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**Research Article**

# Development and Optimization of Apixaban-Loaded Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Enhanced Dissolution and Potential Oral Bioavailability

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## Abstract

Apixaban is an orally active, direct Factor Xa inhibitor used for the prevention and treatment of thromboembolic disorders. Its poor aqueous solubility may limit dissolution and consequently affect oral absorption. The present study aimed to develop and optimize an Apixaban-loaded Self-Nanoemulsifying Drug Delivery System (SNEDDS) using suitable lipid excipients and a Quality-by-Design approach to improve its dissolution characteristics. Solubility screening identified Capryol® 90, Kolliphor® RH40, and Transcutol® P as suitable oil, surfactant, and co-surfactant, respectively. A pseudo-ternary phase diagram was constructed, and a 2:1 Smix ratio was selected based on the largest nanoemulsion region. A Box–Behnken Design comprising 17 experimental runs was employed, with droplet size, polydispersity index (PDI), and drug release selected as responses. Numerical optimization predicted an optimized composition of 20% Capryol® 90, 55% Kolliphor® RH40, and 25% Transcutol® P, with a desirability of 0.987. The optimized formulation exhibited a droplet size of 118.32 ± 2.14 nm, PDI of 0.214 ± 0.01, zeta potential of −24.86 ± 1.12 mV, and drug content of 99.12 ± 0.56%. In vitro dissolution studies demonstrated 95.84 ± 1.35% drug release from the optimized SNEDDS at 60 min compared with 72.48 ± 1.28% for pure Apixaban. The dissolution data showed the best fit to the Korsmeyer–Peppas model ($R^2 = 0.9968$; $n = 0.47$). The optimized formulation also demonstrated good thermodynamic and accelerated stability over 3 months. Overall, the developed Apixaban-loaded SNEDDS significantly improved the in vitro dissolution performance of Apixaban and represents a promising approach for enhancing its oral delivery. Further in vivo pharmacokinetic studies are required to confirm improvement in oral bioavailability.

**Keywords:** Apixaban; SNEDDS; self-nanoemulsifying drug delivery system; lipid-based formulation; Box–Behnken design; nanoemulsion; dissolution; oral bioavailability

## 1. Introduction

Oral administration remains the most convenient and widely accepted route for drug delivery; however, inadequate aqueous solubility is a major obstacle in the development of orally administered drugs. Poorly water-soluble compounds frequently exhibit dissolution-limited absorption and consequently variable or incomplete systemic exposure. [1-3] The Biopharmaceutics Classification System (BCS) identifies solubility and intestinal permeability as major determinants of oral drug absorption. Drugs with low solubility but relatively high permeability are categorized as BCS Class II compounds and are particularly suitable candidates for formulation strategies aimed at improving dissolution.[6] Lipid-based drug delivery systems have received considerable attention for improving the oral performance of poorly water-soluble drugs. These systems can maintain a drug in a solubilized state, reduce dependence on dissolution of the crystalline drug, and facilitate formation of colloidal structures following contact with gastrointestinal fluids.[7-9] Among lipid-based systems, self-emulsifying drug delivery systems (SEDDS), self-microemulsifying drug delivery systems (SMEDDS), and SNEDDS are widely investigated for enhancing the oral delivery of poorly soluble compounds.[4,5,10]

SNEDDS are generally isotropic mixtures containing an oil phase, surfactant, and co-surfactant or co-solvent. Following dilution with an aqueous medium and mild agitation, they spontaneously form fine oil-in-water nanoemulsions. The resulting small droplets provide a large interfacial surface area, which can facilitate drug dissolution

and maintain the drug in a solubilized or dispersed state.[4,5,10,11]

Apixaban is an orally active and selective direct Factor Xa inhibitor. It inhibits Factor Xa and consequently reduces thrombin generation and fibrin clot formation. Clinical studies have established its efficacy in preventing stroke and systemic embolism in patients with atrial fibrillation.[12] Apixaban exhibits predictable pharmacokinetics, with maximum plasma concentrations generally occurring approximately 3–4 h after oral administration and a terminal half-life of approximately 8–15 h depending on the population studied.[13,14]

Despite its favorable pharmacological profile, formulation of Apixaban remains challenging because of its limited aqueous solubility. Improving drug solubilization and dissolution is therefore a rational formulation strategy. Previous pharmaceutical approaches, including solid dispersions and lipid-based nanocarriers, have demonstrated that improving Apixaban solubilization can enhance its dissolution and, in some cases, oral exposure.[15]

The present investigation was therefore designed to develop an Apixaban-loaded SNEDDS using systematic excipient screening, pseudo-ternary phase diagram analysis, and statistical optimization through a Box–Behnken Design. The work focused on improving the in vitro dissolution characteristics of Apixaban while establishing a physically stable and reproducible nanoemulsifying formulation.

## 2. Materials and Methods

### 2.1 Materials

Apixaban was used as the active pharmaceutical ingredient. The lipid excipients, surfactants, co-surfactants, chemicals, and reagents were selected according to their pharmaceutical acceptability, solubilization capacity, and suitability for SNEDDS development. The drug and excipients were obtained from the suppliers documented in the thesis.

**Table 1. Materials Used for the Development of Apixaban-Loaded SNEDDS**

| Category | Material | Supplier/Manufacturer |
|---|---|---|
| Drug | Apixaban | Yarrow Chem Products, Mumbai, India |
| Oil | Capryol® 90 | Gattefossé, France |
| Oil | Labrafac™ Lipophile WL 1349 | Gattefossé, France |
| Oil | Maisine® CC | Gattefossé, France |
| Oil | Oleic acid | Loba Chemie Pvt. Ltd., Mumbai, India |
| Surfactant | Tween® 80 | |
| Co-surfactant | Propylene glycol | |
| Surfactant | Cremophor® EL | BASF SE, Germany |
| Surfactant | Kolliphor® RH40 | BASF SE, Germany |
| Co-surfactant | Transcutol® P | Gattefossé, France |
| Co-surfactant | PEG 400 | Merck Life Science Pvt. Ltd., India |

### 2.2 Instruments

The formulation and evaluation studies were performed using calibrated analytical and laboratory instruments. The principal instruments included UV–visible spectrophotometry, FTIR, DSC, particle-size analysis, zeta-potential measurement, dissolution testing, centrifugation, and routine formulation equipment.

### 2.3 Experimental Design and Formulation Development

The overall experimental strategy consisted of drug characterization, solubility screening, selection of formulation components, pseudo-ternary phase diagram construction, preparation of Apixaban-loaded SNEDDS, statistical optimization using Box–Behnken Design, and evaluation of the optimized formulation.

### 2.4 Characterization of Apixaban

Before formulation development, Apixaban was characterized with respect to its organoleptic properties, melting point, aqueous/solvent solubility, UV absorption characteristics, calibration curve, FTIR spectrum, drug–excipient compatibility, and DSC behavior.[16]

#### 2.4.1 Organoleptic Evaluation

A small quantity of Apixaban was visually examined under normal laboratory conditions for colour, appearance, odour, texture, and physical state. The observations were recorded and compared with reported characteristics.

#### 2.4.2 Melting Point Determination

A small quantity of powdered Apixaban was filled into a sealed capillary tube and placed in a digital melting-point apparatus. The temperature was gradually increased until complete melting occurred, and the melting temperature was recorded.

#### 2.4.3 UV Spectrophotometric Analysis

Approximately 10 mg of Apixaban was accurately weighed and dissolved in methanol. Suitable dilutions were prepared using phosphate buffer pH 6.8. The solution was scanned from 200–400 nm using a Shimadzu UV-1800 spectrophotometer to determine the wavelength of maximum absorption.[17]

#### 2.4.4 Calibration Curve

Appropriate Apixaban standard solutions were prepared in phosphate buffer pH 6.8, and their absorbance values were measured at the predetermined λmax. A plot of absorbance against concentration was constructed to obtain the calibration relationship used for quantitative estimation.

#### 2.4.5 FTIR Spectroscopy

FTIR analysis was performed using a Shimadzu IR Affinity-1S instrument. Spectra of pure Apixaban and the drug–excipient mixture were recorded over 4000–400 $cm^{-1}$ using the KBr pellet technique to assess characteristic functional groups and possible drug–excipient interactions.

### 2.4.6 Differential Scanning Calorimetry

DSC analysis was carried out using a Shimadzu DSC-60 Plus to assess the thermal behavior of Apixaban and the optimized formulation. The thermograms were evaluated for changes in the characteristic endothermic peak and possible evidence of interaction or alteration in crystallinity.

### 2.5 Solubility Screening

Solubility screening was performed to identify the most suitable oil, surfactant, and co-surfactant for the development of Apixaban-loaded SNEDDS. Excess Apixaban was added separately to Capryol® 90, Labrafac™ Lipophile WL 1349, Maisine® CC, oleic acid, Cremophor® EL, Tween® 80, Kolliphor® RH40, Transcutol® P, PEG 400, and propylene glycol. The samples were vortex mixed and equilibrated at 25 ± 2°C for 48 h to attain equilibrium. After equilibration, the samples were centrifuged at 5000 rpm for 15 min and the supernatants were filtered through a 0.45-µm membrane filter. The filtrates were suitably diluted with methanol and analyzed using UV spectrophotometry. Among the tested oils, Capryol® 90 exhibited the highest Apixaban solubility (48.62 ± 0.81 mg/mL) and was therefore selected as the oil phase. Among the surfactants, Kolliphor® RH40 showed the highest drug solubilization (82.47 ± 1.24 mg/mL) and was selected as the surfactant. Among the co-surfactants, Transcutol® P demonstrated the highest solubility (126.84 ± 1.56 mg/mL), followed by PEG 400 (95.61 ± 1.18 mg/mL) and propylene glycol (68.45 ± 0.87 mg/mL). Therefore, Transcutol® P was selected as the co-surfactant. On the basis of the overall solubility screening results, Capryol® 90, Kolliphor® RH40, and Transcutol® P were selected as the oil, surfactant, and co-surfactant, respectively, for further development of the Apixaban-loaded SNEDDS.[18-20]

### 2.6 Selection of Formulation Components

**Table 2. Selected Formulation Components**

| Component | Selected Excipient | Selection Basis |
|---|---|---|
| Oil | Capryol® 90 | Highest Apixaban solubility |
| Surfactant | Kolliphor® RH40 | High solubilization and emulsification efficiency |
| Co-surfactant | Transcutol® P | Highest Apixaban solubility and interfacial flexibility |

### 2.7 Construction of Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed using the water-titration method. Capryol® 90 was used as the oil phase, while Kolliphor® RH40 and Transcutol® P were combined at different Smix ratios. The investigated Smix ratios were 1:1, 2:1, 3:1, 4:1, and 1:2.

For each Smix ratio, the oil and Smix were mixed in different proportions ranging from 1:9 to 9:1. Distilled water was added gradually under continuous stirring at room temperature. The systems were visually examined for transparency, turbidity, phase separation, flowability, and drug precipitation. Clear and stable systems were considered part of the nanoemulsion region.

### 2.8 Preparation of Apixaban-Loaded SNEDDS

Apixaban-loaded SNEDDS were prepared using the simple isotropic mixing method. The selected components were Capryol® 90, Kolliphor® RH40, and Transcutol® P. The required quantities were accurately weighed, and the oil phase was transferred into a clean, dry glass vial. Kolliphor® RH40 was gradually incorporated under continuous stirring, followed by Transcutol® P. Apixaban was then added gradually.

The mixture was stirred at 500–700 rpm for approximately 30–45 min at 40 ± 2°C until a clear and homogeneous isotropic formulation was obtained. The formulation was cooled to room temperature and stored in tightly closed amber-coloured glass vials until evaluation.

### 2.9 Optimization Using Box–Behnken Design

A three-factor, three-level Box–Behnken Design (BBD) was employed using Design-Expert® Software Version 13.0 (Stat-Ease Inc., Minneapolis, MN, USA). Three formulation variables were selected based on the solubility and phase-diagram studies.

**Table 3. Independent Variables and Their Levels**

| Factor | Variable | Low (−1) | Medium (0) | High (+1) |
|---|---|---|---|---|
| A | Capryol® 90 (% w/w) | 15 | 20 | 25 |
| B | Kolliphor® RH40 (% w/w) | 45 | 55 | 65 |
| C | Transcutol® P (% w/w) | 20 | 25 | 30 |

**Table 4. Dependent Variables and Optimization Criteria**

| Response | Parameter | Goal |
|---|---|---|
| $Y_1$ | Droplet size | Minimize |
| $Y_2$ | PDI | Minimize |
| $Y_3$ | Drug release at 60 min | Maximize |

The design contained 17 experimental runs, including five centre-point runs.

The experimental formulations were prepared using the same isotropic-mixing procedure, while the concentrations of oil, surfactant, and co-surfactant were varied according to the design matrix. Apixaban quantity was kept constant.

### 2.10 Evaluation of Optimized SNEDDS

The optimized SNEDDS was evaluated for visual appearance, self-emulsification time, percentage transmittance, drug content, robustness to dilution, cloud point, thermodynamic stability, droplet size, PDI, zeta

potential, refractive index, viscosity, in vitro dissolution, drug-release kinetics, and accelerated stability to assess its physicochemical and formulation performance.[21-23]

**Visual Appearance:** The formulation was examined for colour, clarity, homogeneity, precipitation, and phase separation.

**Self-Emulsification Time:** The time required for the formulation to form a clear or slightly bluish nanoemulsion after dilution with water under gentle agitation was recorded.

**Percentage Transmittance:** Optical clarity of the diluted formulation was determined spectrophotometrically.

**Drug Content:** The formulation was suitably diluted and analyzed by UV spectrophotometry using the Apixaban calibration curve.

**Robustness to Dilution:** The formulation was diluted with distilled water, 0.1 N HCl, and phosphate buffer pH 6.8 and examined for precipitation, turbidity, and phase separation.

Cloud Point: The temperature at which visible cloudiness appeared upon gradual heating of the diluted formulation was recorded.

**Thermodynamic Stability:** The formulation was subjected to heating–cooling, centrifugation, and freeze–thaw cycles and examined for phase separation and precipitation.

**Droplet Size and PDI:** Droplet size and PDI were measured using a Malvern particle-size analyzer/Zetasizer after suitable dilution.

**Zeta Potential:** Zeta potential was determined using a Zetasizer Nano ZS after appropriate dilution.

**Refractive Index and Viscosity:** Refractive index and viscosity were measured using suitable calibrated instruments.

### 2.11 In Vitro Dissolution Study

The dissolution study was performed using USP Dissolution Apparatus II (paddle method). The dissolution medium consisted of 900 mL phosphate buffer pH 6.8, maintained at 37 ± 0.5°C, with a paddle speed of 50 rpm. Samples were withdrawn at predetermined intervals, suitably diluted, and analyzed spectrophotometrically at the predetermined wavelength. The cumulative percentage drug release was calculated and plotted against time.[24-25]

### 2.12 Drug Release Kinetics

The dissolution data of the optimized SNEDDS were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models. The model with the highest $R^2$ value was considered the best fit for the drug-release profile.

### 2.13 Accelerated Stability Study

The optimized Apixaban-loaded SNEDDS was subjected to accelerated stability testing at 40 ± 2°C/75 ± 5% RH for 3 months. Samples were evaluated at initial, 1-, 2-, and 3-month intervals for appearance, drug content, droplet size, PDI, zeta potential, and drug release.

**Table 5. Accelerated Stability Study Design**

| Storage condition | Duration | Sampling intervals |
|---|---|---|
| 40 ± 2°C / 75 ± 5% RH | 3 months | 0, 1, 2 and 3 months |

### 2.14 Statistical Analysis

The experimental data generated from the BBD were analyzed using Design-Expert® Version 13.0. Analysis of variance (ANOVA) was performed to assess the significance of the developed response-surface models. Numerical optimization was performed using a desirability-function approach, with droplet size and PDI minimized and drug release maximized. The optimized formulation was subsequently prepared experimentally and compared with the predicted values. The thesis reports model $R^2$ values of 0.9886 for droplet size, 0.9824 for PDI, and 0.9928 for drug release, demonstrating strong model fitting.

**Table 6. Predicted Optimized Formulation**

| Parameter | Optimized value |
|---|---|
| Capryol® 90 | 20% |
| Kolliphor® RH40 | 55% |
| Transcutol® P | 25% |
| Predicted droplet size | 118.3 nm |
| Predicted PDI | 0.214 |
| Predicted drug release | 96.3% |
| Desirability | 0.987 |

**Table 7. Experimental Validation of Optimized Formulation**

| Response | Predicted | Experimental | % Error |
|---|---|---|---|
| Droplet size (nm) | 118.3 | 119.1 | 0.68 |
| PDI | 0.214 | 0.217 | 1.40 |
| Drug release (%) | 96.3 | 95.8 | 0.52 |

The experimental values were in close agreement with the predicted values, with all prediction errors below 2%.

## 3. Results and Discussion

### 3.1 Characterization of Apixaban

The physicochemical characterization of Apixaban was performed before formulation development to establish its identity, analytical characteristics, thermal behavior, and compatibility with the selected excipients. The characterization results provided the basis for further development of the Apixaban-loaded SNEDDS.

#### 3.1.1 Solubility Characteristics

Apixaban exhibited very low solubility in distilled water, whereas it was soluble in methanol and showed higher solubility in DMSO and PEG 400. The poor aqueous solubility observed for Apixaban supported the need for a lipid-based formulation approach to improve drug solubilization and dissolution.

### 3.1.2 UV Spectrophotometric Analysis

The UV absorption spectrum of Apixaban was recorded in phosphate buffer pH 6.8 over the wavelength range of 200–400 nm. Apixaban exhibited a maximum absorption at 279 nm, which was subsequently used for quantitative estimation during the formulation and dissolution studies.

### 3.1.3 Calibration Curve

A calibration curve was prepared using Apixaban concentrations ranging from 2–12 µg/mL. The regression equation obtained was:

$$Y = 0.0587X + 0.0016$$

with a correlation coefficient of $R^2 = 0.9994$. The high linearity confirmed the suitability of the UV spectrophotometric method for quantitative estimation of Apixaban.

**Table 8. Calibration Curve Data of Apixaban**

| Concentration (µg/mL) | Absorbance |
|---|---|
| 2 | 0.118 |
| 4 | 0.234 |
| 6 | 0.347 |
| 8 | 0.468 |
| 10 | 0.589 |
| 12 | 0.705 |

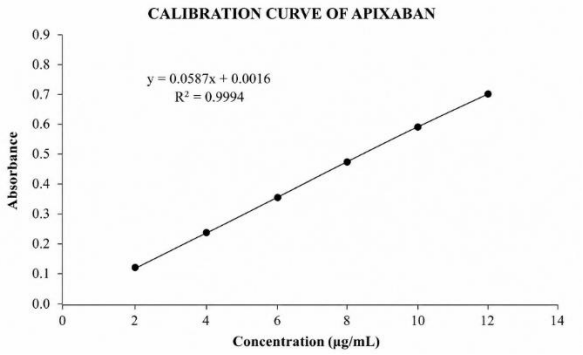


**Figure 1. Calibration Curve of Apixaban**

### 3.1.4 FTIR Spectral Analysis

The FTIR spectrum of pure Apixaban exhibited characteristic peaks at 3308, 3058, 2932, 1683, 1612, and 1248 $cm^{-1}$. These peaks were close to the reported characteristic absorption bands of Apixaban, confirming the identity of the drug. No additional significant peaks were observed.

**Table 9. FTIR Spectrum of Apixaban**

| Reported Peak ($cm^{-1}$) | Observed Peak ($cm^{-1}$) |
|---|---|
| 3310 | 3308 |
| 3060 | 3058 |
| 2935 | 2932 |
| 1685 | 1683 |
| 1615 | 1612 |
| 1250 | 1248 |

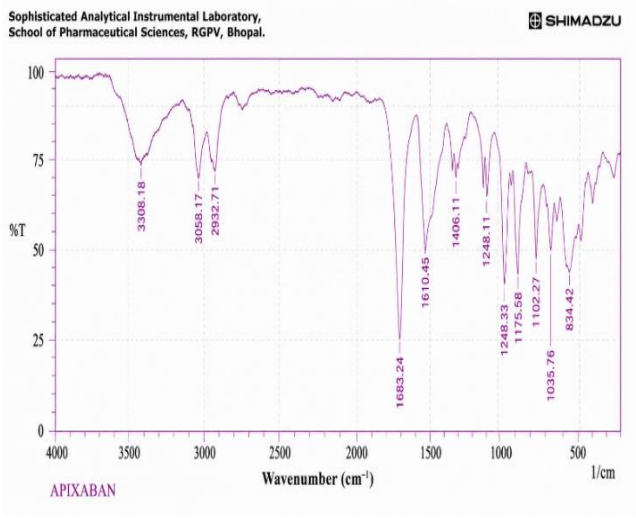


**Figure 2. FTIR Spectrum of Pure Apixaban**

The close agreement between reported and observed peaks indicated that the characteristic chemical groups of Apixaban remained intact.

### 3.1.5 Drug–Excipient Compatibility

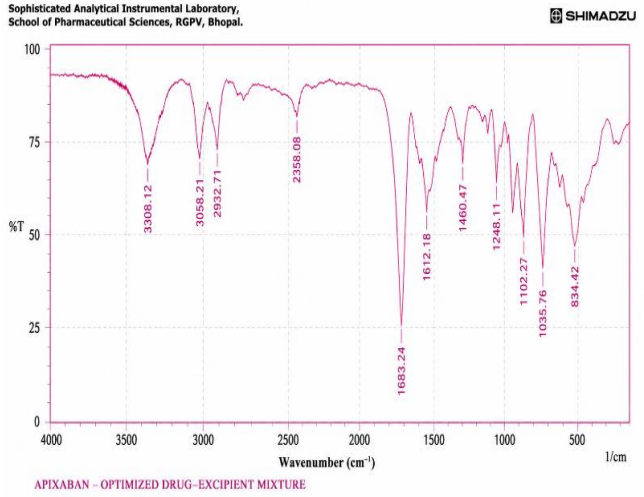


**Figure 3. FTIR Spectrum of Optimized Drug–Excipient Mixture**

FTIR spectra of pure Apixaban and the optimized drug–excipient mixture were compared. The characteristic Apixaban peaks were retained without the appearance of significant new peaks or major shifts, indicating the absence of significant chemical incompatibility between Apixaban and the selected formulation components.

### 3.1.6 Differential Scanning Calorimetry

DSC analysis showed a sharp endothermic peak for pure Apixaban at 236.1°C, while the optimized SNEDDS exhibited a broad, less intense peak at 231.92°C. The slight shift and reduced intensity indicated altered drug crystallinity and successful incorporation of Apixaban into the formulation, with no significant thermal incompatibility observed.

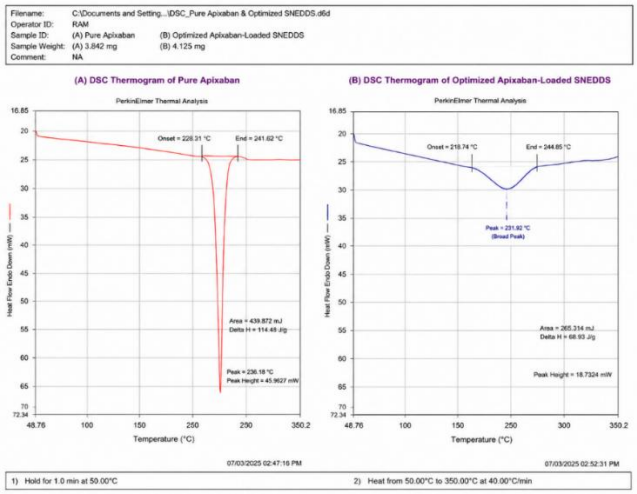


**Figure 4. DSC Thermogram**

**Table 10. DSC Analysis of Apixaban**

| Sample | Endothermic Peak |
|---|---|
| Pure Apixaban | 236.1°C |
| Optimized SNEDDS | 231.92°C |

### 3.2 Solubility Screening of Apixaban

Solubility screening was performed to identify the most appropriate oil, surfactant, and co-surfactant for development of the SNEDDS. The selected components were expected to provide high drug solubilization and minimize the possibility of drug precipitation following aqueous dilution.

#### 3.2.1 Solubility in Oils

Among the investigated oils, Capryol® 90 demonstrated the highest Apixaban solubility of 48.62 ± 0.81 mg/mL, followed by Labrafac™ Lipophile WL1349, Maisine® CC, and oleic acid.

**Table 11. Solubility of Apixaban in Different Oils**

| Oil | Solubility (mg/mL) |
|---|---|
| Capryol® 90 | 48.62 ± 0.81 |
| Labrafac™ Lipophile WL1349 | 35.84 ± 0.67 |
| Maisine® CC | 28.43 ± 0.54 |
| Oleic acid | 18.91 ± 0.42 |

Capryol® 90 was therefore selected as the oil phase because it provided the highest drug-solubilizing capacity among the tested oils.

#### 3.2.2 Solubility in Surfactants

Kolliphor® RH40 exhibited the highest Apixaban solubility among the investigated surfactants, with a value of 82.47 ± 1.24 mg/mL. Cremophor® EL and Tween® 80 showed comparatively lower solubilization capacities.

**Table 12. Solubility of Apixaban in Different Surfactants**

| Surfactant | Solubility (mg/mL) |
|---|---|
| Kolliphor® RH40 | **82.47 ± 1.24** |
| Cremophor® EL | 74.56 ± 1.08 |
| Tween® 80 | 61.73 ± 0.95 |

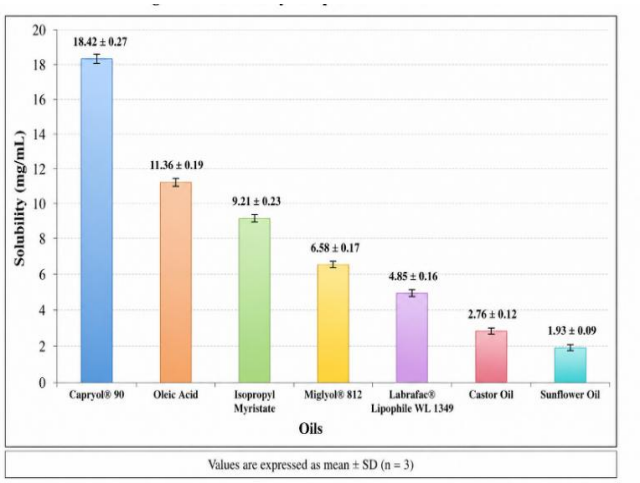


**Figure 5. Solubility of Apixaban in Different Surfactants**

Based on its superior solubilization and emulsification characteristics, Kolliphor® RH40 was selected as the surfactant.

#### 3.2.3 Solubility in Co-surfactants

Among the tested co-surfactants, Transcutol® P showed the highest Apixaban solubility of 126.84 ± 1.56 mg/mL, followed by PEG 400 and propylene glycol.

**Table 13. Solubility of Apixaban in Different Co-surfactants**

| Co-surfactant | Solubility (mg/mL) |
|---|---|
| Transcutol® P | 126.84 ± 1.56 |
| PEG 400 | 95.61 ± 1.18 |
| Propylene glycol | 68.45 ± 0.87 |

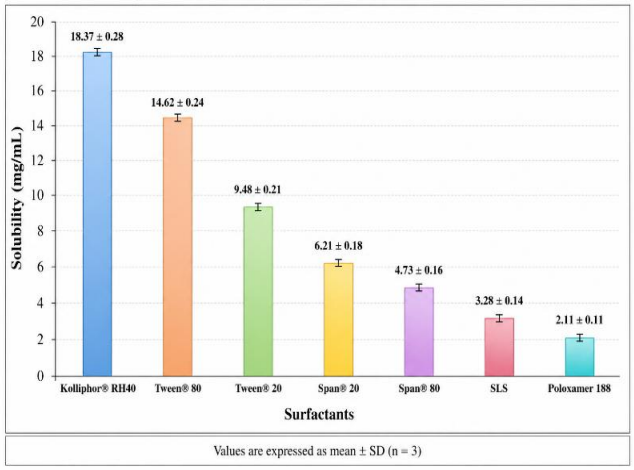


**Figure 6. Solubility of Apixaban in Different Co-surfactants**

Thus, Transcutol® P was selected as the co-surfactant for further formulation development.

### 3.3 Pseudo-Ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed using Capryol® 90 as the oil, Kolliphor® RH40 as the surfactant, and Transcutol® P as the co-surfactant. Smix ratios of 1:1, 2:1, 3:1, 4:1, and 1:2 were investigated.

**Table 14. Observations from Pseudo-Ternary Phase Diagram**

| Smix Ratio | Nanoemulsion Region | Observation |
|---|---|---|
| 1:1 | Moderate | Transparent nanoemulsion |
| 2:1 | Largest | Excellent self-emulsification |
| 3:1 | Large | Stable nanoemulsion |
| 4:1 | Moderate | Smaller nanoemulsion region |
| 1:2 | Small | Limited region and slight turbidity |

The 2:1 Smix ratio produced the largest nanoemulsion region and was therefore selected for subsequent formulation optimization.

### 3.4 Optimization of Apixaban-Loaded SNEDDS Using Box–Behnken Design

The Box–Behnken Design generated 17 experimental formulations using three independent variables: Capryol® 90, Kolliphor® RH40, and Transcutol® P. The responses selected for optimization were droplet size, PDI, and drug release at 60 min.

**Table 15. Experimental Responses of BBD Formulations**

| Formulation | Droplet Size (nm) | PDI | Drug Release (%) |
|---|---|---|---|
| F1 | 152.6 | 0.341 | 86.8 |
| F2 | 168.3 | 0.387 | 82.6 |
| F3 | 118.4 | 0.226 | 95.4 |
| F4 | 132.5 | 0.248 | 92.8 |
| F5 | 140.7 | 0.286 | 89.5 |
| F6 | 158.1 | 0.332 | 84.7 |
| F7 | 112.3 | 0.214 | 96.2 |
| F8 | 124.6 | 0.237 | 94.3 |
| F9 | 149.2 | 0.318 | 87.4 |
| F10 | 128.5 | 0.244 | 91.8 |
| F11 | 136.1 | 0.271 | 90.6 |
| F12 | 109.8 | 0.198 | 97.3 |
| F13 | 118.2 | 0.214 | 96.1 |
| F14 | 117.9 | 0.211 | 96.4 |
| F15 | 118.5 | 0.216 | 95.9 |
| F16 | 118.1 | 0.213 | 96.2 |
| F17 | 118.3 | 0.214 | 96.3 |

F12 exhibited the lowest experimental droplet size and PDI and the highest drug release among the experimental runs. The center-point formulations showed close response values, indicating good reproducibility.

#### 3.4.1 Statistical Analysis

The ANOVA results demonstrated that all three response models were statistically significant ($p < 0.0001$). The $R^2$ values of 0.9886, 0.9824, and 0.9928 for droplet size, PDI, and drug release, respectively, indicated excellent model fitting.

**Table 16. ANOVA Summary**

| Response | Model F-value | p-value | $R^2$ | Significance |
|---|---|---|---|---|
| Droplet size | 42.83 | <0.0001 | 0.9886 | Significant |
| PDI | 31.24 | <0.0001 | 0.9824 | Significant |
| Drug release | 58.16 | <0.0001 | 0.9928 | Significant |

#### 3.4.2 Response Surface and Contour Plot Analysis

The response-surface and contour plots demonstrated that increasing Kolliphor® RH40 and Transcutol® P within the investigated formulation range generally reduced droplet size and PDI and improved drug release. Increasing the oil concentration beyond the optimum level resulted in an increase in droplet size, which was attributed to the increased viscosity of the formulation.

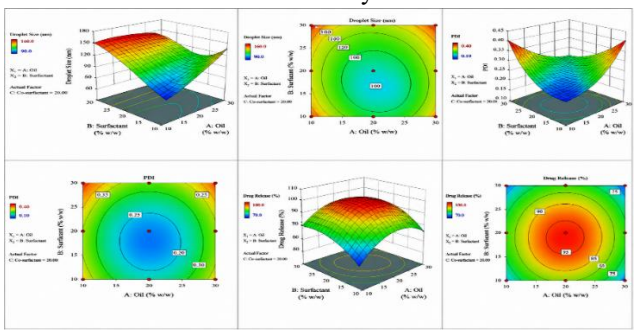

**Figure 7. 3D Response Surface Plot for Droplet Size**

#### 3.4.3 Numerical Optimization

Numerical optimization was performed by minimizing droplet size and PDI while maximizing drug release. The optimized composition predicted by Design-Expert® software consisted of 20% Capryol® 90, 55% Kolliphor® RH40, and 25% Transcutol® P, with a desirability value of 0.987.

**Table 17. Predicted Optimized Formulation**

| Parameter | Optimized Value |
|---|---|
| Capryol® 90 | 20% |
| Kolliphor® RH40 | 55% |
| Transcutol® P | 25% |
| Predicted droplet size | 118.3 nm |
| Predicted PDI | 0.214 |
| Predicted drug release | 96.3% |
| Desirability | 0.987 |

#### 3.4.4 Validation of Optimized Formulation

The predicted formulation was prepared experimentally and evaluated. The experimental values closely agreed with the predicted values, with prediction errors below 2%.

**Table 18. Validation of Optimized Formulation**

| Response | Predicted | Experimental | Error (%) |
|---|---|---|---|
| Droplet size (nm) | 118.3 | 119.1 | 0.68 |
| PDI | 0.214 | 0.217 | 1.40 |
| Drug release (%) | 96.3 | 95.8 | 0.52 |

The low prediction errors demonstrate good agreement between the statistical model and experimental observations, confirming the suitability of the BBD model for optimization.

### 3.5 Evaluation of Optimized SNEDDS

The optimized formulation was evaluated for visual appearance, self-emulsification time, percentage transmittance, drug content, dilution robustness, cloud point, thermodynamic stability, droplet size, PDI, zeta potential, refractive index, and viscosity.

**Table 19. Evaluation of Optimized Apixaban-Loaded SNEDDS**

| Evaluation parameter | Result |
|---|---|
| Appearance | Pale yellow, clear and transparent |
| Homogeneity | Homogeneous |
| Phase separation | Not observed |
| Drug precipitation | Not observed |
| Self-emulsification time | 24.35 ± 1.18 s |
| Percentage transmittance | 98.74 ± 0.42% |
| Drug content | 99.12 ± 0.56% |
| Droplet size | 118.32 ± 2.14 nm |
| PDI | 0.214 ± 0.01 |
| Zeta potential | −24.86 ± 1.12 mV |
| Refractive index | 1.432 ± 0.004 |
| Viscosity | 42.63 ± 1.35 cP |

The optimized formulation was pale yellow, clear, transparent, and homogeneous, without visible precipitation or phase separation. The self-emulsification time of 24.35 ± 1.18 s indicated rapid emulsification after contact with the aqueous medium. The high transmittance value of 98.74 ± 0.42% further indicated good optical clarity.

The mean droplet size of 118.32 ± 2.14 nm and PDI of 0.214 ± 0.01 demonstrated the formation of a nanosized and relatively uniform droplet population. The zeta potential of −24.86 ± 1.12 mV indicated satisfactory electrostatic stability, while the refractive index and viscosity values supported the isotropic and flowable nature of the formulation.

### 3.6 In Vitro Dissolution Study

The optimized SNEDDS demonstrated a substantially faster dissolution profile than pure Apixaban. At 5 min, the SNEDDS released 24.36 ± 0.68%, compared with 8.24 ± 0.52% from pure Apixaban. At 30 min, release reached 84.36 ± 1.18% from the SNEDDS compared with 47.25 ± 1.08% from the pure drug. At 60 min, the optimized SNEDDS achieved 95.84 ± 1.35% drug release, whereas pure Apixaban achieved 72.48 ± 1.28%.

**Table 20. In Vitro Drug Release Profile**

| Time (min) | Pure Apixaban (%) | Optimized SNEDDS (%) |
|---|---|---|
| 0 | 0.00 | 0.00 |
| 5 | 8.24 ± 0.52 | 24.36 ± 0.68 |
| 10 | 15.82 ± 0.74 | 41.75 ± 0.84 |
| 15 | 24.63 ± 0.83 | 58.92 ± 0.91 |
| 20 | 33.41 ± 0.95 | 71.48 ± 1.04 |
| 30 | 47.25 ± 1.08 | 84.36 ± 1.18 |
| 45 | 61.82 ± 1.16 | 92.64 ± 1.22 |
| 60 | 72.48 ± 1.28 | 95.84 ± 1.35 |

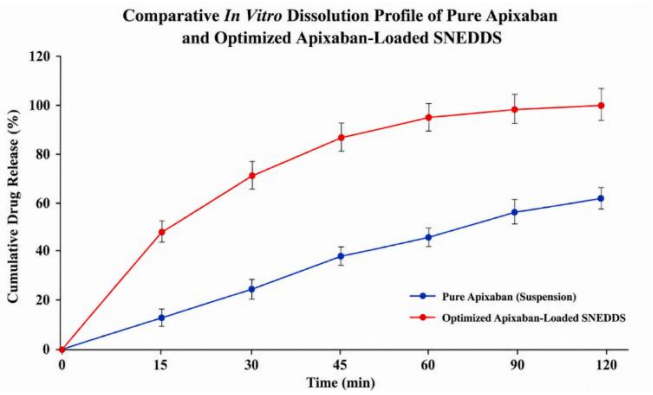


**Figure 23. Comparative In Vitro Dissolution Profile of Pure Apixaban and Optimized Apixaban-Loaded SNEDDS**

The improved dissolution was attributed in the thesis to spontaneous formation of nanosized droplets, increased surface area, and maintenance of Apixaban in a solubilized state by the selected lipid excipients.

### 3.7 Drug Release Kinetics

The dissolution data were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models.

**Table 21. Drug Release Kinetic Parameters**

| Kinetic Model | Regression Equation | $R^2$ |
|---|---|---|
| Zero-order | y = 1.581x + 4.214 | 0.9412 |
| First-order | y = −0.018x + 2.012 | 0.9726 |
| Higuchi | y = 13.482x + 6.118 | 0.9884 |
| Korsmeyer–Peppas | y = 0.564x + 1.326 | 0.9968 |
| Hixson–Crowell | y = −0.009x + 4.658 | 0.9679 |

The Korsmeyer–Peppas model exhibited the highest $R^2$ (0.9968) and was therefore the best-fitting model. The release exponent was n = 0.47, which, according to the thesis interpretation, indicated predominantly Fickian diffusion. The relatively high $R^2$ obtained with the Higuchi model (0.9884) also supported a diffusion-associated release process.

### 3.8 Accelerated Stability Study

The optimized SNEDDS was subjected to accelerated stability testing at 40 ± 2°C/75 ± 5% RH for 3 months. The formulation remained clear throughout the

study, with no visible phase separation, creaming, or drug precipitation.

**Table 22. Accelerated Stability Study of Optimized SNEDDS**

| Parameter | Initial | 1 Month | 2 Months | 3 Months |
|---|---|---|---|---|
| Appearance | Clear | Clear | Clear | Clear |
| Drug content (%) | 99.12 ± 0.56 | 98.84 ± 0.48 | 98.41 ± 0.62 | 97.96 ± 0.71 |
| Droplet size (nm) | 118.32 ± 2.14 | 119.45 ± 2.26 | 120.81 ± 2.43 | 122.18 ± 2.56 |
| PDI | 0.214 ± 0.01 | 0.218 ± 0.01 | 0.223 ± 0.02 | 0.229 ± 0.02 |
| Zeta potential (mV) | −24.86 ± 1.12 | −24.42 ± 1.06 | −23.94 ± 1.18 | −23.58 ± 1.24 |
| Drug release at 60 min (%) | 95.84 ± 1.35 | 95.42 ± 1.28 | 94.86 ± 1.21 | 94.18 ± 1.32 |

Only minor changes were observed during storage. Drug content decreased from 99.12 ± 0.56% to 97.96 ± 0.71%, while droplet size increased slightly from 118.32 ± 2.14 to 122.18 ± 2.56 nm. PDI showed a small increase from 0.214 ± 0.01 to 0.229 ± 0.02, whereas zeta potential remained sufficiently negative. Drug release at 60 min decreased from 95.84 ± 1.35% to 94.18 ± 1.32%. These results indicate good short-term accelerated stability of the optimized formulation.

**3.9 Overall Discussion**

The results demonstrate that systematic excipient screening successfully identified Capryol® 90, Kolliphor® RH40, and Transcutol® P as suitable components for Apixaban-loaded SNEDDS. Capryol® 90 provided the highest solubility among the investigated oils, while Kolliphor® RH40 and Transcutol® P provided superior solubilization among the surfactants and co-surfactants, respectively.

The pseudo-ternary phase diagram further demonstrated that the 2:1 Smix ratio generated the largest nanoemulsion region, providing a suitable basis for formulation optimization.

The BBD results showed that formulation variables significantly influenced the critical quality attributes of the SNEDDS. All three response models were statistically significant, with $R^2$ values greater than 0.98. The numerical optimization produced a formulation containing 20% Capryol® 90, 55% Kolliphor® RH40, and 25% Transcutol® P, with a desirability of 0.987. The experimental validation showed close agreement with predicted values, with prediction errors below 2%.

The optimized formulation exhibited nanosized droplets, low PDI, rapid self-emulsification, high drug content, and good physical stability. Most importantly, the formulation substantially improved the dissolution of Apixaban, achieving 95.84 ± 1.35% release at 60 min compared with 72.48 ± 1.28% for pure Apixaban.

The release kinetics showed the highest correlation with the Korsmeyer–Peppas model ($R^2$ = 0.9968; n = 0.47), indicating predominantly diffusion-associated drug release according to the thesis interpretation. Finally, the optimized formulation retained satisfactory physicochemical characteristics during 3 months of accelerated storage.

## 4. Conclusion

The present study successfully developed and optimized an Apixaban-loaded SNEDDS using a systematic formulation-development strategy. Solubility screening identified Capryol® 90, Kolliphor® RH40, and Transcutol® P as suitable formulation components, while pseudo-ternary phase analysis identified a 2:1 surfactant/co-surfactant ratio as providing the largest nanoemulsion region.

Application of a Box–Behnken Design enabled systematic optimization of oil, surfactant, and co-surfactant concentrations. The optimized composition consisted of 20% Capryol® 90, 55% Kolliphor® RH40, and 25% Transcutol® P, with a desirability of 0.987. The optimized formulation demonstrated a droplet size of approximately 118 nm, low PDI, satisfactory zeta potential, rapid self-emulsification, high drug content, and good thermodynamic stability.

Most importantly, the optimized SNEDDS produced 95.84 ± 1.35% Apixaban release at 60 min, compared with 72.48 ± 1.28% for pure Apixaban, demonstrating a substantial improvement in dissolution performance. The formulation also remained stable during 3 months of accelerated storage.

Therefore, the developed Apixaban-loaded SNEDDS is a promising formulation approach for improving the dissolution and solubilization of Apixaban. However, the claim of enhanced oral bioavailability remains prospective, because pharmacokinetic/in vivo bioavailability studies were not performed in the present investigation. Future studies should therefore include in vivo pharmacokinetic evaluation, in vitro lipolysis, and ultimately comparative bioavailability studies.

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