RESEARCH PAPER

Systematic AQbD-Driven Formulation of Clarithromycin SNEDDS for Enhanced Biopharmaceutical Performance

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ABSTRACT

The therapeutic efficacy of clarithromycin, a BCS Class II medication, is limited by its poor water solubility, acid instability, and variable oral bioavailability. Analytical Quality by Design (AQbD) was used to build a Self-Nanoemulsifying Drug Delivery System (SNEDDS) in order to get around these restrictions. To choose an appropriate oil, surfactant, and co-surfactant, solubility tests were carried out. Based on maximal solubility and emulsification efficiency, Tween 80 and Propylene Glycol were chosen as surfactants and co-surfactants, respectively, while Capryol 90 was chosen as the oil phase. Utilizing a 3^2 complete factorial design, formulation variables were optimized. Globule size, PDI, and drug release were chosen as dependent factors, whereas Capryol 90 concentration (X1) and Smix concentration (X2) were chosen as independent variables. With a globule size of 181.09 nm, a PDI of 0.269, and a drug release of 94.45%, the modified formulation demonstrated effective nanoemulsion production and improved dissolving behavior. When compared to the pure drug, the produced SNEDDS showed enhanced dissolving profile, rapid emulsification, high transparency, and strong thermodynamic stability. Clarithromycin SNEDDS formulation development and optimization for improved oral administration were successfully made possible by the AQbD technique.

Keywords: Clarithromycin; SNEDDS; Quality by Design (QbD); Solubility Enhancement; Oral Bioavailability; Nanoemulsion.

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1. Introduction

Oral drug delivery is the most preferred route of administration because of its convenience, patient compliance, cost-effectiveness, and ease of manufacturing. However, the successful delivery of many therapeutic agents through the oral route is often limited by poor aqueous solubility and inadequate bioavailability.[1] In recent years, a large number of newly developed drug molecules have been categorized under Biopharmaceutical Classification System (BCS) class II and IV, which exhibit poor water solubility and variable absorption characteristics. Since dissolution is the rate-limiting step for the absorption of such drugs, inadequate solubility significantly affects therapeutic efficacy and clinical performance.[2]

Clarithromycin is a semi-synthetic macrolide antibiotic widely used in the treatment of respiratory tract infections, skin infections, and Helicobacter pylori associated gastric ulcers. Clarithromycin has poor aqueous solubility, acid instability, and variable oral bioavailability despite its broad-spectrum antibacterial action. These factors lead to unpredictable plasma drug concentrations and decreased therapeutic efficacy.[3]. Clarithromycin's systemic availability is further restricted by substantial first-pass metabolism and degradation in the acidic stomach environment.

Conventional dosage forms such as tablets and suspensions are unable to adequately overcome these drawbacks. Therefore, development of an advanced formulation approach is essential to enhance the solubility, stability, and bioavailability of clarithromycin.[4]

Several techniques have been investigated for enhancing the solubility of poorly water-soluble drugs, including micronization, solid dispersions, inclusion complexation, nanosuspensions, and lipid-based drug delivery systems. Among these strategies, lipid-based formulations have gained considerable attention because of their ability to improve drug dissolution and intestinal absorption.[5]

Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) are one of the most promising lipid-based approaches for enhancing the oral delivery of lipophilic drugs. SNEDDS are isotropic mixtures of oils, surfactants, co-surfactants, and drug substances that spontaneously form fine oil-in-water nanoemulsions upon mild agitation in

gastrointestinal fluids. These systems produce droplets generally below 100 nm in size, thereby providing a large interfacial surface area for rapid drug release and absorption.[6]

The enhanced performance of SNEDDS is attributed to improved drug solubilization, increased membrane permeability, reduced variability associated with food effects, and possible lymphatic transport, which helps bypass hepatic first-pass metabolism. Additionally, SNEDDS offer more stable bioavailability profiles and shield acid-labile medications from gastrointestinal tract degradation. Surfactants and co-surfactants lower interfacial tension and stabilize the nanoemulsion system, while oils utilized in SNEDDS enhance drug solubilization and lymphatic absorption. In order to achieve desired physicochemical attributes and therapeutic effectiveness, these formulation components must be carefully chosen and optimized.[6,7]

Despite the benefits of SNEDDS, formulation development is still difficult because of problems such drug precipitation on dilution, stability challenges, and inconsistent emulsification efficiency. Therefore, to guarantee the creation of a reliable and repeatable distribution system, a methodical and scientific formulation approach is needed.In this sense, the Analytical Quality by Design (AQbD) method provides a logical framework for optimizing formulations. Critical quality attributes (CQAs), risk assessment, and optimization through Design of Experiments (DoE) are all part of AQbD.

which helps in understanding the influence of formulation variables on product performance. This method guarantees enhanced formulation robustness, scalability, reproducibility, and process comprehension.[9]

In order to increase the aqueous solubility, stability, and oral bioavailability of clarithromycin-loaded SNEDDS, the current study focuses on their development and optimization utilizing an AQbD strategy. When treating bacterial infections, the optimized formulation is anticipated to increase patient compliance, therapeutic efficacy, and clinical outcomes.[10]

2.1 Materials

Clarithromycin was obtained from Century Pharmaceuticals Ltd., Mumbai, India. Capryol 90 (Propylene glycol monocaprylate) was procured from Loba Chem, Mumbai, India and used as the oil phase. Tween 80 (Polysorbate 80) was obtained from Loba Chem, Mumbai, India and used as surfactant. Propylene glycol was procured from Loba Chem, Mumbai, India and used as co-surfactant. Methanol, phosphate buffer pH 6.8, potassium bromide, chloroform, acetic acid, sodium hydroxide, potassium hydroxide, sodium thiosulfate, carbon tetrachloride, starch indicator, and all other analytical grade chemicals and reagents used in the study were obtained from standard commercial sources and used without further purification. Double distilled water was freshly prepared whenever required throughout the study.

2.2 Solubility Studies

The solubility of Clarithromycin in various oils, surfactants, and co-surfactants was determined by using the shake flask method. Briefly, an excess amount of Clarithromycin was added separately into glass vials containing 2 mL of selected vehicles including oils, surfactants, and co-surfactants. Oils screened included Capryol 90, Oleic acid, Castor oil, Olive oil, and Soybean oil, while surfactants and co-surfactants screened included Tween 80, Cremophor RH40, Labrasol, Kolliphor EL, Tween 20, Span 80, PEG 400, Propylene Glycol, Transcutol P, and PEG 200. The mixtures were vortexed for 10 min using a cyclomixer to facilitate proper mixing of Clarithromycin with the vehicles.[11] The mixtures were then shaken for 48 h in a mechanical shaker maintained at 37 ± 0.5 °C to attain equilibrium. After equilibration, the samples were centrifuged at 3000 rpm for 15 min followed by filtration through a 0.45 µm membrane filter. The filtrates were suitably diluted with methanol and the amount of dissolved Clarithromycin in various vehicles was quantified using a UV-visible spectrophotometer at λmax 210 nm. The solubility of Clarithromycin in each vehicle was calculated in mg/mL, and the excipients showing maximum solubility were selected for further formulation development of SNEDDS.[12]

2.3 Screening of Surfactants for Emulsifying Ability

The emulsification ability of various surfactants was screened to identify suitable surfactants for preparation of Clarithromycin SNEDDS. Briefly, 300 mg of selected surfactant was added to 300 mg of selected oil phase (Capryol 90). The mixture was gently heated at 40–45 °C for homogenization of components and formation of isotropic mixture.

About 50 mg of the isotropic mixture was accurately weighed and diluted with double distilled water up to 50 mL to produce fine emulsion. The ease of emulsification was evaluated by noting the number of flask inversions required to obtain uniform emulsion. The resulting emulsions were visually observed for transparency and turbidity. The emulsions were allowed to stand for 2 h and percentage transmittance was measured at 650 nm using a UV-visible spectrophotometer taking double distilled water as blank. Surfactants producing rapid emulsification with maximum transparency were selected for further studies.[13-14]

2.4 Screening of Co-surfactants

The turbidimetric method was employed to evaluate the relative efficiency of co-surfactants in improving nanoemulsification ability of selected surfactants. Briefly, 0.2 g of surfactant was mixed with 0.1 g of co-surfactant. Capryol 90 (0.3 g) was added to this mixture and homogenized with gentle heating at 40–45 °C to obtain isotropic mixture. About 50 mg of the mixture was accurately weighed and diluted to 50 mL with double distilled water to form nanoemulsion.[15] The ease of emulsification was assessed by recording the number of flask inversions required to produce uniform emulsion. The emulsions were visually observed for turbidity and clarity and allowed to stand for 2 h. Percentage transmittance was measured at 650 nm using a UV-visible spectrophotometer taking double distilled water as blank. The co-surfactant producing clear nanoemulsion with high transmittance and minimum turbidity was selected for formulation optimization.[16]

2.5 Construction of Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed using the water titration method to identify the nanoemulsion region and optimize concentrations of oil, surfactant, and co-surfactant. The selected surfactant and co-surfactant were mixed in different weight ratios such as 1:1, 2:1, 3:1, and 4:1 to prepare Smix. Various combinations of oil and Smix were prepared in glass vials and titrated dropwise with distilled water under continuous magnetic stirring at room temperature. After each addition of water, the formulations were visually examined for clarity, transparency, phase separation, turbidity, and flowability. The compositions producing clear and transparent nanoemulsions were identified as nanoemulsion regions in the pseudo-ternary phase diagram. The optimized nanoemulsion region was selected for preparation and optimization of Clarithromycin-loaded SNEDDS formulations.[17-18]

2.6 Risk Assessment and Factor Selection

Risk assessment and factor selection were carried out to identify the Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) affecting the quality and performance of Clarithromycin-loaded Self-Nanoemulsifying Drug Delivery System (SNEDDS). A systematic Quality by Design (QbD) approach was employed for optimization of formulation variables and achievement of desired product quality attributes.[19,20] Initially, formulation variables such as oil concentration, surfactant concentration, and co-surfactant concentration were evaluated based on their influence on globule size, emulsification efficiency, drug release, and percentage transmittance. Risk assessment was performed using literature survey, preliminary trials, Ishikawa fishbone diagram, and Failure Mode and Effects Analysis (FMEA). Based on the risk evaluation, Capryol 90 concentration and Smix ratio were selected as independent variables, whereas globule size, polydispersity index (PDI), and drug release were selected as dependent variables for optimization studies.[21] A 3^2 full factorial design was further applied to optimize the formulation variables and to study their effect on the responses of Clarithromycin SNEDDS formulation.[20,21]

2.7 Spontaneous Emulsification Method

Clarithromycin-loaded SNEDDS were prepared by spontaneous emulsification method using Capryol 90 as oil phase and optimized Smix ratio. Accurately weighed Clarithromycin (100 mg) was dissolved in Capryol 90 (5–10 mL) with continuous magnetic stirring. The required quantity of Smix containing Tween 80 and Propylene Glycol (20–40 mL) was added slowly with continuous stirring to obtain a clear isotropic mixture. Distilled water (50–75 mL) was then added gradually under gentle stirring to allow spontaneous nanoemulsion formation. The prepared formulations were vortexed or mildly sonicated to obtain uniform dispersion and finally stored in airtight containers for further evaluation studies.[19,21]

Table 1: Independent Variables and Their Levels

Factor	Independent Variable	Low Level (-1)	Medium Level (0)	High Level (+1)

X1	Capryol 90 (mL)	5	7	10
X2	Smix Ratio (Tween 80 : Propylene Glycol) (mL)	20	30	40

Table 2: Experimental Batches of Clarithromycin SNEDDS

Batch Code	Clarithromycin (mg)	Capryol 90 (mL)	Smix (mL)	Distilled Water (mL)
F1	100	5	20	75
F2	100	5	30	65
F3	100	5	40	55
F4	100	7	20	73
F5	100	7	30	63
F6	100	7	40	53
F7	100	10	20	70
F8	100	10	30	60
F9	100	10	40	50

2.8 Evaluation of prepared liquid SNEDDS

Rheological Study

The rheological behavior of optimized Clarithromycin-loaded SNEDDS was evaluated using a Brookfield viscometer to determine viscosity and flow characteristics of the formulation. Measurements were carried out at room temperature using suitable spindle at different rotational speeds, and viscosity values were recorded in centipoise (cP).[22,23]

Globule Size and Zeta Potential

The globule size and zeta potential of optimized Clarithromycin SNEDDS were determined using Dynamic Light Scattering (DLS) technique with zeta sizer after suitable dilution with distilled water. Average globule size, polydispersity index (PDI), and zeta potential values were recorded to evaluate uniformity and physical stability of nanoemulsion droplets.[22,24]

Dispersibility Test

The dispersibility test of optimized SNEDDS was performed by adding 1 mL formulation into 500 mL distilled water maintained at 37 ± 0.5 °C under gentle agitation. The formulations were visually observed for rate of emulsification, clarity, and phase separation. Formulations producing clear nanoemulsion rapidly were considered acceptable.[23,25]

Percentage Transmittance

The optimized SNEDDS formulation was diluted with distilled water (1:100) and analyzed for percentage transmittance using UV-visible spectrophotometer at 650 nm using distilled water as blank. Higher transmittance values indicated formation of transparent nanoemulsion with smaller globule size.[22,26]

Cloud Point Determination

Cloud point determination was carried out to evaluate thermal stability of optimized Clarithromycin SNEDDS. The diluted formulation was heated gradually, and the temperature at which the formulation became cloudy was recorded as cloud point. Formulations having cloud point above physiological temperature were considered stable for oral administration.[23,27]

Emulsification Time

Emulsification time was determined using USP dissolution apparatus II containing 500 mL distilled water maintained at 37 ± 0.5 °C under gentle agitation. The time required for complete formation of nanoemulsion after addition of formulation was recorded visually.[24,28]

Drug Content

Drug content of optimized Clarithromycin SNEDDS was determined by dissolving formulation equivalent to 10 mg drug in methanol followed by suitable dilution with phosphate buffer pH 6.8. The absorbance was measured spectrophotometrically at 210 nm and percentage drug content was calculated.[22,29]

FTIR Study

FTIR study was carried out to evaluate compatibility between Clarithromycin and selected excipients used in SNEDDS formulation. FTIR spectra of pure drug, physical mixture, and optimized formulation were recorded by potassium bromide pellet method in the range of 4000–400 cm^{-1} and compared for any significant interaction.[25,30]

***In-vitro* Drug Release Study**

In-vitro dissolution study of optimized Clarithromycin SNEDDS was performed using USP dissolution apparatus II (paddle type) containing 900 mL phosphate buffer pH 6.8 maintained at 37 ± 0.5 °C and stirred at 50 rpm. Samples were withdrawn at predetermined intervals, filtered, diluted suitably, and analyzed spectrophotometrically at 210 nm. The cumulative percentage drug release of SNEDDS was compared with pure Clarithromycin.[24,29]

Thermodynamic Stability Study

Thermodynamic stability studies were carried out to evaluate physical stability of optimized Clarithromycin-loaded SNEDDS under stress conditions. Formulations showing phase separation, precipitation, creaming, or cracking were rejected.[22,26]

a. Heating–Cooling Cycle

The optimized formulation was subjected to heating–cooling cycles between 4 °C and 45 °C with storage at each temperature for 48 h. The cycle was repeated three times and formulations were observed for instability or phase separation.[23,27]

b. Centrifugation Study

The optimized SNEDDS formulation was centrifuged at 3500 rpm for 30 min and examined visually for phase separation, creaming, or drug precipitation.[24,28]

c. Freeze–Thaw Cycle

The optimized formulation was subjected to freeze–thaw cycles between −20 °C and +25 °C with storage for 48 h at each temperature. After completion of cycles, formulations were evaluated for clarity and phase stability.[25,29]

3. Results and discussion

Solubility Study

The solubility study demonstrated that Clarithromycin showed maximum solubility in Capryol 90 (85.4 ± 2.3 mg/ml) among the selected oils, indicating excellent drug solubilization capacity. Oleic acid exhibited good solubility, whereas Castor oil and Olive oil showed moderate solubility. The least soluble oil was soybean. Because of its excellent solubilization efficiency, Capryol 90 was chosen as the oil phase for the creation of Clarithromycin-loaded SNEDDS. Transcutol P showed the greatest solubility for Clarithromycin (112.7 ± 3.1 mg/ml) among the examined surfactants and co-surfactants, followed by Propylene Glycol (96.2 ± 2.8 mg/ml). Additionally, Tween 80 demonstrated outstanding emulsification and solubility. Because of their superior drug solubilization and emulsification qualities, Tween 80 and Propylene Glycol/Transcutol P were chosen for additional SNEDDS formulation research based on the results.

Table 3: Solubility of Clarithromycin in Various Oils, Surfactant/ Co-surfactant

Sr. No.	Vehicle Type	Vehicle	Solubility (mg/ml)	Observation
1	Oil	Capryol 90	85.4 ± 2.3	Excellent solubility
2	Oil	Oleic acid	62.8 ± 1.9	Good solubility
3	Oil	Castor oil	48.6 ± 1.7	Moderate solubility
4	Surfactant	Tween 80	64.8 ± 2.1	Excellent solubility
5	Surfactant	Cremophor RH40	59.6 ± 1.8	Very good solubility
6	Co-surfactant	Propylene Glycol	96.2 ± 2.8	Excellent solubility
7	Co-surfactant	Transcutol P	112.7 ± 3.1	Highest solubility

Selection and Screening of Oil

In order to choose the best oil phase for SNEDDS formulation, the solubility of clarithromycin was assessed in a variety of oils. Capryol 90 demonstrated the best emulsification qualities and the maximum solubility of Clarithromycin (85.4 ± 2.3 mg/ml) among the studied oils. Its medium-chain glyceride structure, which offers better affinity for lipophilic medications, may be responsible for the increased solubility. Consequently, Capryol 90 was chosen as the oil phase for the creation of SNEDDS loaded with clarithromycin.

Selection and Screening of Surfactant

The solubilization capacity and emulsification efficiency of the chosen oil phase were used to screen the surfactants.Among the evaluated surfactants, Tween 80 exhibited excellent solubility (64.8 ± 2.1 mg/ml) and rapid self-emulsification behavior due to its high HLB value. Tween 80 produced transparent nanoemulsion with improved stability and minimum emulsification time. Hence, Tween 80 was selected as surfactant for SNEDDS formulation.

Selection and Screening of Co-surfactant

Co-surfactants were evaluated based on drug solubilization and miscibility with oil and surfactant system. Propylene Glycol showed excellent solubility of Clarithromycin (96.2 ± 2.8 mg/ml) along with good miscibility and emulsification performance. It effectively improved flexibility of interfacial film and promoted

spontaneous nanoemulsification. Therefore, Propylene Glycol was selected as co-surfactant for development of Clarithromycin SNEDDS.

Construction of Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed using water titration method to identify nanoemulsion region and optimize concentration of oil, surfactant, and co-surfactant. Different Smix ratios of Tween 80 and Propylene Glycol (1:1, 2:1, and 3:1) were evaluated with Capryol 90. The Smix ratio of 2:1 demonstrated the largest nanoemulsion region with faster self-emulsification and superior transparency among the ratios examined. The study demonstrated that stable Clarithromycin-loaded SNEDDS require the right ratio of oil, surfactant, and co-surfactant.

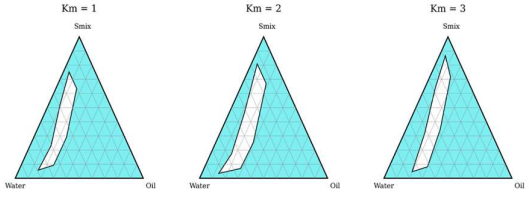


Fig 1: Pseudo-ternary phase diagrams

Risk Assessment and Factor Selection

To determine the Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) influencing the performance and quality of the Clarithromycin-loaded SNEDDS formulation, a risk assessment was conducted.Various formulation variables such as concentration of Capryol 90, Tween 80, Propylene Glycol, and Smix ratio were evaluated for their influence on globule size, polydispersity index (PDI), emulsification time, percentage transmittance, and drug release.

Among the evaluated variables, oil concentration and Smix ratio showed significant effect on nanoemulsion characteristics. Increase in surfactant and co-surfactant concentration improved emulsification efficiency, reduced globule size, and enhanced transparency of the formulation. Based on the risk assessment study, Capryol 90 concentration (X1) and Smix ratio (X2) were selected as independent variables for optimization of Clarithromycin SNEDDS formulation.

Formulation and optimization of CLA-SNEDDS using DoE

Table 4: Experimental Results of 3² Full Factorial Design

Std	Run	Factor 1	Factor 2	Response 1	Response 2	Response 3
		A: Capryol 90	**B: Smix Ratio**	**Globule size**	**PDI**	**Drug release**
		mL	**mL**	**nm**		**%**
9	1	5	20	242.6	0.389	78.42
4	2	5	30	218.4	0.341	84.65
3	3	5	40	196.8	0.302	81.71
1	4	7	20	228.5	0.352	82.16
7	5	7	30	178.9	0.274	95.29
8	6	7	40	186.3	0.281	91.42
6	7	10	20	251.7	0.401	76.35
2	8	10	30	224.2	0.336	85.14
5	9	10	40	208.6	0.315	89.87

Optimization of Clarithromycin-loaded SNEDDS Using DoE

Optimization of Clarithromycin-loaded SNEDDS was carried out using a 3² full factorial design to evaluate the effect of independent variables, namely Capryol 90 concentration (A) and Smix ratio (B), on globule size of the formulation. The experimental results indicated that both variables significantly influenced nanoemulsion characteristics.

Table 5: Fit Summary for Globule Size

Source	Sequential p-value	Adjusted R^2	Predicted R^2	Remark
Linear	0.0497	0.5099	0.3680	—
2FI	0.9523	0.4123	-0.1869	—
Quadratic	0.0631	0.8448	0.5777	Suggested
Cubic	0.9953	0.5389	-10.8604	Aliased

The quadratic model was selected as the most suitable model for optimization of globule size due to higher adjusted R^2 and predicted R^2 values. The model showed good agreement with the experimental data and was found to be statistically significant.

Table 6: ANOVA for Quadratic Model – Globule Size

Source	F-value	p-value	Remark
Model	9.71	0.0452	Significant
A – Capryol 90	1.25	0.3444	Not significant
B – Smix Ratio	29.92	0.0120	Significant

AB	0.0150	0.9103	Not significant
A^2	12.94	0.0368	Significant
B^2	3.00	0.1819	Not significant

The ANOVA results demonstrated that the quadratic model was significant with Model F-value of 9.71 and p-value of 0.0452. Among the formulation variables, Smix ratio (B) and quadratic term of oil concentration (A^2) showed significant effect on globule size. Increase in Smix ratio resulted in reduction of globule size due to efficient reduction in interfacial tension and better stabilization of nano-sized droplets.

Table 7: Fit Statistics for Globule Size

Parameter	Value
Std. Dev.	9.73
Mean	215.11
C.V. %	4.53
R^2	0.9418
Adjusted R^2	0.8448
Predicted R^2	0.5777
Adeq Precision	9.2362

The model showed high R^2 value (0.9418), indicating good correlation between predicted and experimental values. Adeq Precision value of 9.236 indicated adequate signal and confirmed suitability of the model for navigating the design space.

Polynomial Equation for Globule Size

The polynomial equation generated for globule size in terms of coded factors was as follows:

$$\text{Globule Size} = 189.81 + 4.45A - 21.81B + 0.5921AB + 25.97A^2 + 11.92B^2$$

The polynomial equation indicated that increase in Smix ratio reduced globule size, whereas higher oil concentration increased globule size of the nanoemulsion system. The obtained results confirmed that optimized concentration of oil and surfactant system is essential for development of stable Clarithromycin-loaded SNEDDS with smaller globule size and improved stability.

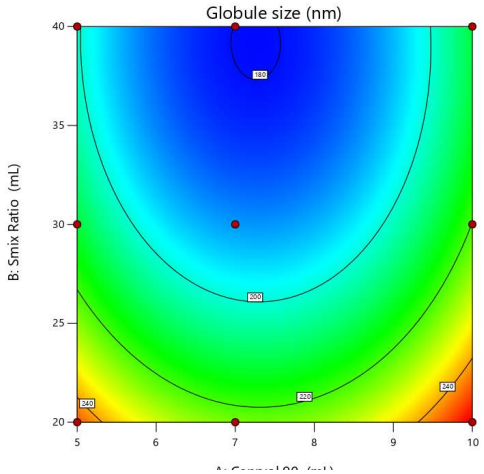


Figure 2: Response Surface Plot Showing Effect of Capryol 90 and Smix Ratio on Globule Size.

Response 2: Polydispersity Index (PDI)

Optimization of Clarithromycin-loaded SNEDDS was further evaluated for polydispersity index (PDI) using 3^2 full factorial design. PDI is an important parameter indicating uniformity of droplet distribution within nanoemulsion system. Lower PDI values indicate formation of homogeneous and stable nanoemulsion.

Table 8: Fit Summary for PDI

Source	Sequential p-value	Adjusted R^2	Predicted R^2	Remark
Linear	0.0433	0.5317	0.3395	—
2FI	0.9879	0.4381	-0.4349	—
Quadratic	0.0327	0.9042	0.7151	Suggested
Cubic	0.8657	0.7847	-4.5383	Aliased

The quadratic model was selected as the most suitable model for optimization of PDI due to higher adjusted and predicted R^2 values. The model adequately explained the effect of formulation variables on PDI response.

Table 9: ANOVA for Quadratic Model – PDI

Source	F-value	p-value	Remark
Model	16.11	0.0224	Significant
A – Capryol 90	0.3556	0.5929	Not significant
B – Smix Ratio	52.63	0.0054	Significant
AB	0.0015	0.9716	Not significant
A^2	20.69	0.0199	Significant
B^2	5.64	0.0980	Not

			significant

The ANOVA results showed that the quadratic model was statistically significant with F-value of 16.11 and p-value of 0.0224. Among the formulation variables, Smix ratio (B) and quadratic effect of oil concentration (A^2) significantly influenced PDI values. Increase in Smix concentration resulted in reduction of PDI due to improved stabilization and uniform distribution of nano-sized droplets.

Polynomial Equation for PDI

$PDI=0.2858+0.0033A-0.0407B-0.0003AB+0.0462A^2+0.0230B^2$

The polynomial equation indicated that increase in Smix ratio reduced PDI, whereas higher oil concentration slightly increased PDI values. The optimized formulation showed lower PDI, indicating formation of uniform and stable nanoemulsion system.

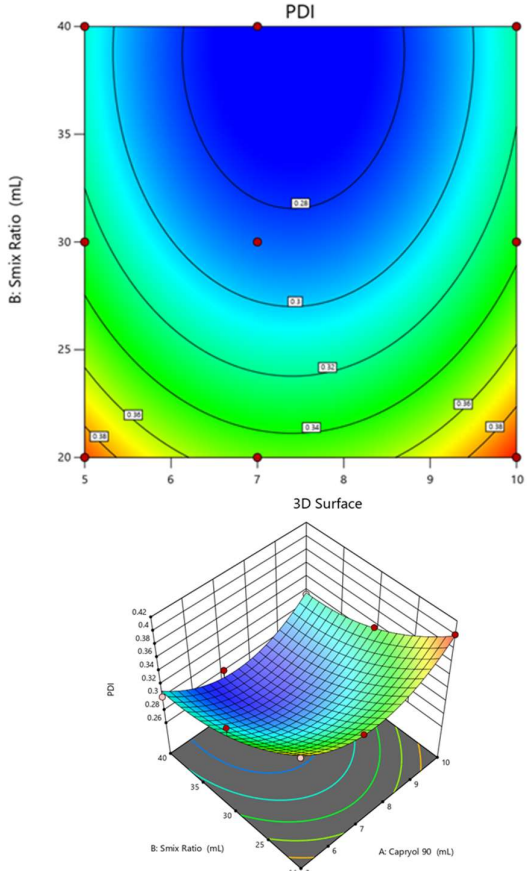


Figure 3: Response Surface Plot Showing Effect of Capryol 90 and Smix Ratio on PDI.

Response 3: Drug Release

The effect of formulation variables on percentage drug release of Clarithromycin-loaded SNEDDS was evaluated using quadratic model generated through 3^2 full factorial design. Drug release is an important parameter indicating dissolution enhancement capability of SNEDDS formulation.

Table 10: Fit Summary for Drug Release

Source	Sequential p-value	Adjusted R^2	Predicted R^2	Remark
Linear	0.2435	0.1674	-0.2812	—
2FI	0.4245	0.1320	-1.0947	—
Quadratic	0.0278	0.8674	0.5093	Suggested
Cubic	0.8354	0.7223	-6.1428	Aliased

The quadratic model was selected as the best fitted model for drug release response due to satisfactory adjusted and predicted R^2 values. The model demonstrated good agreement between experimental and predicted results.

Table 11: ANOVA for Quadratic Model – Drug Release

Source	F-value	p-value	Remark
Model	11.46	0.0360	Significant
A – Capryol 90	1.41	0.3201	Not significant
B – Smix Ratio	23.76	0.0165	Significant
AB	4.94	0.1127	Not significant
A^2	19.77	0.0212	Significant
B^2	9.94	0.0511	Slightly significant

The ANOVA results indicated that the quadratic model was significant with F-value of 11.46 and p-value of 0.0360. Smix ratio (B) and quadratic term of oil concentration (A^2) significantly affected drug release. Increase in surfactant concentration enhanced drug release due to improved solubilization and spontaneous formation of nano-sized droplets with larger surface area.

Table 12: Fit Statistics for Drug Release

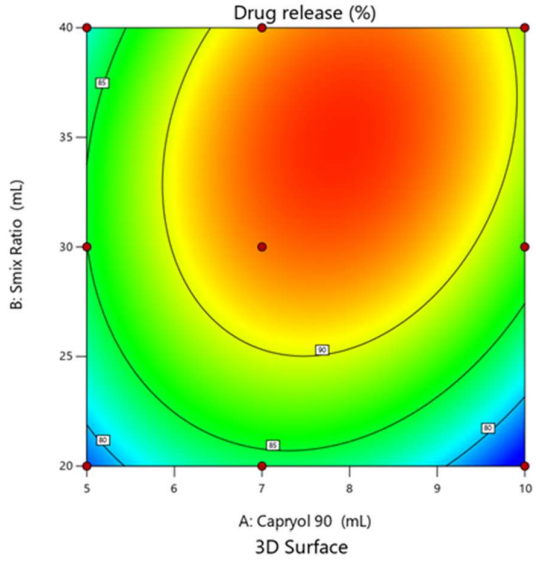
Parameter	Value
Std. Dev.	2.26
Mean	85.00
C.V. %	2.66
R^2	0.9503
Adjusted R^2	0.8674
Predicted R^2	0.5093
Adeq Precision	9.6918

The high R^2 value (0.9503) indicated excellent correlation between predicted and observed responses. Adeq Precision value greater than 4 confirmed adequate signal and suitability of the model for optimization studies.

Polynomial Equation for Drug Release

Drug Release=93.50+1.10A+4.51B+2.50AB−7.45 A^2−5.04B^2

The polynomial equation demonstrated that increase in Smix ratio significantly enhanced drug release of Clarithromycin from SNEDDS formulation. The optimized formulation showed maximum drug release due to improved nanoemulsification and enhanced dissolution behavior.



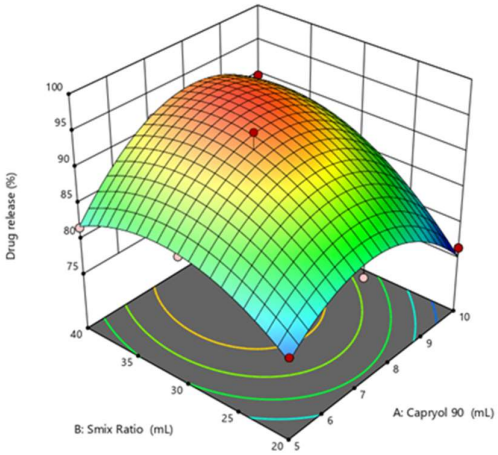


Figure 4: Response Surface Plot Showing Effect of Capryol 90 and Smix Ratio on Drug Release.

Optimization of Clarithromycin-loaded SNEDDS was successfully carried out using Design of Experiments (DoE) approach by evaluating the effect of Capryol 90 concentration (A) and Smix ratio (B) on globule size, PDI, and drug release. The results demonstrated that Smix ratio significantly influenced all responses, where increase in surfactant concentration reduced globule size and PDI while enhancing drug release due to improved emulsification and drug solubilization.

The optimized formulation showed minimum globule size, low PDI, rapid self-emulsification, and maximum drug release, indicating formation of stable and uniform nanoemulsion system. Statistical analysis confirmed significance of the quadratic model with adequate precision values greater than 4, indicating suitability of the model for optimization studies.

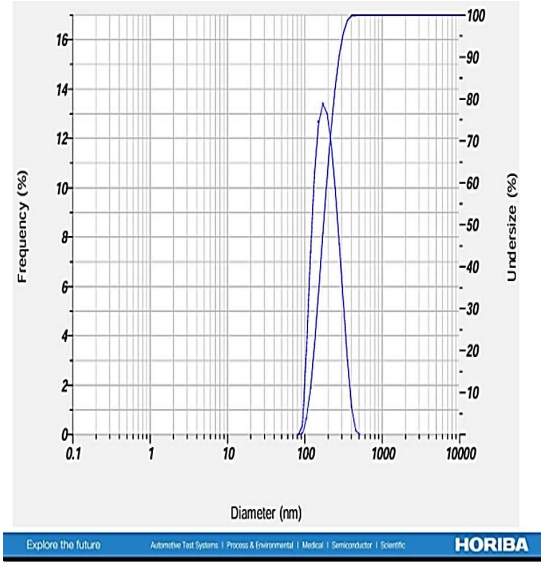
Among all formulations, batch F5 was selected as optimized formulation due to its superior physicochemical properties, excellent clarity, improved dissolution characteristics, and enhanced self-emulsification efficiency. The optimized SNEDDS formulation exhibited stable nanoemulsion formation without phase separation or precipitation, confirming its suitability for oral delivery of Clarithromycin.

Characterization and Evaluation of Optimized SNEDDS

The optimized Clarithromycin-loaded SNEDDS batch F5 was found to be clear to pale yellow, free-flowing, and physically stable without phase separation or precipitation. SEM analysis revealed spherical and uniformly distributed nano-sized droplets with smooth surface morphology, confirming successful nanoemulsion formation.

Table 13: Evaluation Parameters of Optimized SNEDDS (F5)

Parameter	Result
Appearance	Clear to pale yellow
Viscosity	42.6 ± 1.4 cP
Globule Size	178.9 nm
PDI	0.274
Zeta Potential	−23 mV
% Transmittance	96.8 ± 0.6 %
Emulsification Time	38 ± 2 sec
Drug Content	98.42 ± 0.56 %
Flow Behavior	Newtonian flow
Phase Separation	Not observed



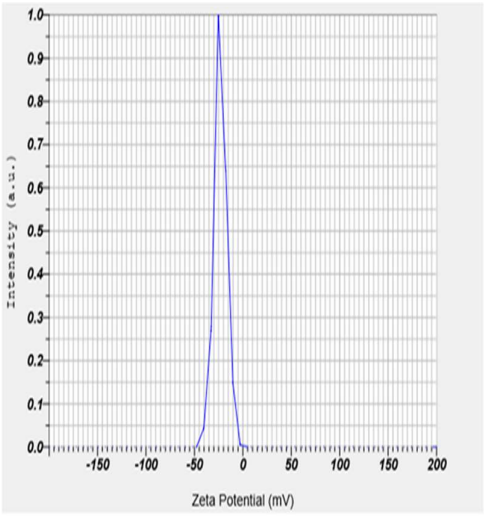


Figure 5: Particle size and zeta potential of optimized batch

The production of a transparent and stable nanoemulsion system was indicated by the improved formulation's low viscosity, quick self-emulsification, and high percentage transmittance. The zeta potential value showed that the formulation had acceptable physical stability, and the globule size and low PDI verified uniform droplet distribution.

Dispersibility and Cloud Point Study

The improved batch F5 quickly generated a clear nanoemulsion without precipitation or aggregation and demonstrated outstanding dispersibility upon dilution. The cloud point was discovered to be higher than physiological temperature, indicating that the formulation is suitable for oral administration and has acceptable thermal stability.

FTIR Study

The optimized batch F5's FTIR spectrum displayed all of the distinctive peaks of clarithromycin without any notable shifting or disappearance, demonstrating the lack of a chemical interaction between the medication and excipients and verifying the formulation's component compatibility.

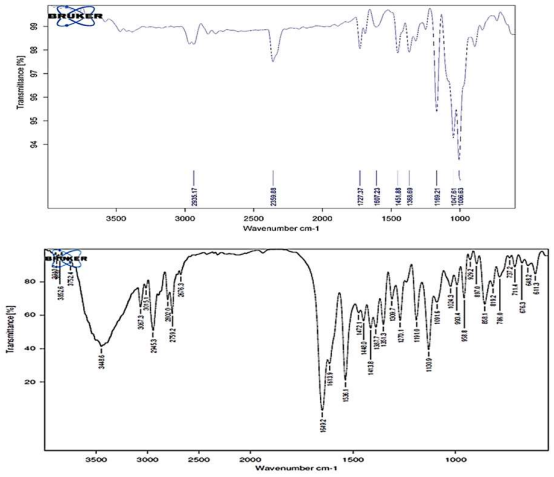


Figure 6: FTIR Spectrum of Drug and Optimized SNEDDS Batch F5.

***In-vitro* Drug Release Study**

When compared to pure clarithromycin, the optimized SNEDDS formulation (F5) demonstrated noticeably better dissolving behavior. Due to the spontaneous development of nano-sized droplets with a greater surface area and better drug solubilization, Batch F5 showed a total drug release of 98.64 ± 1.42% within 120 minutes.

Table 14: *In-vitro* Drug Release of Optimized SNEDDS Batch F5

Formulation	% Drug Release at 120 min
Pure Clarithromycin	Lower drug release
Optimized SNEDDS (F5)	98.64 ± 1.42 %

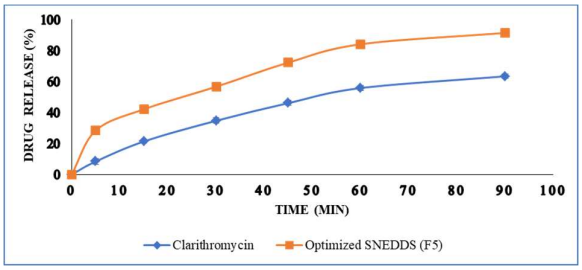


Figure 7: Comparative *In-vitro* Drug Release of Pure Drug and Optimized Batch F5.

Thermodynamic Stability Study

Excellent stability of optimized SNEDDS batch F5 under stress conditions was demonstrated by thermodynamic stability experiments. Phase separation, creaming, cracking, or precipitation did not occur during the freeze-thaw cycle, centrifugation study, or heating-cooling cycle.

Table 15: Thermodynamic Stability Study of Optimized Batch F5

atch	Heating–Cooling Cycle	Centrifugation Study	Freeze–Thaw Cycle
5	No phase separation	No creaming/cracking	Stable formulation observed.

The results showed that optimized batch F5 was suitable as a stable oral Clarithromycin-loaded SNEDDS formulation due to its superior physical stability, effective self-emulsification, and improved dissolving characteristics.

4. Conclusion

In order to improve the solubility and dissolution of clarithromycin, the current work effectively created and improved a Clarithromycin-loaded Self Nano Emulsifying Drug Delivery System (SNEDDS) employing Capryol 90, Tween 80, and Propylene Glycol. When compared to pure drug, the optimized formulation showed nano-sized globules, a low polydispersity index, quick self-emulsification, superior physical stability, and improved drug release. Globule size, PDI, and drug release were all significantly impacted by formulation variables, according to optimization using Design of Experiments (DOE). The acquired results demonstrated that SNEDDS is a viable and efficient lipid-based method for enhancing the oral administration and dissolving of medications that are poorly soluble in water, such as clarithromycin.

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