	85.3 ± 2.4	84.7 ± 3.1

4. Conclusion

The present study successfully developed and optimized quercetin-loaded solid lipid nanoparticles (SLNs) to overcome the limitations of poor solubility and low bioavailability of quercetin. Preformulation studies confirmed the suitability of quercetin for lipid-based delivery, and FTIR analysis indicated compatibility between the drug and excipients.

The optimized formulation obtained using Box–Behnken design showed a particle size of 143.97 ± 1.62 nm, entrapment efficiency of 85.30 ± 1.07%**,** and 85.92 ± 1.51% drug release at 12 hours. The PDI value (<0.3) indicated uniform particle distribution, while the zeta potential (−28.23 ± 1.05 mV) confirmed good stability. SEM analysis revealed spherical and uniformly distributed nanoparticles.

The in-vitro diffusion study demonstrated enhanced drug diffusion (88.9 ± 2.6%) compared to pure quercetin (43.8 ± 2.1%)**.** The permeation parameters further confirmed improved drug transport. Release kinetics followed the Higuchi model, indicating diffusion-controlled release. Statistical analysis showed significant improvement in drug diffusion ($p < 0.0001$).

Stability studies confirmed that the optimized SLNs remained stable for 3 months with minimal variations. Overall, the developed SLNs improved solubility, stability, and drug release of quercetin, demonstrating their potential as an effective drug delivery system.

5. Acknowledgment

The authors would like to express their sincere gratitude to the Department of Pharmaceutics, Santhiram College of Pharmacy, Nandyal, for providing necessary facilities to carry out this research work. The authors also thank the management and faculty members for their continuous support and encouragement during the study.

6. Funding

The authors declare that no specific funding was received for this research work.

7. Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

8. Author Contributions

Conceptualization and study design were performed by the authors. Experimental work, data analysis, and manuscript preparation were carried out by the authors. All authors reviewed and approved the final manuscript.

9. Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

10. References

1. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S. Quercetin, inflammation and immunity. Nutrients. 2023;15(3):657–671.
2. Anand David AV, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacogn Rev. 2022;16(31):84-92.
3. Paliwal R, Paliwal SR, Vyas SP. Solid lipid nanoparticles: A review on recent perspectives and patents. Expert Opin Ther Pat. 2023;33(2):125-146.
4. Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev. 2022;187:114321.
5. Kakkar V, Kaur IP. Recent advances in solid lipid nanoparticles for drug delivery. J Drug Deliv Sci Technol. 2024;85:104673.
6. Ferreira SL, Bruns RE, Ferreira HS, Matos GD, David JM, Brandão GC. Box–Behnken design: An alternative for optimization in analytical chemistry. Anal Chim Acta. 2022;597(2):179-186.
7. Shah R, Eldridge D, Palombo E, Harding I. Optimisation and stability assessment of lipid nanoparticles using statistical design. Colloids Surf B Biointerfaces. 2023;221:112958.
8. Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: A modern formulation approach in drug delivery system. Indian J Pharm Sci. 2022;84(4):723-736.
9. Gupta A, Sharma R, Kumar S. Nanoparticle-based delivery systems for improving bioavailability of quercetin. Int J Biol Macromol. 2024;252:127164.
10. Patel S, Patel N, Patel M. Statistical optimization of solid lipid nanoparticles using Box–Behnken design. Drug Dev Ind Pharm. 2023;49(6):1043-1054.
11. Shah R, Eldridge D, Palombo E, Harding I. Lipid nanoparticles: Production, characterization and stability assessment. Colloids Surf B Biointerfaces. 2023;221:112958.
12. Kakkar V, Kaur IP. Solid lipid nanoparticles: Current advances in drug delivery systems. J Drug Deliv Sci Technol. 2024;85:104673.
13. Gupta A, Sharma R, Kumar S. Development and optimization of solid lipid nanoparticles for improved drug delivery: Recent advances. Int J Pharm. 2023;635:122687.
14. Zhang H, Liu X, Li Y. Drug–excipient compatibility evaluation using FTIR spectroscopy. Int J Pharm. 2024;652:123850.
15. Sharma P, Gupta S, Verma R. FTIR-based compatibility studies in lipid drug delivery systems. J Drug Deliv Sci Technol. 2023;84:104537.
16. Kumar R, Singh B. Application of FTIR in pharmaceutical compatibility studies. J Mol Struct. 2023;1280:134998.
17. Patel M, Shah T, Amin A. Design of experiments for optimization of lipid nanoparticles using Box–Behnken design. J Drug Deliv Sci Technol. 2023;84:104520.
18. Singh B, Kumar R. Statistical optimization of pharmaceutical formulations using Box–Behnken design. Int J Pharm Investig. 2024;14(1):12–20.
19. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology in pharmaceutical optimization. Talanta. 2022;76(5):965–977.
20. Ekinci M, Ilem-Ozdemir D, Gundogdu E, Asikoglu M, Yilmaz S. Development and evaluation of radiolabeled 5-fluorouracil-loaded solid lipid nanoparticles for colorectal cancer imaging. J Drug Deliv Sci Technol. 2026;
21. Ravindra Yadav, Rauchi Jakhmola mani, Arun K. Sharma, Ashish Kumar, Deepshikha Pande katare, Formulation and optimization of Metformin-Berberin Loaded solid lipid nanoparticles for their neuroprotective effects in the brain. Journal of Applied pharmaceutical Research. 2025; 13(5):114-133.
22. Jamous, Y.F.; Altwaijry, N.A.; Saleem, M.T.S.; Alrayes, A.F.; Albishi, S.M.;

Almeshari, M.A. Formulation and Characterization of Solid Lipid Nanoparticles Loaded with Troxerutin. Processes 2023, 11, 3039.

23. Cassayre, M.; Teles de Souza, D.; Claeys-Bruno, M.; Altié, A.; Piccerelle, P.; Sauzet, C. Optimization of Solid Lipid Nanoparticle Formulation Using Design of Experiments, PART I: Strategic Tool for the Determination of Critical Parameters Regarding Formulation and Process. Nanomaterials 2025, 15, 1034.
24. Nagaraja sreeharsha, Samathoti Prasanti, Gudhanti Siva Naga Koteswara Rao, Lakshmi Radhika Gajula, Nikitha Birdar, Prakash Goudanavar, Nimbagal Raghavendran Naveen, Prdeepkumar Narayanappa Shiroorkar. Formulation optimization of chitosan surface coated solid lipid nanoparticles of Griseofulvin: A Box Behnken design and in vivo pharmacokinetic study. European Journal of Pharmaceutical Sciences 204 (2023) 106951.
25. Gaddam Suvarsha, Ramaiyan Velmurugan, Aduri Prakash Reddy. Development and Optimization of Solid Lipid Nanoparticle Formulation for Enhanced Solubility of Ceritinib Using Box–Behnken Design. Asian Journal of Pharmaceutics 2020; 14 (1): 123-132.
26. Ashwini G. Dhome, Sanjeevani S. Deshkar, Satish V. Shirolkar. Gliclazide Solid Lipid Nanoparticles: Formulation, Optimization and in Vitro Characterization. Pharmaceutical Resonance.2018;1(1): 8-16.
27. Mohammad. Gulshan, Prof.S.Joshna Rani. Formulation and Evaluation of Quercetin-Loaded Solid Lipid Nanoparticles Using Central Composite Design: A Strategic Approach for Optimized Delivery Systems. Afr. J. Biomed. Res. 2025;28(1): 114-130.
28. Shawky, S.; Makled, S.; Awaad, A.; Boraie, N. Quercetin Loaded Cationic Solid Lipid Nanoparticles in a Mucoadhesive In Situ Gel—A Novel Intravesical Therapy Tackling Bladder Cancer. Pharmaceutics 2022, 14, 2527.
29. Sonali Bose, Yuechao Du, Paul Takhistov, Bozena Michniak-Kohn. Formulation optimization and topical delivery of quercetin from solid lipid based nanosystems. International Journal of Pharmaceutics 441 (2013) 56– 66.
30. Konatham S, Patangay S. Development and Optimization of Apixaban Loaded Solid Lipid Nanoparticles by Central Composite Design, in vitro and ex vivo Characterization. Int J Pharm Sci Drug Res. 2022;581–8.
31. Vutti Nagendra Babu, Gudhanti S. N. Koteswara Rao, Roja Rani Budha, Rajasekhar Reddy Alavala, Prasanna Kumar Desu, Govada Kishore Babu, Arja Durga Prasad. Development, characterization and optimization of solid lipid nanoparticles of alpha-mangostin by central composite design approach. Journal of Applied Pharmaceutical Science. 2023;13(08):140-150.
32. Chokshi NV, Khatri HN, Patel MM. Formulation, optimization and characterization of rifampicin loaded solid lipid nanoparticles for the treatment of tuberculosis. Drug Dev Ind Pharm. 2018;44(12):1975–1989.
33. Ahmed SS, Baqi MA, Baba MZ, Jawahar N. Formulation, characterization and optimization of folic acid-tailored daidzein solid lipid nanoparticles for improved cytotoxicity against colon cancer cells. Int J Appl Pharm. 2024;16(2):320–328.
34. Rajoriya V, Kashaw V, Kashaw SK. Folate conjugated solid lipid nanoparticle: Formulation development, optimization, and characterization. Curr Nanomed. 2021;11(3):232–245.
35. Dodia R, Shirsat MK. 5-fluorouracil solid lipid nanoparticles (SLNs): Formulation and evaluation for the treatment of skin disorders. Nat Volatiles Essent Oils. 2021;8(5):10315–10331.