Research Article

Enhancement of Solubility and Dissolution of Ticagrelor Using SMEDDS by Scheffe's Mixture Design

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ABSTRACT

The primary focus of this study was to enhance the solubility of Ticagrelor by developing a Self-Microemulsifying Drug Delivery System. Ticagrelor, classified as a BCS class IV drug due to its poor aqueous solubility and permeability, prompted this investigation. To determine the saturated solubility of Ticagrelor in various oils, surfactants, and co-surfactants, HPLC was employed. Excipients were carefully chosen based on their maximum solubility and compatibility with Ticagrelor. Several SMEDDS formulations of Ticagrelor were created using different combinations of oils, surfactants, and co-surfactants (at ratios of 4:1 and 3:1). Pseudo ternary phase diagrams were constructed, aiding in the evaluation of the Nanoemulsification area. Utilizing these diagrams, formulations were designed with varying proportions of oil (Linseed Oil), surfactant (Tween 20), and co-surfactant (PEG-300). From these formulations, one batch was optimized and further assessed through dispersibility tests, self-emulsification time assessments, phase separation and stability tests, thermodynamic stability studies, droplet size and zeta potential measurements, and in vitro drug release studies. The outcomes of this study suggest that the Ticagrelor SMEDDS developed holds promise as a method to significantly enhance the solubility of Ticagrelor.

Keywords: Ticagrelor, SMEDDS, Scheffe's Mixture Design, Solubility, Dissolution.

INTRODUCTION

A growing number of newly discovered drug compounds struggle with poor solubility in water, resulting in limited absorption post oral administration. Roughly 35-40% of these fresh chemical entities (NCEs) face this issue due to a shift in their properties towards higher molecular weight and increased lipophilicity, consequently decreasing their solubility in water. This poor solubility often prevents potentially effective drugs from reaching the market, despite showing promising pharmacodynamic activity. Moreover, these drugs with low water solubility often necessitate much higher individual doses than optimal to achieve required plasma levels[1, 2]. The effectiveness and availability of any drug hinge significantly on its solubility, a crucial factor in achieving the desired drug concentration in systemic circulation for the intended pharmacological response. Consequently, there's an ongoing pursuit of strategies to enhance both the aqueous solubility and release rate of drugs. Numerous techniques have been explored for solubility enhancement, such as particle size reduction, pH adjustment, co-solvency, complexation, solid dispersions, and SMEDDS, among others. Each technique presents its advantages and limitations. Among these methods, SMEDDS emerge as a promising approach due to their ease of formulation and evaluation [3, 4]. SMEDDS are recognized for their potential in improving the solubility of hydrophobic drugs. They comprise isotropic mixtures of an oily vehicle, surfactants, co-surfactants, and thickening agents. One notable advantage is their minimal energy requirement for emulsification, leading to spontaneous emulsification within the gut upon dilution in an aqueous phase under gentle gastrointestinal motility. Consequently, the microemulsions formed are readily absorbed from the through the villi in the gastrointestinal tract as chylomicrons[5, 6]. Ticagrelor, identified as a cyclopentyl-triazolo-pyrimidine, falls within a category of chemically noncompetitive and reversible antagonists targeting the platelet P2Y12 ADP receptor [1-4]. Its approval for treating acute coronary syndrome stemmed from a phase III study (Platelet Inhibition and Patient Outcomes), demonstrating significant advantages over clopidogrel [5, 6]. Despite these positive outcomes, Ticagrelor's solubility remains notably low (approximately 10 µg/mL) across all pH levels, coupled with limited intestinal membrane permeability, categorizing it as a Biopharmaceutical Classification System (BCS) class IV compound. Consequently, the absolute bioavailability of TICAGRELOR post-oral administration stands at around 36% [7]. While some formulations have emerged to improve Ticagrelor's bioavailability and antiplatelet activity, such as solid dispersion and cocrystallization[9], these approaches have seen limited exploration in research studies.

MATERIALS AND METHODS

Chemicals & Reagents

Ticagrelor was obtained as a gift sample from Kopran Pvt Ltd. k-Tween 20, k-Tween 40, k-Tween 60, k-Tween 80, PEG-300, PEG 400, PEG 600 and Propyline glycol was purchased from FINAR Chemicals. Castor Oil, Linseed Oil, Groundnut Oil, Mustard Oil, Olive Oil, Sesame Oil and Sunflower oil was purchased from LOBA Chemicals. Methanol and other solvents were purchased from Merk

Chem. Distilled water was used throughout the experiments.

Determination of Partition coefficient (log P)

Partition coefficient of Ticagrelor was analyzed by performing shake flask method at 22.0± 0.1°C. Two different phases were prepared composing of 1-octanol as organic phase and sodium di-hydrogen phosphate (NaH2 PO4) buffer of 100mM (pH 5) strength as aqueous phase. The mixture of two phases in the ratio of 1:1 was taken in a separating funnel. The known amount (750 µg) of Ticagrelor was loaded in to the mixture such that the concentration of final dilution lies in the range of pre-developed analytical HPLC method. After loading of Ticagrelor, the mixture was vortexed for 5 min and allowed to stand for 24 hours. After saturation, the 1-octanol phase and buffer phase were separated and transferred into conical flask and flask was shaken in thermostatic shaker at 100 rpm and 25.0± 0.1°C for 30 min. the concentration of the Ticagrelor in each phase was determined before and after partitioning by using HPLC at the respective λmax (254nm) to get the partition coefficient. Each of the values was an average of three parallel measurements. The log P value was calculated using following formula.

$$\text{Log P} = \log10\ (P)$$

where,

Partition coefficient (P) = (concentration in organic phase)/(concentration in aqueous phase)

HPLC Analysis of Ticagrelor

The analysis of Ticagrelor via HPLC involved adapting a previously described method. An Agilent 1260 infinity series (Agilent Technologies Inc., Santa Clara, CA, USA), complete with a pump, autosampler, thermostat, and UV-Vis detector, was utilized for this analysis. Employing an Xterra RP18 column (250×4.6 mm, 5 µm; Waters, Milford, MA, USA), the TCG analysis was conducted. The mobile phase, maintained isocratically, consisted of acetonitrile and 50 nM ammonium acetate buffer (58:42, v/v), with the pH adjusted to 8.2 using 6 M ammonium hydroxide. A constant flow rate of 1.0 mL/min was maintained, with a 20 µL sample injection volume, and the column temperature held at a steady 40°C. Detection of Ticagrelor via ultraviolet light was set at a wavelength of 254 nm.

Screening of excipients for SMEDDS

Solubility Studies

An excessive quantity of Ticagrelor was introduced into different oils, surfactants, and co-surfactants, followed by thorough mixing using a cyclo-mixer. This mixture was left at room temperature for 72 hours to reach equilibrium. After achieving equilibrium, the sample was centrifuged at 100rpm for 10 minutes to eliminate any insoluble drug particles. A portion of the resulting supernatant was diluted with methanol, and the quantification of Ticagrelor was conducted using a HPLC analysis.

Pseudo ternary Phase Diagram

The solubility studies utilized specific components—Linseed oil (oil), Tween 20 (surfactant), and PEG-300 (co-surfactant)—to construct pseudo ternary phase diagrams. These diagrams were established via the water titration method at room temperature, aiming to pinpoint regions conducive to self-emulsification and select optimal concentrations of oils, surfactants, and co-surfactants for SMEDDS formulation.

Various ratios of surfactant to co-surfactant (S mix) within groups (1:3, 3:1, 4:1) were combined by weight, along with different weight ratios of oil and specific S mix ratios (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9). Each blend underwent titration with water, followed by a 2-minute vortex and subsequent equilibration. The transition in physical state, from transparent to turbid, was visually monitored and marked on a three-component ternary phase diagram. Each axis of this diagram represents oil, S mix, and water, facilitating the plotting of the phase diagram.

Formulation of Liquid SMEDDS of Ticagrelor

Various ratios of oil, surfactant, and co-surfactant were chosen based on a ternary phase diagram. A range of SMEDDS formulations was created by adjusting the proportions of the selected components: Linseed Oil, Tween 20, and PEG-300. The S mix, consisting of the desired quantity of surfactant and co-surfactant, was prepared separately. Keeping the drug (Ticagrelor) constant at 90mg across all formulations, Ticagrelor was gradually added to the oily phase while stirring continuously until a clear solution was achieved. This Ticagrelor-containing oil phase was then combined with the S mix, and the mixture underwent continuous stirring until a uniform, homogeneous blend was achieved. Finally acquired formulations were stored at ambient temperature.

Assessment of emulsification efficiency and phase separation

The evaluation of emulsification was carried out for the preselected excipients to determine the most appropriate surfactant and cosurfactant. Based on the requirement for a self-forming microemulsion, a type IV composition was used, which dispersed very finely and formed small droplets. The SMEDDS formulations were prepared with a composition of 5 % oil, 63.2 % surfactant, and 31.6 % cosurfactant, and 100 mg SMEDDS was diluted in 10 mL of distilled water or media at pH 1.2, pH 4.0, or pH 6.8. Subsequently, the mixture was homogenized for 30 minutes and then evaluated for emulsification and phase separation. The emulsification grade of SMEDDS was classified by the droplet size, transmittance, and phase separation by modifying the previously reported grading system. The phase separation was visually confirmed by the presence or absence of precipitation.

Experimental optimization of Ticagrelor-SMEDDS formulation

The SMEDDS formulation's composition underwent optimization utilizing Scheffé's mixture design, a favorable approach for employing response surface methodology in refining intricate formulations. The study involved experiments with three factors and four responses (see Table 1). Design-Expert 13 (Stat-Ease Inc., Minneapolis, MN, USA) facilitated both the experimental design and statistical analysis.

Design-Expert 13 (Stat-Ease Inc.) was utilized for the experimental design and statistical analysis. The percentages of Linseed Oil (X1, w/w %), Tween 20 (X2, w/w %), and PEG 300 (X3, w/w %) were established based on pseudoternary phase diagrams, falling within ranges of 10%–40%, 10%–80%, and 10%–80%, respectively. The total of X1, X2, and X3 always amounted to 100% in all experiments. To optimize the SMEDDS composition, factors such as Ticagrelor solubility in SMEDDS (Y1), Ticagrelor precipitation (Y2), droplet size (Y3), and transmittance (Y4) were considered as responses. Seventeen experiments were designed and fitted to various polynomial models including linear, cubic, quadratic, and special cubic/quadratic forms. The suitable fitting model for each response was determined by evaluating ANOVA parameters such as sequential P-values, lack of fit, squared correlation coefficients (R^2), adjusted R2, and adequate precision. Subsequently, the desirability function was employed to optimize factors linked to desirable responses following the statistical model fitting.

Table 1: Factors and responses used in Scheffé's mixture design

Factors	Range	
	Low limit (w/w %)	High limit (w/w %)
X_1: Capmul MCM (oil)	10	40
X_2: Cremophor EL (surfactant)	10	80
X_3: Transcutol P (cosurfactant)	10	80
Responses	Goal	
Y_1: Solubility (mg/mL)	Maximize	
Y_2: Precipitation (%)	Minimize	
Y_3: Droplet size (nm)	Minimize	
Y_4: Transmittance (%)	Maximize	

Assessment of Ticagrelor solubility within SMEDDS (Y1)

The investigation aimed to formulate an SMEDDS capable of dissolving a substantial amount of Ticagrelor in the smallest volume possible. Solubility assessments involved adapting the excipient screening method (refer to method: Preliminary screening of excipients for SMEDDS). Essentially, excess Ticagrelor was introduced to 1 g of prepared SMEDDS, and after 72 hours of stirring, solubility was measured. The procedure included centrifuging the samples at 15,000 × g for 15 minutes at 25°C, diluting the supernatant with methanol, and quantifying Ticagrelor using HPLC.

Precipitation Evaluation (Y2)

To ensure the formation of homogeneous microemulsion droplets below 200 nm in diameter, a precipitation test was conducted. In summary, 10 mL of distilled water was combined with 100 mg of Ticagrelor SMEDDS, vortexed for 30 minutes to create a uniform microemulsion, and filtered through a 0.22 µm PVDF membrane filter. The filtered mixture was promptly diluted with methanol and analyzed using HPLC. Precipitation was quantified by comparing Ticagrelor concentrations between Ticagrelor SMEDDS in distilled water and Ticagrelor SMEDDS in isopropanol using the equation: Precipitation (%) = × 100 1− Cw/Cp, where Cw represents Ticagrelor concentration in Ticagrelor SMEDDS diluted with distilled water and Cp signifies Ticagrelor concentration in Ticagrelor SMEDDS diluted in isopropanol. A minimal percentage indicates negligible Ticagrelor precipitation.

Droplet Size Measurement (Y3)

After the reconstitution process, SMEDDS droplet size was gauged. One hundred milligrams of SMEDDS were combined with 10 mL of distilled water to create a reconstituted microemulsion. This mixture was allowed to incubate at room temperature for 30 minutes before measurement. And droplet size measured.

Transmittance Assessment (Y4)

The transmittance of each formulation was determined by assessing the absorbance at 620 nm using a microplate reader comparison to distilled water used as a control. To determine absorbance, 100 mg of each blend was introduced to 10 mL of distilled water to create the microemulsion. The transmittance was computed using the formula: Transmittance (%) = 100 - 10 × A, where A represents the absorbance of the microemulsion. A transmittance value nearing 100% signifies a clear and transparent microemulsion.

Characterization of Ticagrelor SMEDDS

The optimized Ticagrelor SMEDDS formulation involved dissolving 90 mg of Ticagrelor in 400 mg of the refined SMEDDS. To assess the morphology of Ticagrelor SMEDDS, a transmission electron microscope (JEM 1400; JEOL Ltd, Tokyo, Japan) operating at 120 kV was utilized. The Ticagrelor SMEDDS was dispersed in distilled water, where 10 µL of the sample was applied directly onto a copper grid and air-dried before observation under the microscope. For measuring droplet sizes, 100 mg of Ticagrelor SMEDDS was gently mixed in 10 mL of distilled water for 30 minutes and then analyzed.

Comparative in vitro dissolution analysis of Ticagrelor within the optimized Ticagrelor SMEDDS and Brilinta® 90 mg (a commercial product) was conducted employing the United States Pharmacopeia (USP) apparatus II method, utilizing a dissolution tester (Electro Lab). Dissolution

media conforming to USP guidelines—distilled water and media at pH 1.2, pH 4.0, and pH 6.8—were prepared. The experiment involved 900 mL of dissolution media maintained at 37°C±0.5°C, with a constant paddle speed of 50 rpm. Ticagrelor SMEDDS containing 90 mg of TICAGRELOR were encapsulated in size 00 hard gelatin capsules. At specific time intervals (5, 10, 15, 30, 45, 60, 90, 120, and 180 minutes), 5 mL samples were withdrawn, filtered through a 0.45 µm membrane filter, and diluted with methanol. The concentration of Ticagrelor in each formulation was then assessed using HPLC.

Evaluation of Ticagrelor SMEDDS Formulation

Globule Size and Zeta Potential

The liquid SMEDDS formulations of Ticagrelor were diluted with distilled water at a ratio of 1:100, stirred on a cyclo mixer for 1 minute, and left to stand for 1 hour. Subsequently, the globule size and zeta potential of the resulting formulation were determined using DLS spectroscopy at a 90-degree angle with a Zeta sizer ZS 90 (Malvern Instruments). The size of the liquid SMEDDS formulation of Ticagrelor was measured by placing the diluted solution in disposable cuvettes at 25°C.

Self-Emulsification Time

The emulsification time, indicating the duration for the formulation to form a homogeneous mixture upon dilution, was evaluated. The pre-concentrated emulsion liquid SMEDDS Ticagrelor formulation was added dropwise to a beaker containing distilled water and stirred continuously at 100 rpm on a magnetic stirrer. The time taken for the self-emulsification process was visually assessed.

Dispersibility Test

The dispersibility test of SMEDDS aimed to assess its ability to dispense into an emulsion and categorize the resulting globule size. A preconcentrate of SMEDDS was added to distilled water, stirred at ~100 rpm using a magnetic stirrer, and the time required for emulsion formation was noted. The type of emulsion formed was graded based on its appearance and the time taken for emulsification.

Phase Separation and Stability Test

To assess phase separation, a formulation of Ticagrelor liquid SMEDDS was added to distilled water maintained at 3°C, agitated, and left for 24 hours. The formulation was observed visually for any signs of phase separation.

Effect of Dilution

Formulations were diluted with excess water, 0.1 N HCl, and phosphate buffer (pH 6.8) and stored for 24 hours. Stability was determined by checking for any precipitation or phase separation.

Centrifugation

Mixtures of distilled water and Ticagrelor SMEDDS formulations were subjected to centrifugation and observed for any physical changes such as precipitation or phase separation. Stable formulations post-centrifugation was selected for further evaluation.

Thermal Stability Studies

To evaluate the physical stability of the formulations, they underwent freeze-thaw stress cycles, followed by assessments for phase separation, cracking, or creaming. The formulations passing this test were subsequently evaluated for their ability to produce emulsions independently.

Heating and Cooling Cycle

The Ticagrelor SMEDDS formulation and distilled water were combined in a ratio of (1:50) and subjected to six consecutive cooling and heating cycles. These cycles alternated between refrigerator temperature (4°C) and a higher temperature (45°C), with each temperature exposure lasting no more than 48 hours. Centrifugation testing is conducted on the formulations that successfully pass the stability test.

pH

The pH of the Ticagrelor SMEDDS formulation is determined using a pH meter.

Drug Loading Efficacy

Drug loading efficiency was evaluated spectrophotometrically using HPLC analysis. 50mg of each formulation was precisely weighed and diluted to 100mL with methanol. The resulting solutions underwent spectroscopic analysis following suitable dilution. Drug loading efficiency was calculated using the formula:

Drug loading efficiency =Amt. of drug is known amt of formulation/ Initial drug load *100

FT-IR studies

FT-IR studies were performed using an FT-IR spectrophotometer to obtain spectra of the pure drug, solid SMEDDS, and liquid SMEDDS formulation. The spectra, accumulated over 24 scans with a resolution of 4cm^{-1} across the range of 400-4000 cm^{-1}, were compared to detect any interactions within the formulation.

Formulation of Solid-SMEDDS

Based on the evaluation studies conducted on various Ticagrelor SEDDS formulations, one batch was chosen for its favorable stability, robust self-nanoemulsification property, smaller particle size, and lower PDI. Solid-SMEDDS was prepared by combining the liquid SMEDDS containing Ticagrelor with nuselin as carrier at a ratio of 1:2. The liquid SEDDS was added dropwise onto the nuselin in a porcelain dish. After each addition, the contents were mixed thoroughly using a glass rod to ensure uniform distribution of the formulation. The resulting damp mass was sieved through sieve no. 120, dried at room temperature, and stored for future use.

Characterization of S-SMEDDS:

Flow Properties of S-SMEDDS:

Angle of Repose: The angle of repose for S-SMEDDS was determined via the funnel method. The funnel's height was adjusted so that its tip barely touched the highest point of the powdered heap. A precisely weighed sample was allowed to freely flow through the funnel onto a surface. The diameter of the resulting powder cone was measured, and the angle of repose was calculated using the equation:

$$\tan \theta = h/r$$

Where 'h' and 'r' denote the height and radius of the powder cone.

Bulk Density and Tapped Density: A 2g quantity of S-SMEDDS was introduced into a 10mL measuring cylinder. The initial volume was noted, and the cylinder was allowed to fall under its weight from a height of 2.5 cm at 2-second intervals. Tapping continued until no further change in volume was observed. Bulk density (BD) and tapped density (TD) were calculated using the following formulas:

Bulk density (BD) = Weight of powder blend/ volume of the packing

Tapped density (TD) = Weight of powder blend/ tapped volume of the packing

Compressibility Index: The blend's compressibility index was determined by Carr's compressibility index equation:

Carr's compressibility index (%) = (TD -BD)/ TD *100

Hausner's Ratio: Hausner's Ratio, indicative of a powder or granular material's flowability, was calculated using the equation:

Hausner's Ratio = TD/ BD

Drug Content: S-SMEDDS of Ticagrelor, equivalent to 10mg, was precisely weighed and dissolved in adequate methanol. The solution underwent sonication for 10 minutes to extract the drug, followed by filtration. The absorbance of the filtrate was measured at 254 nm using a HPLC.

RESULTS AND DISCUSSION

Determination of Partition coefficient (log P)

Prior to choosing the afore mentioned components, it's essential to take into account the drug's lipophilicity and dosage for the SMEDDS formulation development. The drug ought not to exhibit significant first-pass metabolism and should possess a low log P, necessitating a lower dosage. Partition Coefficient of Ticagrelor was found to be 2.44.

Screening of excipients for SMEDDS

In the process of creating a SMEDDS for the dissolution of a Ticagrelor unit dose (90 mg) with a minimal formulation volume, the initial phase involved choosing suitable excipients to maximize Ticagrelor solubility. Specifically, Linseed Oil exhibited notably superior solubility (0.8220 mg/mL).

The selection of oil components in SMEDDS holds significant importance as oils possess the ability to dissolve hydrophobic drugs and enhance the drug's lipophilicity. Hence, Linseed oil, exhibiting the highest solubility, was utilized as the primary oil in the SMEDDS formulation. Among the screened surfactants—Cremophor 40, Tween 20, Tween 40, Tween 60 And Tween 80, Tween 20 showed highest solubility. Typically, surfactants reduce surface energy, facilitating the dispersion of oil into small droplets within the aqueous phase. Among the screened cosurfactants, Tween 20 showcased the highest TCG solubility (0.09890mg/mL). Cosurfactants play a role in augmenting drug solubility within micelles, forming stable micelles with surfactants in the aqueous phase, thereby preventing drug precipitation and maintaining micelle stability. Among the selected co surfactants PEG 300, PEG 400, PEG 600 and propylene glycol, propylene glycol showed highest solubility but PEG 300 selected due to better compatibility with surfactant selected. In methanol the highest solubility of ticagrelor was found.

In the present study pseudo ternary phase diagrams were constructed against oil, water and surfactant/ co-surfactant using water titration methods. The results are shown in the Figure

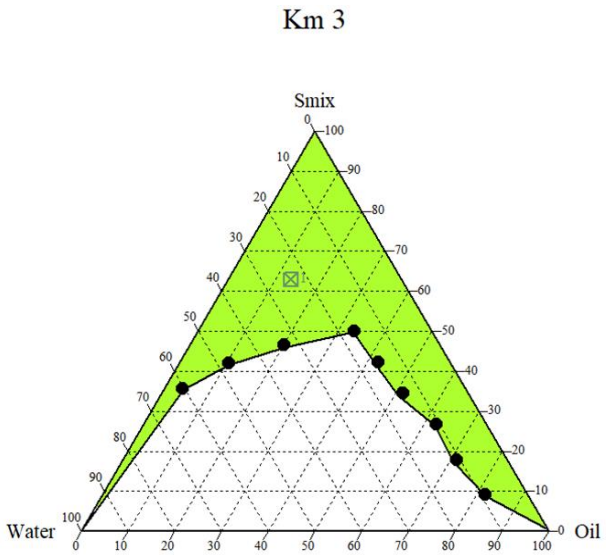

Figure 1: Pseudo ternary phase diagram for Ticagrelor

Design of experiments for optimizing TCG-SM

The optimization of Ticagrelor SMEDDS through Scheffé's mixture design was executed, utilizing Design-Expert 13 software to fit response results. Statistical analysis suggested various fitting models and their relationships to the variables involved. Key variables such as TCG solubility in SMEDDS (Y1), precipitation (Y2), droplet size (Y3), and transmittance (Y4) played crucial roles in formulating SMEDDS, ensuring stable formulations and enhancing the oral absorption of poorly soluble drugs.

High Ticagrelor solubility within SMEDDS allowed for greater drug content in a stable formulation with minimal volume, mitigating precipitation risks. Understanding the relationship between precipitation and the variables was imperative as it affected drug encapsulation in micelles. Smaller droplet sizes increased micelle surface area, aiding drug absorption and dissolution upon contact with the intestinal membrane. Transmittance served as a monitoring parameter to achieve a clear, homogeneous microemulsion when dispersed in an aqueous phase.

The statistical models for Y1, Y2, Y3, and Y4 responses were fitted using linear, quadratic, cubic, and special quadratic models, respectively (Table 2). Parameters such as sequential P-values, lack of fit, R^2, and adjusted R^2 were assessed to gauge the models' significance and appropriateness. All suggested models showed sequential P-values below 0.05, indicating parameter significance at a 95% confidence level. Lack-of-fit P-values were above 0.05, validating the appropriateness of the fitted models. R2 and adjusted R^2 were scrutinized to measure the variability explanation within the models The models effectively reflected the experimental data, with all R^2 and adjusted R2 values for Y1, Y3, and Y4 surpassing 0.9, indicating close alignment between the data and the fitted values. While the R^2 value for Y2 was 0.7732, its precision at 8.0892 suggested adequacy, indicating potential use of the Y2 model within the design space (adequate precision >4). The similarity between R^2 and adjusted R^2 values (with a difference <0.2) signified a suitable goodness-of-fit indicator.

The interrelation between factors was depicted through three-dimensional response surface plots and coefficient equations. These illustrations highlighted the ranges for Ticagrelor solubility in SMEDDS (Y1) from 126.51 to 330.59 mg/mL and Ticagrelor precipitation (Y2) from 0.2% to 67.7% (Figure 2A and B). Droplet size (Y3) ranged from 54.7 to 1,023.6 nm, while transmittance (Y4) varied between 49.5% and 99.2%.

Ticagrelor solubility in SMEDDS (Y1) and precipitation (Y2) increased with X3. There was no significant interaction effect among X1, X2, and X3 regarding Ticagrelor solubility. For droplet size (Y3), the interaction between X2 and X3 was noteworthy, indicating a decrease in Y3 as X2 increased and X3 decreased. Transmittance (Y4) was influenced by the interaction of X2 and X3, apart from the main effect, with Y4 tending to increase as X2 increased and X3 decreased. Utilizing the desirability function considering all responses, factors were optimized. Y1 and Y4 were set for maximization, while Y2 and Y3 were aimed for minimization. The desirability plot depicted the impact of different variables on the four responses. he optimized values for X1, X2, and X3 stood at 10.0%, 53.8%, and 36.2%, respectively, yielding a desirability value of 0.766 at that specific point. To validate the prediction accuracy, the percentage difference between predicted and actual values was computed for each response. Though the percentage errors were slightly elevated for Y1 (6.25%) and Y3 (5.61%), they remained quite low for Y2 (2.50%) and Y4 (1.36%). These errors, all under 10%, confirmed the success of the Ticagrelor SMEDDS optimization process.

Transmission electron microscopy data of the optimized Ticagrelor SMEDDS revealed spherical microemulsion droplets within the nanometer scale, akin in size to those obtained through ELS measurement (116.4±5.7 nm), as depicted.

Measurement of Droplet Size and Zeta Potential

The formulations underwent assessment for droplet size, PDI (Polydispersity Index), and Zeta potential. Droplet size ranged from 49.52 to 51.86 nm, and the PDI across all formulations remained below 0.5, indicating a uniform distribution of particle sizes. Zeta potential values ranged from -38.1 to -22.7 mV. Specifically, formulation exhibited a smaller droplet size compared to the other formulations based on the findings.

Table 2: Summary of model fitting and statistical analysis

Responses	Suggested model	Model *P*-value	Lack-of-fit *P*-value	R^2	Adjusted R^2	Adequate precision
Y_1: Solubility	Linear	0.0001	0.7558	0.9646	0.9596	37.7012
Y_2: Precipitation	Quadratic	0.0432	0.4149	0.7732	0.6701	8.0892
Y_3: Droplet size	Cubic	0.0018	0.2559	0.9882	0.9731	23.3584
Y_4: Transmittance	Special quartic	0.0022	0.0606	0.9879	0.9757	23.4122

Table 3: Predicted values and actual values of optimized Ticagrelor SMEDDS

Optimized factors	Response	Goal	Importance	95% CI low predicted value	Predicted value	95% CI high predicted value	Actual value	Error percentage (%)
X_1: 10.0% X_2: 53.8% X_3: 36.2%	Y_1: Solubility (mg/mL)	Maximize	+++	212.01	222.32	232.62	236.2±3.6	6.25
	Y_2: Precipitation (%)	Minimize	+++	–4.0	10.4	24.7	10.6±0.3	2.50
	Y_3: Droplet size (nm)	Minimize	+++	25.6	116.7	207.8	110.2±2.6	5.61
	Y_4: Transmittance (%)	Maximize	+++	90.7	95.3	99.8	96.6±0.2	1.36

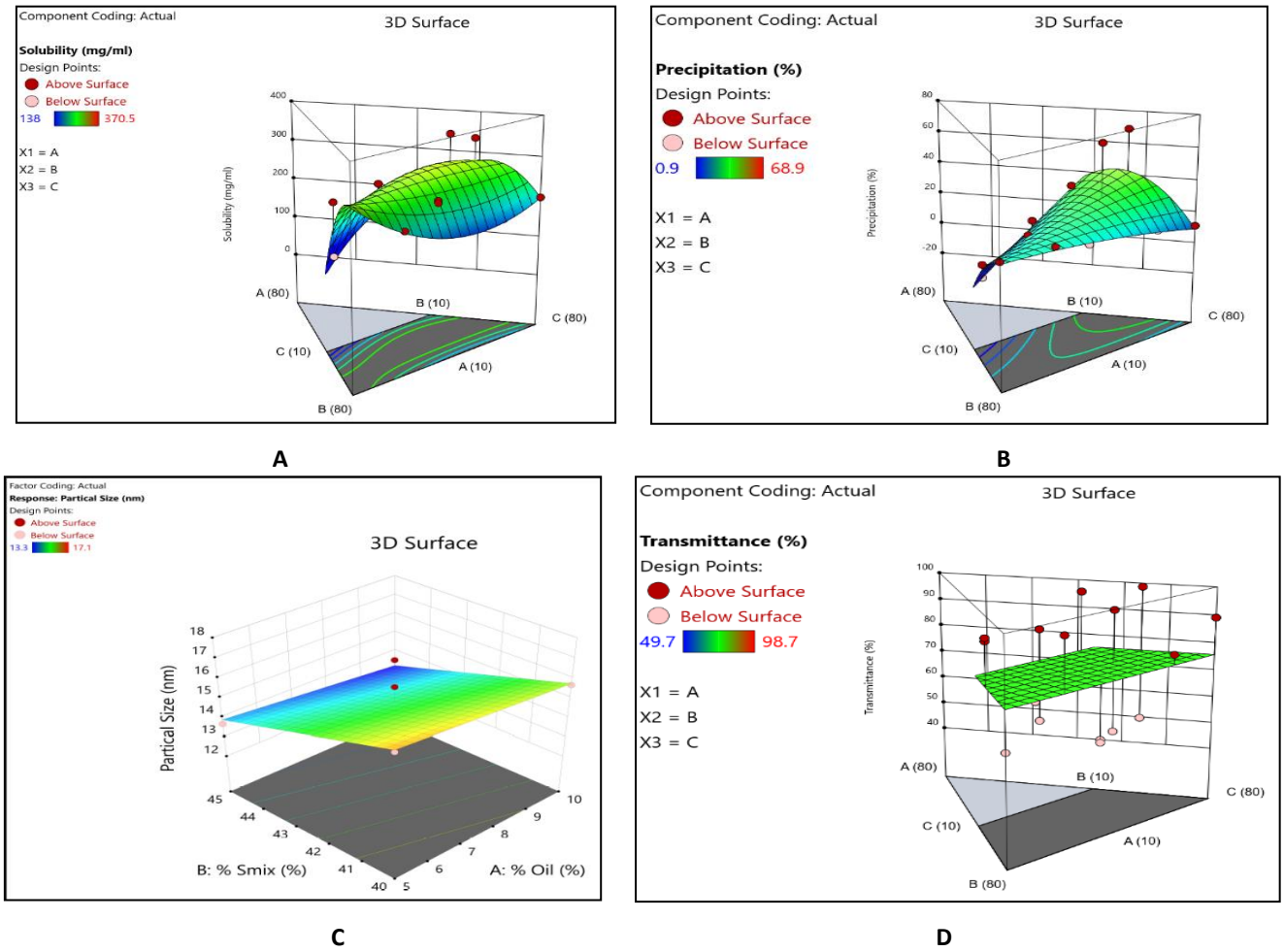

Figure 2: Desirability plot using numerical optimization and Three-dimensional surface plots of responses A) Y1: Solubility of TCG in SMEDDS. (B) Y2: Precipitation. (C) Y3: Droplet size. (D) Y4: Transmittance

FT-IR studies

FT-IR Spectrum of pure drug and SMEDDS formulation were obtained by FTIR Spectrophotometer. The drug was found to be pure.

XRD studies

X-ray diffractograms for bulk Ticagrelor and it's SMEDDS are represented. XRD studies of pure Ticagrelor and SMEDDS reinforced our hypothesis that crystallinity of Ticagrelor reduces in SMEDDS formulation.

Assessment of Self-Emulsification

The evaluation of self-emulsification involves grading formulations based on their ability to rapidly disperse in an aqueous medium through mild shaking. Notably, all formulations exhibited emulsification within 22 to 43 seconds, indicating excellent performance across the board.

Dispersibility Test

Upon conducting the dispersibility test, observations for all Ticagrelor SMEDDS formulations. These formulations demonstrated slight clarity. Formulation 1 rapidly formed a slightly less clear emulsion with a bluish appearance, graded as B, while 2 resulted in a bright milky emulsion graded as C.

Phase Separation and Stability Assessment

The stability of the prepared SMEDDS formulations was monitored for precipitation and phase separation of the drug over intervals of 2, 4, 6, 8, 12, and 24 hours. Study shows that none of the formulations exhibited precipitation or phase separation during these time points.

Robustness to Dilution

Evaluation of the formulations' robustness to dilution involved their dilution with excess water, 0.1N HCl, and phosphate buffer of pH 6.8. Study demonstrates that no precipitation or phase separation was observed in any of the diluted samples after 24 hours, indicating the robustness of all formulations.

Thermodynamic Stability Studies

The stability of the SMEDDS formulations was further assessed through thermodynamic stability studies involving centrifugation, freeze-thaw cycles, and heating-cooling cycles. Study reveals that all formulations remained stable during centrifugation at 3,500 rpm and through alternate

temperature cycles of -20°C and +25°C, as well as 4°C and 45°C.

Overall, the results from these assessments indicate the excellent self-emulsification, dispersibility, stability, and robustness to dilution of the Ticagrelor SMEDDS formulations

Efficiency of Drug Loading

The drug loading efficiency assessment revealed that formulation 1 achieved a loading efficiency exceeding 98.2%, whereas 1 contained 87.4% loading.

Evaluation of Solid SMEDDS of Ticagrelor

The flow properties of solid SMEDDS (s-SMEDDS) of Ticagrelor were comprehensively assessed, encompassing parameters such as Angle of Repose, Bulk Density, Tapped Density, Compressibility Index, and Hausner's Ratio. Notably, the prepared s-SMEDDS exhibited favorable flow properties, classified as "Good," as outlined in Table 4.

Table 4: Flow Properties of s-SMEDDS of Ticagrelor (n=3)

Flow properties	Results
Angle of repose	27.998 ± 1.302
Bulk density(g/mL)	0.367 ± 0.015
Tapped density (g/mL)	0.41 ± 0.015
Compressibility index (%)	9.85 ± 0.38
Hausner‟s ratio	1.11 ± 0.006

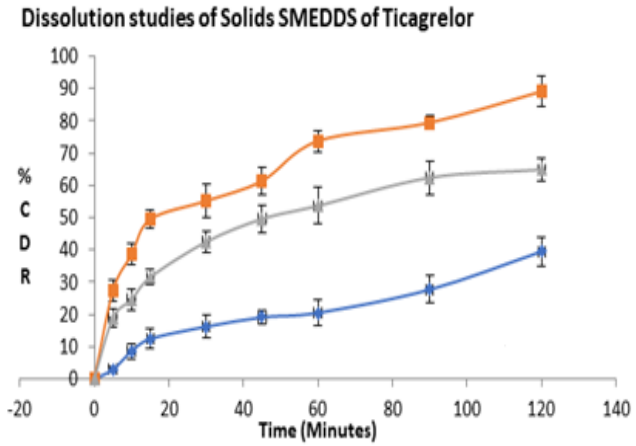

Figure 3: *In vitro* dissolution of SMEDDS Ticagrelor

A. Orange line- Solid-SMEDDS; **B.** Grey Line- Liquid-SMEDDS;

C. Blue Line- Brillinta Tablet

CONCLUSION

A refined SMEDDS composition, containing Ticagrelor, Linseed oil, Tween 20, and Polyethylene Glycol (PEG) 300, was successfully developed. These formulations notably increased Ticagrelor's solubility, dissolution rate, and bioavailability. Moreover, they demonstrated thermodynamic stability against dilution, freeze-thaw cycles, and centrifugation without any drug precipitation or phase separation. The dissolution profiles of the selected formulations exhibited a drug release exceeding 70% within 120 minutes, with showcasing the highest release of 90.2% within the same timeframe. This study validates the potential of SMEDDS formulations as an effective alternative to conventional oral forms, particularly for enhancing the solubility and dissolution of poorly soluble drugs like Ticagrelor.

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