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# OPTIMISATION OF QUERCETIN SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM COMPONENTS AS AN ANTIOXIDANT

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**ABSTRACT**

Oxidative stress is implicated in ageing and a range of degenerative disorders, and quercetin is a flavonol with well-documented radical-scavenging activity. Its pharmaceutical use is nevertheless limited because it belongs to class II of the Biopharmaceutics Classification System, with low aqueous solubility. A self-nanoemulsifying drug delivery system (SNEDDS) keeps such a compound molecularly dissolved in an isotropic oil–surfactant–cosurfactant mixture that disperses spontaneously into a nanoemulsion. To optimise the proportions of oleic acid, Tween 80 and PEG 400 in a quercetin SNEDDS and to verify the physical characteristics and antioxidant activity of the optimum formula. A laboratory experimental study using a D-optimal mixture design in Design-Expert® version 13, with oleic acid 10–20%, Tween 80 70–80% and PEG 400 10–20% constrained to a total of 100%, generating 16 runs. Preconcentrates were sonicated, homogenised, saturated with quercetin and centrifuged. Responses were drug load, emulsification time, particle size, polydispersity index, transmittance, zeta potential and DPPH $IC_{50}$. Quercetin was quantified by a validated UV-Vis method at 373 nm. Numerical optimisation minimised particle size, emulsification time, polydispersity index and $IC_{50}$ while maximising drug load, and the optimum formula was verified in triplicate. Responses varied widely across the 16 runs: drug load 1.043–16.755 mg/mL, emulsification time 18.6–34.8 s, particle size 90–220 nm, polydispersity index 0.138–0.245, transmittance 90.106–99.379%, zeta potential −20.5 to −38.0 mV and $IC_{50}$ 40.49–146.13 ppm. All five optimisation models were significant ($p < 0.05$) with non-significant lack of fit. Raising the oleic acid proportion enlarged droplets, lengthened emulsification and widened the size distribution, whereas Tween 80 acted in the opposite direction. The optimum formula was oleic acid 10%, Tween 80 80% and PEG 400 10%, with a desirability of 0.898. Verification gave a particle size of 90 ± 5 nm, polydispersity index 0.138 ± 0.006, drug load 13.377 ± 0.554 mg/mL and $IC_{50}$ 67.637 ± 0.957 ppm, none differing significantly from prediction ($p > 0.05$; prediction errors ⩽ 0.85%). The optimised quercetin SNEDDS forms a nanometric, homogeneous and rapidly self-emulsifying system with strong antioxidant activity, and the mixture model is sufficiently predictive to guide further development.

Keywords: antioxidant; D-optimal mixture design; nanoemulsion; quercetin; self-nanoemulsifying drug delivery system

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## INTRODUCTION

Oxidative stress arises when the generation of reactive oxygen species outpaces the endogenous antioxidant defence. The excess radicals damage lipids, proteins and DNA, disturbing cellular function and tissue homeostasis, and this imbalance has been linked to premature ageing, chronic inflammation, cardiovascular disease, metabolic disorders and cancer (Pooja et al., 2025). Developing pharmaceutical preparations that deliver antioxidant compounds effectively is therefore one strategy for preventing and managing conditions associated with oxidative damage. Quercetin is a polyphenolic flavonol abundant in fruit and vegetables and is among the most extensively studied dietary antioxidants. It scavenges free radicals directly, inhibits lipid peroxidation and modulates endogenous antioxidant enzymes such as superoxide dismutase and catalase, so that oxidative stress within the cell is reduced (Aghababaei & Hadidi, 2023). Its activity rests on the phenolic hydroxyl groups of the flavonol skeleton, which donate a hydrogen atom or an electron to

a radical and then stabilise the resulting species through electron delocalisation across the conjugated ring system (Mirza et al., 2023).

The pharmaceutical use of quercetin is nevertheless constrained by its physicochemical behaviour. It belongs to class II of the Biopharmaceutics Classification System, combining low aqueous solubility with high permeability, so dissolution rather than membrane transport limits its availability (Aghababaei & Hadidi, 2023). Poor solubility, extensive presystemic metabolism and rapid conjugation together keep systemic exposure low after oral administration (Frenț et al., 2024; Liu et al., 2025). Formulation approaches that keep the molecule dissolved before dispersion are therefore central to any attempt to exploit its antioxidant potential.

A self-nanoemulsifying drug delivery system (SNEDDS) is a lipid-based platform consisting of an isotropic mixture of oil, surfactant and cosurfactant that forms a nanoemulsion spontaneously on contact with an aqueous medium under mild agitation. The droplets produced are smaller than 200 nm, which greatly enlarges the interfacial area between the compound and the dissolution medium, accelerates dissolution of lipophilic molecules and can promote lymphatic transport (Chauhan et al., 2024). Component selection governs the outcome. Oleic acid was chosen as the oil phase for its high solubilising capacity towards lipophilic compounds and its ability to form nanodroplets spontaneously (Lestari & Sukmawati, 2024); Tween 80 as a high-HLB non-ionic surfactant suited to oil-in-water nanoemulsions; and PEG 400 as a cosurfactant that increases the flexibility of the interfacial film.

Because the three components must sum to 100%, their proportions cannot be varied independently, and a mixture design is the appropriate optimisation framework. The D-optimal mixture design distributes experimental points efficiently across a constrained region, accommodates replication for pure-error estimation and supports higher-order models describing component interaction (Podder & Mukherjee, 2024; Habib et al., 2022). Previous work has applied this approach to SNEDDS of naringenin and to mixture-design topical formulations such as niosomal gels (Candra et al., 2025; Habib et al., 2022), and quercetin SNEDDS have been developed with dissolution as the principal outcome (Wildan, 2022). What has been examined less often is the inclusion of antioxidant activity itself as an optimisation response alongside the physical characteristics, even though it is the property the preparation is intended to deliver. This study therefore optimised the proportions of oleic acid, Tween 80 and PEG 400 in a quercetin SNEDDS using a D-optimal mixture design in which drug load, emulsification time, particle size, polydispersity index and DPPH $IC_{50}$ were treated together as responses, and verified the resulting optimum formula experimentally.

## METHOD

This was a laboratory experimental study carried out between January and July 2026. Quercetin (Sigma-Aldrich, ≥ 95%) served as the active compound, with oleic acid as the oil phase, Tween 80 as the surfactant and PEG 400 as the cosurfactant. DPPH (2,2-diphenyl-1-picrylhydrazyl), HPLC-grade methanol, analytical-grade ethanol and purified water were used for analysis. Instrumentation comprised a magnetic stirrer, probe sonicator, UV-Vis spectrophotometer (Shimadzu 1800), particle size analyser and zetasizer (Malvern Zetasizer Nano ZS), analytical balance and centrifuge. Because the study involved neither human subjects nor animals, ethical approval was not required. The identity of the raw material was confirmed by attenuated total reflectance FTIR over 4000–400 $cm^{-1}$ at 4 $cm^{-1}$ resolution with 32 scans. A quercetin stock solution was prepared in ethanol, scanned over 300–400 nm to establish the maximum wavelength, and read repeatedly to establish the operating time. A calibration curve was constructed at 4.02, 6.03, 8.04, 10.05 and 12.06 ppm. The analytical method was validated for linearity, precision, accuracy, limit of detection and limit of quantification in accordance with ICH Q2(R2) (International Council for Harmonisation, 2023; Muhtadi et al., 2022).

Formula optimisation used a D-optimal mixture design in Design-Expert® version 13 with oleic acid constrained to 10–20%, Tween 80 to 70–80% and PEG 400 to 10–20%, the three summing to 100% (w/w). The design generated 16 runs covering edge points, interior points and replicates so that pure error could be estimated. For each run the components were pipetted to a total volume of 3 mL, sonicated for 10 minutes and homogenised on a magnetic stirrer at 500 rpm until a clear single-phase isotropic system was obtained. Quercetin was then added incrementally until saturation, indicated by the appearance of turbidity, and the saturated system was centrifuged at 5000 rpm for 45 minutes. The supernatant was stored in light-protected vials at room temperature and used for characterisation.

Drug load was determined by diluting 0.1 mL of the preconcentrate to 5 mL with analytical-grade methanol, taking 1 mL of that solution into a 10 mL volumetric flask, diluting to volume and reading the absorbance at the maximum wavelength against a methanol blank; the concentration was calculated from the calibration equation. Emulsification time was measured by adding 0.1 mL of preconcentrate to 10 mL of distilled water at 37 ± 1 °C under stirring at 100 rpm and recording the time to a homogeneous dispersion (Chauhan et al., 2024). Percentage transmittance was measured at 650 nm after diluting 0.1 mL of preconcentrate to 100 mL with distilled water (Fitriah et al., 2023). Droplet size and polydispersity index were measured by dynamic light scattering at 25 ± 1 °C after 1:100 (v/v) dilution, and zeta potential was measured on the same instrument in electrophoresis mode, each in triplicate.

Antioxidant activity was assessed by the DPPH method. Serial dilutions covering 10–100 µg/mL were prepared in methanol, and 1 mL of each was mixed with 1 mL of 0.04 mM DPPH solution. The mixture was shaken gently and incubated in the dark at room temperature for the established operating time, after which absorbance was read at 517 nm (Dikdayani, 2021; Gulcin & Alwasel, 2023). Inhibition was calculated as the proportional reduction in absorbance relative to the DPPH control, plotted against concentration, and $IC_{50}$ was obtained from the linear regression equation. Determinations were performed in triplicate and reported as mean ± SD. Activity was interpreted using the conventional bands in which $IC_{50}$ below 50 ppm denotes very strong activity, 50–100 ppm strong, 101–150 ppm moderate and above 150 ppm weak.

Model fitting and analysis of variance were performed in Design-Expert®, with model significance judged at $p < 0.05$, non-significant lack of fit taken as evidence of adequate fit, and $R^2$, adjusted $R^2$ and adequate precision used as fit diagnostics. Numerical optimisation set particle size, emulsification time, polydispersity index and $IC_{50}$ to be minimised and drug load to be maximised, with the three components kept within their design ranges; transmittance and zeta potential were retained as characterisation parameters but not entered as optimisation responses. The solution with the highest desirability was reformulated in triplicate, and observed responses were compared with predicted values through prediction error and a one-sample test at the 5% significance level (Azcarate et al., 2023).

## RESULT

The FTIR spectrum of the raw material showed a broad band at 3362–3368 $cm^{-1}$ from hydrogen-bonded hydroxyl stretching, a carbonyl band at 1653 $cm^{-1}$, aromatic C=C bands at 1599 and 1511 $cm^{-1}$, aromatic ether stretching at 1306 and 1239 $cm^{-1}$ and the strongest band at 1155 $cm^{-1}$ from phenolic C–O stretching, a pattern consistent with published quercetin spectra and confirming the identity of the material (Sah et al., 2023). Scanning gave a maximum wavelength of 373 nm with an absorbance of 0.743, and absorbance stabilised at the 29th to 33rd reading so the 30th minute was adopted as the operating time. The calibration curve gave $y = 0.02522x + 0.14500$ with $r = 0.99965$. Validation returned a linearity equation of $y = 0.02270x + 0.20460$ ($r = 0.99463$; $R^2 = 0.98929$), a precision RSD of 0.40% from six replicate readings at 8 ppm, recovery of 98.68–101.94% at 6, 8 and 10 ppm, a limit of detection of 0.706 ppm and a limit of quantification of 2.355 ppm, all within

ICH acceptance expectations. The composition and full set of responses for the 16 runs are given in Table 1. Drug load ranged from 1.043 to 16.755 mg/mL, the highest values occurring in runs 6, 10 and 5 and the lowest in run 3. Emulsification time ranged from 18.6 to 34.8 s, so every formula emulsified within one minute; the fastest were runs 14 and 5 and the slowest run 12. Particle size ranged from 90 to 220 nm, with 15 of the 16 runs below 200 nm; runs 5 and 14, both at the 10:80:10 composition, gave the smallest droplets at 90 nm, while run 12 with 20% oleic acid gave the largest. Polydispersity index ranged from 0.138 to 0.245 and remained below 0.30 throughout, indicating relatively uniform distributions. Transmittance exceeded 90% in every run, and zeta potential was negative throughout at −20.5 to −38.0 mV.

Table 1.
Composition and responses of the 16 runs of the D-optimal mixture design

| Run | Oleic acid (%) | Tween 80 (%) | PEG 400 (%) | Emulsification time (s) | Transmittance (%) | Drug load (mg/mL) | Particle size (nm) | PDI | Zeta potential (mV) | $IC_{50}$ (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 14.979 | 70.000 | 15.021 | 27.8 | 90.106 | 11.395 | 165 | 0.182 | −28.5 | 75.21 |
| 2 | 11.653 | 76.619 | 11.728 | 20.5 | 90.257 | 2.584 | 115 | 0.145 | −24.0 | 108.99 |
| 3 | 10.000 | 70.000 | 20.000 | 24.2 | 90.156 | 1.043 | 140 | 0.172 | −22.0 | 146.13 |
| 4 | 15.010 | 74.990 | 10.000 | 23.7 | 92.257 | 11.028 | 135 | 0.151 | −29.0 | 79.68 |
| 5 | 10.000 | 80.000 | 10.000 | 18.9 | 92.116 | 15.580 | 90 | 0.138 | −20.5 | 56.30 |
| 6 | 16.763 | 71.734 | 11.503 | 28.5 | 92.297 | 16.755 | 180 | 0.164 | −32.5 | 40.49 |
| 7 | 14.979 | 70.000 | 15.021 | 28.1 | 97.784 | 13.377 | 165 | 0.182 | −28.5 | 67.91 |
| 8 | 12.458 | 70.000 | 17.542 | 25.9 | 91.119 | 8.825 | 145 | 0.205 | −25.5 | 135.44 |
| 9 | 10.130 | 74.909 | 14.961 | 22.1 | 93.984 | 1.997 | 110 | 0.158 | −22.5 | 121.49 |
| 10 | 13.384 | 73.318 | 13.298 | 24.6 | 94.448 | 16.167 | 130 | 0.169 | −27.0 | 40.99 |
| 11 | 10.130 | 74.909 | 14.961 | 22.4 | 90.314 | 2.217 | 110 | 0.158 | −22.5 | 121.38 |
| 12 | 20.000 | 70.000 | 10.000 | 34.8 | 95.235 | 10.881 | 220 | 0.245 | −38.0 | 68.29 |
| 13 | 15.010 | 74.990 | 10.000 | 23.9 | 90.297 | 1.924 | 135 | 0.151 | −29.0 | 132.26 |
| 14 | 10.000 | 80.000 | 10.000 | 18.6 | 91.457 | 11.542 | 90 | 0.138 | −20.5 | 78.33 |
| 15 | 15.010 | 74.990 | 10.000 | 24.1 | 92.305 | 4.934 | 135 | 0.151 | −29.0 | 101.25 |
| 16 | 10.000 | 72.456 | 17.544 | 23.5 | 99.379 | 1.777 | 125 | 0.196 | −22.0 | 137.18 |
| Mean | — | — | — | 24.48 | 92.72 | 8.25 | 136.9 | 0.169 | −26.3 | 94.46 |

Note: PDI = polydispersity index; $IC_{50}$ = concentration inhibiting 50% of DPPH radicals. Mean values are arithmetic means of the 16 runs. Source: research data (2026).

Table 2.
Analysis of variance and fit diagnostics of the mixture models for each optimisation response

| Response | Model | F-value | p-value | Lack of fit (p) | $R^2$ | Adjusted $R^2$ | Adequate precision |
|---|---|---|---|---|---|---|---|
| Drug load | Special quartic | 7.05 | 0.0092 | 0.8958 | 0.8896 | 0.7633 | 7.7319 |
| Emulsification time | Special quartic | 812.86 | < 0.0001 | 0.6174 | 0.9989 | 0.9977 | 110.7631 |
| Particle size | Special quartic | 282.64 | < 0.0001 | 0.3260 | 0.9969 | 0.9934 | 63.8183 |
| Polydispersity index | Cubic | 203.30 | < 0.0001 | 0.2365 | 0.9967 | 0.9918 | 53.1262 |
| $IC_{50}$ (DPPH) | Special quartic | 5.58 | 0.0177 | 0.3463 | 0.8644 | 0.7094 | 7.9726 |

Note: Adequate precision compares the signal to the noise; a value above 4 is desirable. Source: research data (2026).

Antioxidant activity varied over more than a threefold range, from 40.49 to 146.13 ppm with a mean of 94.46 ppm. Two runs fell in the very strong band (runs 6 and 10, at 40.49 and 40.99 ppm), six in the strong band (runs 1, 4, 5, 7, 12 and 14) and eight in the moderate band. Runs 6 and 10, which produced the two lowest $IC_{50}$ values, were also the two runs with the highest drug load, suggesting that solubilising capacity governs how much quercetin is available to react with the radical in the test medium. Analysis of variance showed that the fitted models were significant for all five optimisation responses, with non-significant lack of fit in every case (Table 2). Fit was excellent for emulsification time, particle size and polydispersity index, with $R^2$ above 0.99 and adequate precision far above the threshold of 4. Drug load and $IC_{50}$ were fitted less tightly, with $R^2$

of 0.8896 and 0.8644 and adequate precision near 8; predicted $R^2$ was negative for drug load, emulsification time, particle size and $IC_{50}$, so these models describe the experimental data well but extrapolate poorly, and their predictions required experimental confirmation.

The fitted models in pseudocomponent form, where A is oleic acid, B is Tween 80 and C is PEG 400, were:

$$\text{Drug load (mg/mL): } Y = 10.80A + 13.49B + 1.61C - 24.77AB + 25.18AC - 21.32BC + 663.94A^2BC - 695.27AB^2C + 808.45ABC^2$$

$$\text{Emulsification time (s): } Y = 34.78A + 18.75B + 24.29C - 11.51AB - 6.02AC + 2.66BC - 4.46A^2BC - 86.46AB^2C + 113.23ABC^2$$

$$\text{Particle size (nm): } Y = 220.44A + 90.08B + 138.25C - 76.18AB - 55.37AC - 0.20BC + 1057.20A^2BC + 715.86AB^2C - 2225.68ABC^2$$

$$\text{Polydispersity index: } Y = 0.2452A + 0.1381B + 0.1725C - 0.1564AB - 0.0948AC + 0.0010BC + 0.3603ABC - 0.2322AB(A-B) - 0.3320AC(A-C) - 0.3259BC(B-C)$$

$$IC_{50}\text{ (ppm): } Y = 65.39A + 67.56B + 153.95C + 151.84AB - 116.25AC + 47.20BC - 3668.08A^2BC + 2622.90AB^2C - 4034.36ABC^2$$

The linear coefficients place oleic acid highest for particle size, emulsification time and polydispersity index, and Tween 80 lowest for all three, so increasing the oil phase enlarges droplets, slows emulsification and widens the distribution while increasing the surfactant does the reverse. For drug load the largest positive linear coefficient belongs to Tween 80, but the negative AB and BC interaction terms show that raising any single component does not improve solubilisation unless the others are balanced. For $IC_{50}$ the largest linear coefficient belongs to PEG 400 and is positive, meaning that a higher cosurfactant proportion tends to weaken antioxidant activity, while the negative AC interaction indicates that particular oleic acid–PEG 400 combinations can lower $IC_{50}$. Non-parametric testing was applied to the two characterisation parameters that were not normally distributed. Transmittance differed significantly among the 16 runs (Kruskal–Wallis $H = 46.847$; $p < 0.001$), with Dunn–Bonferroni post hoc comparisons identifying differences between runs 1 and 7, runs 1 and 16, and runs 3 and 16. Zeta potential likewise differed among runs ($H = 43.918$; $p < 0.001$), run 12 at −38.0 mV differing significantly from runs 5 and 14 at −20.5 mV.

Numerical optimisation produced two candidate solutions. The selected solution consisted of oleic acid 10.000%, Tween 80 80.000% and PEG 400 10.000%, with a desirability of 0.898 and predicted responses of 90.080 nm, 18.745 s, 13.491 mg/mL, a polydispersity index of 0.138 and an $IC_{50}$ of 67.563 ppm. The alternative solution, at 12.893 : 73.097 : 14.010, was predicted to give a higher drug load of 16.819 mg/mL and a markedly lower $IC_{50}$ of 40.480 ppm, but with a larger droplet size of 120.731 nm, a polydispersity index of 0.179, a longer emulsification time of 24.830 s and a lower desirability of 0.766. The first solution was selected because it offered the better overall compromise across the response set. Verification of the optimum formula in triplicate gave results closely matching the model (Table 3). All four responses fell within a prediction error of 0.85%, all relative standard deviations were below 6%, and none of the observed values differed significantly from the predicted values ($p > 0.05$). The particle size of 90 ± 5 nm confirms formation of nanometric droplets, the polydispersity index of 0.138 ± 0.006 a narrow and homogeneous distribution, and the $IC_{50}$ of 67.637 ± 0.957 ppm places the optimised system in the strong antioxidant band.

Table 3.
Comparison of predicted and observed responses for the optimum quercetin SNEDDS formula

| Response | Predicted | Observed (mean ± SD) | RSD (%) | Prediction error (%) | p |
|---|---|---|---|---|---|
| Particle size (nm) | 90.080 | 90 ± 5 | 5.56 | 0.09 | 0.978 |
| Polydispersity index | 0.138 | 0.138 ± 0.006 | 4.03 | ≈ 0 | 1.000 |
| Drug load (mg/mL) | 13.491 | 13.377 ± 0.554 | 4.14 | 0.85 | 0.734 |
| $IC_{50}$ (ppm) | 67.563 | 67.637 ± 0.957 | 1.41 | 0.11 | 0.896 |

Note: Observed values are means of three replicates; RSD = relative standard deviation; prediction error = |observed − predicted| / predicted × 100%. Source: research data (2026).

## DISCUSSION

The direction of the component effects is consistent across the physical responses and has a straightforward interfacial explanation. Tween 80 is a high-HLB non-ionic surfactant that lowers interfacial tension and adsorbs rapidly at the newly created oil–water interface, so a surfactant-rich composition produces smaller droplets more quickly and with a narrower size distribution. Raising the oleic acid proportion increases the volume of dispersed phase that the available surfactant must cover; once the interfacial film is no longer sufficient, droplets coalesce and both mean size and polydispersity rise, and more energy and time are needed for dispersion (Chauhan et al., 2024; Reddy & Gubbiyappa, 2022). Run 12, with the maximum oil content of 20%, illustrates the limit of the design space, being the only run to exceed 200 nm and the only one with a polydispersity index above 0.20.

Drug load behaved differently, being governed by interaction rather than by any single component. Quercetin partitions between the lipid phase and the surfactant–cosurfactant interfacial region, so oleic acid supplies the lipophilic environment while Tween 80 and PEG 400 create the interfacial region in which a considerable fraction of the flavonol is accommodated. Because the negative AB and BC terms outweigh part of the positive linear contributions, an excess of any component reduces capacity, and the observed maximum lies inside the simplex rather than at a vertex. This is the expected pattern for a poorly water-soluble compound solubilised by a mixed system (Chauhan et al., 2024).

The antioxidant results deserve the closest attention, since $IC_{50}$ is the response that expresses the purpose of the preparation. Quercetin scavenges DPPH by donating a hydrogen atom from its phenolic hydroxyl groups, and the resulting radical is stabilised by delocalisation over the conjugated flavonol system (Mirza et al., 2023; Aghababaei & Hadidi, 2023). Because the assay measures what is available to react, the amount of quercetin held in solution matters as much as the intrinsic reactivity of the molecule, and the two runs with the highest drug load were also the two with the lowest $IC_{50}$. The positive linear coefficient of PEG 400 is consistent with this reading: the runs richest in cosurfactant were also those with the lowest solubilising capacity, run 3 combining the lowest drug load of the series with the highest $IC_{50}$.

This creates a genuine tension in the optimisation that should be stated plainly rather than smoothed over. The selected formula is not the one with the strongest predicted antioxidant activity; the alternative solution was predicted to reach an $IC_{50}$ of 40.480 ppm, in the very strong band, but at the cost of larger, less uniform droplets and slower emulsification. Desirability weighted all five responses together and favoured the physically superior system, and the verified $IC_{50}$ of 67.637 ppm still falls in the strong band. Whether that trade-off is the right one depends on the intended route: where droplet size and distribution drive subsequent performance, the selected formula is preferable, whereas an application judged primarily on radical-scavenging capacity would justify revisiting the weighting. Reporting both solutions allows that judgement to be made explicitly.

Zeta potential offers a further nuance. The optimum formula carried the least negative surface charge of the series at −20.5 mV, which in a purely electrostatic account would suggest weaker resistance to aggregation than run 12 at −38.0 mV. Tween 80 is non-ionic, however, and stabilises largely through the steric barrier formed by its polyoxyethylene chains, so charge magnitude alone is an incomplete indicator of stability in this system (Rehman et al., 2022). The consistently high transmittance, above 90% in every run and reflecting low light scattering, supports the formation of fine and relatively clear dispersions across the whole design space (Fitriah et al., 2023).

Several limitations should temper the interpretation. Predicted $R^2$ was negative for four of the five responses, so the models describe the experimental region well but should not be used to extrapolate beyond it; the close agreement between predicted and observed values at the optimum confirms interpolation within the design space rather than general predictive power. Antioxidant activity was assessed by a single in vitro method, and DPPH measures radical scavenging under conditions that do not reproduce a biological environment, so complementary assays such as ABTS or FRAP would strengthen the characterisation. Long-term and accelerated stability, thermodynamic stability testing under freeze–thaw and centrifugation stress, and in vivo evaluation were outside the scope of this work and remain necessary before the system can be developed further.

## CONCLUSION

The proportions of oleic acid, Tween 80 and PEG 400 significantly affected every characteristic of the quercetin SNEDDS. Increasing oleic acid enlarged droplets, lengthened emulsification time and widened the size distribution, whereas increasing Tween 80 produced the opposite effects, and drug load and antioxidant activity were determined by component interaction rather than by any single constituent. The optimum composition was oleic acid 10%, Tween 80 80% and PEG 400 10%, with a desirability of 0.898. Experimental verification gave a particle size of 90 ± 5 nm, a polydispersity index of 0.138 ± 0.006, a drug load of 13.377 ± 0.554 mg/mL and an $IC_{50}$ of 67.637 ± 0.957 ppm, none differing significantly from the predicted values, so the optimised system is nanometric, homogeneous, rapidly self-emulsifying and a strong antioxidant.

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