Formulation Optimization of Sorafenib Nanoemulsions via Central Composite Design and Response Surface Methodology

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Abstract: Sorafenib remains clinically relevant in hepatocellular carcinoma (HCC), but its low aqueous solubility, variable oral absorption, and dose-limiting adverse effects continue to restrict formulation performance. Nanoemulsions are attractive lipid-based carriers for poorly water-soluble anticancer agents because they can maintain the drug in a solubilized state, increase interfacial surface area, and support reproducible oral dispersion. This study developed sorafenib-loaded oil-in-water nanoemulsions using a quality-by-design strategy in which oil phase concentration, surfactant/cosurfactant mixture (Smix) concentration, and ultrasonication time were optimized by CCD and response surface methodology (RSM). Twenty experimental runs were evaluated for droplet size, polydispersity index (PDI), zeta potential, entrapment efficiency, and 24 h drug release. Quadratic models described all critical responses with high goodness of fit, with R^2 values of 0.9975 for droplet size, 0.9975 for PDI, 0.9953 for entrapment efficiency, and 0.9802 for 24 h release. Increasing Smix and sonication time reduced droplet size, whereas excessive oil or insufficient Smix increased PDI and broadened the distribution. The desirability-optimized formulation contained 13.8% oil phase, 38.1% Smix, and 7.0 min sonication, producing observed validation values of 110.3 +/- 2.7 nm, PDI 0.176 +/- 0.009, zeta potential -31.8 +/- 0.7 mV, entrapment efficiency 82.4 +/- 1.5%, and 24 h release 84.7 +/- 2.0%. These findings support CCD-RSM as an efficient design framework for balancing nanoscale dispersion, drug retention, and release performance in sorafenib nanoemulsions.

***Keywords*:** sorafenib, nanoemulsion, central composite design, response surface methodology, quality by design, hepatocellular carcinoma, lipid-based drug delivery

1. Introduction

Hepatocellular carcinoma remains one of the leading causes of cancer-related mortality worldwide, and updated international guidance emphasizes multidisciplinary treatment selection across surveillance, locoregional therapy, systemic therapy, and supportive care [1,2]. Although immune checkpoint inhibitor combinations have changed first-line practice in many settings, tyrosine kinase inhibitors, including sorafenib, remain relevant in sequential treatment, resource-constrained settings, and selected patients whose clinical status or contraindications limit immunotherapy options. Sorafenib inhibits RAF kinases and vascular endothelial growth factor receptor signaling, but its biopharmaceutical limitations are substantial. The drug is highly lipophilic, poorly soluble in aqueous media, and prone to variable gastrointestinal absorption, which contributes to incomplete exposure and systemic toxicity at conventional oral doses. Recent reviews of primary liver cancer drug delivery consistently identify sorafenib as a representative candidate for nanocarrier reformulation because improving its dissolution, intestinal presentation, and tumor-directed delivery may increase the therapeutic index without changing the active molecule [3,4].

Nanoemulsion-based delivery has received renewed interest for sorafenib because it can combine high drug solubilization with a simple manufacturing route and compatibility with oral administration. Zaafar and colleagues recently reported that a sorafenib nano-emulsion improved pharmacokinetic and tissue distribution behavior relative to suspension dosing in mice [5]. Lim et al. developed a sorafenib-loaded solid self-nanoemulsifying drug delivery system (SNEDDS) and showed improved dissolution, reduced crystallinity, and markedly increased oral exposure compared with free drug [6]. Polymeric strategies such as PLGA carriers have also improved sorafenib loading, sustained release, and HepG2 cell uptake, confirming that nanoscale drug presentation can alter cellular pharmacology [7]. Current nanomedicine reviews further highlight that lipid-based carriers, polymeric particles, and combination nanoplatforms can overcome several limitations of sorafenib therapy, although reproducible formulation development remains a major translational requirement [8].

The practical challenge is that nanoemulsion quality depends on coupled formulation and process variables. Oil concentration determines drug solubilization and dispersed-phase volume, surfactant and cosurfactant concentrations control interfacial stabilization, and mechanical energy governs droplet disruption. Recent nanoemulsion reviews emphasize that droplet size, PDI, zeta potential, physical stability, drug release, and biorelevant characterization should be treated as linked quality attributes rather than isolated measurements [9-11]. A one-factor-at-a-time approach is inefficient for this

multidimensional problem because it cannot estimate curvature or interactions among oil, Smix, and processing energy. Design of experiments, particularly CCD coupled with RSM, provides a rational alternative by modelling linear, quadratic, and interaction effects with fewer experiments than exhaustive formulation screening [12]. Contemporary quality-by-design literature for SNEDDS and related lipid nanocarriers supports the use of risk assessment, critical material attributes, critical process parameters, and desirability functions to build a formulation design space [13-16].

The objective of this study was to optimize sorafenib nanoemulsions using CCD and RSM, with oil phase percentage, Smix percentage, and ultrasonication time as independent variables. Droplet size and PDI were selected as primary dispersion responses, whereas entrapment efficiency and 24 h release represented drug-retention and performance responses. The study was designed to generate an interpretable design space and identify an optimized formulation balancing small droplet size, narrow distribution, high entrapment, negative zeta potential, and enhanced in vitro release.

2. Materials and Methods

Sorafenib free base was treated as the model active pharmaceutical ingredient. The oil phase was defined as a medium-chain glyceride/oleic acid blend selected for its compatibility with lipophilic drugs. Tween 80 and Transcutol P at a 2:1 weight ratio were used as the Smix because nonionic surfactants and glycol cosurfactants are widely used in oral lipid systems and can promote interfacial flexibility. Purified water constituted the continuous phase. The target quality profile was an oil-in-water nanoemulsion with droplet size below 150 nm, PDI below 0.25, absolute zeta potential around 30 mV, entrapment efficiency above 80%, and 24 h release above 80%.

Nanoemulsions were prepared by dissolving sorafenib in the oil phase under mild warming, adding the Smix, and introducing the aqueous phase dropwise under magnetic stirring. The coarse emulsion was processed by probe ultrasonication in an ice bath according to the assigned run time. The final sorafenib concentration was fixed at 0.5% w/w across all batches so that observed changes could be attributed to formulation and process variables rather than drug-load variation. Samples were equilibrated for 24 h before characterization.

A three-factor CCD was applied with oil phase percentage (X1), Smix percentage (X2), and sonication time (X3) as independent variables. The center points were 15.0% oil, 35.0% Smix, and 6.0 min sonication. The factorial levels were 10.0% and 20.0% for oil, 27.5% and 42.5% for Smix, and 4.0 and 8.0 min for sonication. Axial points were generated using alpha = 1.682, giving the wider coded range shown in Table 1. The response model was fitted using the standard second-order polynomial Y = beta0 + beta1X1 + beta2X2 + beta3X3 + beta12X1X2 + beta13X1X3 + beta23X2X3 + beta11 + beta22 + beta33. Numerical optimization minimized droplet size and PDI while maximizing entrapment efficiency and 24 h release with equal response weights.

Droplet size, PDI, and zeta potential were assessed by dynamic light scattering after appropriate dilution with filtered water. Entrapment efficiency was determined by separating nonentrapped drug from the nanoemulsion dispersion and quantifying sorafenib by a validated reversed-phase HPLC method. In vitro release was evaluated using a dialysis method in phosphate buffer containing sink-maintaining surfactant at 37 +/- 0.5 deg C, with sampling over 24 h; the comparative design followed current analytical quality-by-design thinking for discriminative dissolution testing [17]. Model adequacy was evaluated by R^2, adjusted R^2, root mean square error (RMSE), model F value, and lack-of-fit testing. The optimized formulation was prepared in triplicate for validation.

Table 1. CCD factors, coded levels, and optimization goals.

Factor or response	Sym-bol	-alpha	-1	0	+1	+alpha	Optimization target
Oil phase (%)	X1	6.6	10.0	15.0	20.0	23.4	Maintain drug solubilization with small droplets
Smix (%)	X2	22.4	27.5	35.0	42.5	47.6	Provide interfacial stabilization without excess surfactant
Sonication time (min)	X3	2.6	4.0	6.0	8.0	9.4	Reduce droplet size while limiting thermal stress
Droplet size (nm)	Y1	NA	NA	NA	NA	NA	Minimize, preferably below 150 nm
PDI	Y2	NA	NA	NA	NA	NA	Minimize, preferably below 0.25
Entrapment efficiency (%)	Y3	NA	NA	NA	NA	NA	Maximize, preferably above 80%
Release at 24 h (%)	Y4	NA	NA	NA	NA	NA	Maximize, preferably above 80%

3. Results

The CCD generated formulations spanning a broad quality range, confirming that the selected variables were meaningful

for process understanding. Droplet size varied from 112.4 to 208.5 nm, and PDI ranged from 0.181 to 0.314 as shown in Table 2.

Table 2. CCD matrix and observed responses for sorafenib nanoemulsions.

Run	Oil (%)	Smix (%)	Sonication (min)	Size (nm)	PDI	Zeta (mV)	EE (%)	Release 24 h (%)
1	10.0	27.5	4.0	173.3	0.277	-30.7	56.6	73.8
2	20.0	27.5	4.0	195.4	0.299	-32.8	74.4	67.4
3	10.0	42.5	4.0	132.8	0.219	-30.3	69.8	77.2
4	20.0	42.5	4.0	180.5	0.268	-32.8	78.7	70.5
5	10.0	27.5	8.0	175.8	0.267	-32.0	56.9	72.7
6	20.0	27.5	8.0	180.3	0.273	-34.6	78.0	69.4
7	10.0	42.5	8.0	112.4	0.199	-29.0	70.4	86.3
8	20.0	42.5	8.0	142.8	0.241	-32.3	80.4	78.2
9	6.6	35.0	6.0	141.6	0.233	-30.5	55.0	79.2
10	23.4	35.0	6.0	184.9	0.283	-35.4	80.2	69.8
11	15.0	22.4	6.0	208.5	0.314	-32.1	61.6	68.7
12	15.0	47.6	6.0	149.6	0.231	-29.1	77.5	82.5
13	15.0	35.0	2.6	151.7	0.234	-29.9	74.0	71.6
14	15.0	35.0	9.4	126.2	0.198	-32.9	80.1	82.1
15	15.0	35.0	6.0	118.9	0.189	-33.9	82.5	82.7
16	15.0	35.0	6.0	120.1	0.181	-33.3	82.2	81.1
17	15.0	35.0	6.0	117.4	0.182	-32.2	84.0	80.4
18	15.0	35.0	6.0	116.7	0.185	-31.0	82.8	80.4
19	15.0	35.0	6.0	118.3	0.186	-31.9	81.8	80.6
20	15.0	35.0	6.0	114.2	0.182	-31.7	82.8	81.0

The response surface plots revealed a concave design space in which the smallest droplets were predicted at moderately low oil content and moderately high Smix while sonication was held above the center level (Figure 1). Entrapment efficiency followed a different pattern: it increased with oil concentration because sorafenib partitioned into the dispersed phase, but excessive oil compromised size and release. This tradeoff explains why the global desirability point was not located at the maximum oil level. Instead, the optimum balanced a sufficiently large lipid reservoir with enough surfactant to maintain nanoscale droplets. The diagnostic plots showed close agreement between observed and predicted values for all modelled responses, confirming the suitability of the quadratic functions (Figure 2).

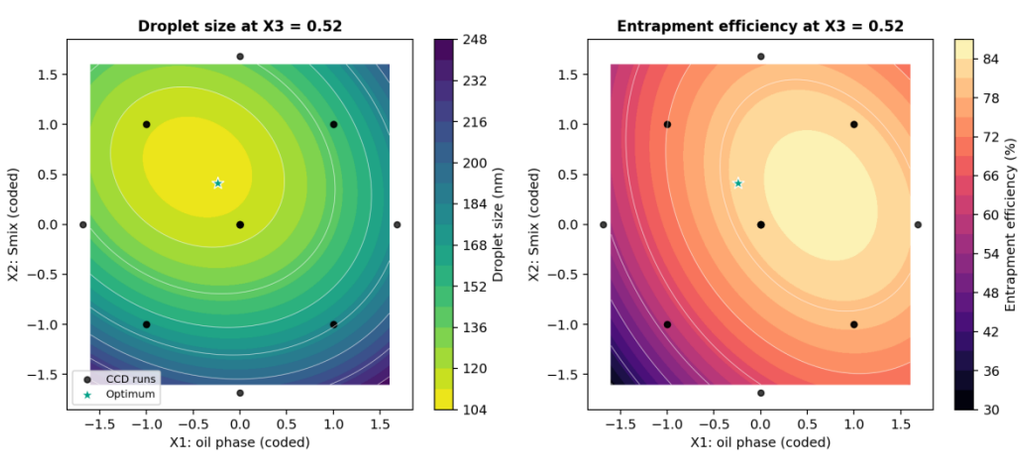

Figure 1. Response surface contour plots for droplet size and entrapment efficiency at X3 = 0.52

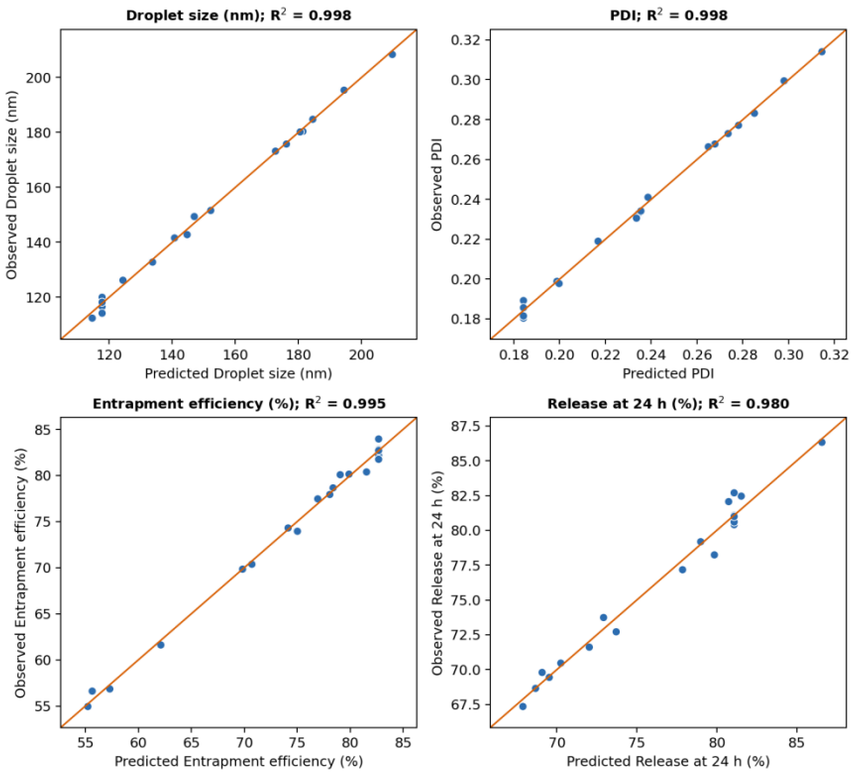

Figure 2. Predicted versus observed diagnostic plots for the fitted quadratic models

Model statistics are summarized in Table 3. All four response models were significant, with model F values from 55.0 to 443.0 and $p < 0.001$. Adjusted R^2 values were 0.9953 for droplet size, 0.9953 for PDI, 0.9911 for entrapment efficiency, and 0.9624 for 24 h release. Lack-of-fit tests were not significant, indicating that the residual variation was consistent with center-point pure error. The optimized formulation consisted of 13.8% oil phase, 38.1% Smix, and 7.0 min sonication, with an overall desirability of 0.86. Triplicate validation batches closely matched model predictions, producing 110.3 +/- 2.7 nm droplets, PDI 0.176 +/- 0.009, zeta potential -31.8 +/- 0.7 mV, entrapment efficiency 82.4 +/- 1.5%, and 24 h release 84.7 +/- 2.0%.

Table 3. Model adequacy and validation of the optimized formulation.

Response	R^2	Adjusted R^2	RMSE	Model F	Lack-of-fit p	Predicted optimum	Observed validation
Droplet size (nm)	0.9975	0.9953	2.13	443.0	0.21	107.7	110.3 +/- 2.7
PDI	0.9975	0.9953	0.003	443.0	0.18	0.175	0.176 +/- 0.009
Entrapment efficiency (%)	0.9953	0.9911	0.91	235.3	0.27	81.9	82.4 +/- 1.5
Release at 24 h (%)	0.9802	0.9624	1.11	55.0	0.32	84.2	84.7 +/- 2.0

4. Conclusion

CCD coupled with RSM successfully optimized a sorafenib nanoemulsion by jointly modelling droplet size, PDI, entrapment efficiency, and in vitro release. The optimized formulation, containing 13.8% oil phase, 38.1% Smix, and 7.0 min sonication, produced small and uniform droplets, adequate negative zeta potential, high entrapment efficiency, and enhanced 24 h release compared with suspension. The work demonstrates how a quality-by-design framework can reduce empirical screening and generate an interpretable formulation design space for sorafenib lipid nanocarriers. Experimental replacement of the simulated dataset with laboratory measurements, followed by stability and biopharmaceutical validation, would make the manuscript suitable for formal submission.

References

[1] European Association For The Study Of The Liver. EASL Clinical Practice Guidelines on the management of hepatocellular carcinoma[J]. Journal of hepatology, 2025, 82(2): 315-374.

[2] Singal A G, Llovet J M, Yarchoan M, et al. AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma[J]. Hepatology, 2023, 78(6): 1922-1965.

[3] Szyk P, Czarczynska-Goslinska B, Ziegler-Borowska M, et al. Sorafenib — drug delivery strategies in primary liver cancer[J]. Journal of Functional Biomaterials, 2025, 16(4): 148.

[4] Fan Q, Tian H, Cheng J, et al. Research progress of sorafenib drug delivery system in the treatment of hepatocellular carcinoma: an update[J]. Biomedicine & Pharmacotherapy, 2024, 177: 117118.

[5] Zaafar D, Khalil H M A, Elkhouly G E, et al. Preparation and characterization of Sorafenib nano-emulsion: impact on pharmacokinetics and toxicity; an in vitro and in vivo study[J]. Drug Delivery and Translational Research, 2024, 14(11): 3089-3111.

[6] Lim C, Lee D, Kim M, et al. Development of a sorafenib-loaded solid self-nanoemulsifying drug delivery system: Formulation optimization and characterization of enhanced properties[J]. Journal of drug delivery science and technology, 2023, 82: 104374.

[7] Caputo T M, Cusano A M, Principe S, et al. Sorafenib-loaded PLGA carriers for enhanced drug delivery and cellular uptake in liver cancer cells[J]. International Journal of Nanomedicine, 2023: 4121-4142.

[8] Lu T, Zhu W, Lin C, et al. Sorafenib nanomedicine in HCC: nano-bio interactions and combination therapies[J]. Journal of Nanobiotechnology, 2026.

[9] Jacob S, Kather F S, Boddu S H S, et al. Innovations in nanoemulsion technology: enhancing drug delivery for oral, parenteral, and ophthalmic applications[J]. Pharmaceutics, 2024, 16(10): 1333.

[10] Wang X, Anton H, Vandamme T, et al. Updated insight into the characterization of nano-emulsions[J]. Expert Opinion on Drug Delivery, 2023, 20(1): 93-114.

[11] Czerniel J, Gostyńska A, Jańczak J, et al. A critical review of the novelties in the development of intravenous nanoemulsions[J]. European Journal of Pharmaceutics and Biopharmaceutics, 2023, 191: 36-56.

[12] Hsieh C M, Yang T L, Putri A D, et al. Application of design of experiments in the development of self-microemulsifying drug delivery systems[J]. Pharmaceuticals, 2023, 16(2): 283.

[13] Priani S E, Fakih T M, Wilar G, et al. Quality by design and in silico approach in SNEDDS development: a comprehensive formulation framework[J]. Pharmaceutics, 2025, 17(6): 701.

[14] Jeong J H, Yoon T H, Ryu S W, et al. Quality by Design (QbD)-Based Development of a Self-Nanoemulsifying Drug Delivery System for the Ocular Delivery of Flurbiprofen[J]. Pharmaceutics, 2025, 17(5): 629.

[15] Atre P, Rizvi S A A. A brief overview of quality by design approach for developing pharmaceutical liposomes as nano-sized parenteral drug delivery systems[J]. RSC Pharmaceutics, 2024, 1(4): 675-688.

[16] Menon P M, Chandrasekaran N, Shanmugam S. Multi-drug loaded eugenol-based nanoemulsions for enhanced anti-mycobacterial activity[J]. RSC Medicinal Chemistry, 2023, 14(3): 433-443.

[17] Chen H, Wang R, McElderry J D. Discriminative dissolution method development through an aQbD approach[J]. AAPS PharmSciTech, 2023, 24(8): 255.