

Original Research Article

Preparation and *in vitro* - *ex vivo* evaluation of ticagrelor solid lipid nanoparticles

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Abstract

Purpose: To investigate the physicochemical properties of Ticagrelor solid lipid nanoparticles (TCG-SLN).

Methods: Formulations were developed based on a 3-level factorial design and subsequently produced using the microemulsion technique. Quality-by-design technique was used to establish correlations between formulation parameters (surfactant ratio, surfactant type, and sonication time) and key quality attributes such as particle size (PS), polydispersity index (PDI), and entrapment efficiency (EE %).

Results: Optimized TCG-SLN showed PS of 67 nm, PDI of 0.165, and EE of 87.5 % with 88 % drug release achieved in 24 h in a controlled manner. *Ex vivo* permeability showed a 3.3-fold increase in TCG-SLNs permeation compared to Ticagrelor suspension. Scanning electron microscope (SEM), Fourier Transform Infrared spectroscopy (FTIR), and Powder X-ray Diffraction (PXRD) showed that Ticagrelor was effectively encapsulated in the nanoparticulate system, having a non-aggregated, spherical surface with no distinct change in functional groups.

Conclusion: This study shows that TCG-SLN appears as non-aggregated, spherical SLNs with various sizes, exhibits a biphasic release profile, exhibits high intestinal permeability, restores histological structure resembling normal morphology, with short-term stability.

Keywords: Entrapment efficiency, *In vitro*, Nanoparticles, Ticagrelor, Solid lipid nanoparticles

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INTRODUCTION

Ticagrelor (TCG) is a non-thienopyridine oral antiplatelet drug. It resembles adenosine due to the presence of cyclopentyl triazolopyrimidine, a configuration in which a triazole ring is joined to a pyrimidine ring [1]. It is preferred over earlier thienopyridine agents like clopidogrel and prasugrel due to its non-prodrug status and the reversible inhibition of adenosine diphosphate (ADP) receptors, enabling platelets to completely regain their function within three days following therapy cessation [2]. However, TCG has low solubility, characterized as class IV in the

Biopharmaceutics Classification System (BCS) [3]. Reports indicated that the bioavailability of TCG is around 36 % and may be immediately transported from the liver to the bile [4]. Solid lipid nanoparticles (SLN) have gained significant interest as innovative colloidal drug carriers for the delivery of pharmaceuticals via many routes, including oral, topical, parenteral, ocular, and rectal administration [5]. Solid lipid nanoparticles possess an average size ranging from 40 to 1000 nm with a spherical morphology, comprising a solid lipid phase and surfactant.

The dispersed phase consists of solid fat, while a surfactant serves as the emulsifier. The lipid constituents of SLNs remain solid at both physiological and environmental temperatures [6]. It consists of highly purified triglycerides, complicated glyceride combinations, or even waxes [7]. Surfactants are utilised between 0.5 and 5 % to improve stability. Solid lipid nanoparticles are synthesised by several techniques, which include high-pressure homogenisation, ultrasonication/high-speed homogenisation, solvent emulsification-diffusion, solvent evaporation, double emulsion, and microemulsion [8].

This study investigated the physicochemical properties of optimized TCG- SLN produced by microemulsion method.

EXPERIMENTAL

Preparation of SLN formulations

Ticagrelor (TCG: Anhui Hailkan PH Co Ltd, China) solid lipid nanoparticles were prepared using microemulsion template techniques [9]. Compritol 888 (BDH Chemicals Ltd., Poole, England) was melted at 80 °C, and 20 mg TCG was dispersed in the lipid phase. Cremophor EL (BDH Chemicals Ltd., Poole, England) solution was prepared in distilled water (4 mL) to create an aqueous phase. The primary emulsion was formed by adding the aqueous phase to the lipid phase, and ethanol was added to 0.5 mL. The resultant solution was dispersed in cooled distilled water (2-4 °C) to form the microemulsion and sonicated.

Optimization of SLN

Initial studies were conducted to identify potential factors that affect the characteristics of SLN for oral administration. Upon identification, Design Expert (version 11 software) was used to construct a three- level factorial design. Independent variables include the effect of lipid-to-surfactant ratio, surfactant type, and sonication time, while response variables include PS, PDI, and EE (Table 1).

Table 1: Optimization characteristics

Variables		Levels		
Independent variables	Lipid: Surfactant	1:1	1:2	1:3
	Surfactant type	Tw 60	Cr	Tw 60 & Cr
Dependent variables	Sonication time (min)	0	7.5	15
	PS			
	PDI			
	EE %			

Tween 60 (Tw), Cremophor EL (Cr), particle size (PS), polydispersity index (PDI), entrapment efficiency (EE)

Particle size (PS) and polydispersity index (PDI)

The PS and PDI of TCG-SLN were detected using Zetasizer NanoZS® (Malvern Instruments, UK) with a dynamic scattering light approach. The formulated SLN was diluted in distilled water in a polystyrene cuvette and subjected to a 173 ° laser light diffraction at 25 °C.

Entrapment efficiency

Drug encapsulation efficiency (EE) was assessed using ultrafiltration (utilizing centrifuge tubes equipped with an ultrafilter (Amicon Ultra, MWCO 3 kDa, Millipore Company)). The TCG-SLN (1 mL) was placed in the upper chamber, and the tubes were centrifuged for 20 min at 4 °C and 2000 rpm [10]. Entrapment efficiency was calculated using Eq 1.

$$EE (\%) = (T_a - T_b / T_a) 100 \dots\dots\dots (1)$$

Where T_a is the total amount of TCG and T_b is the amount of free TCG.

Characterization of TCG-SLN optimum formula

Morphology of the particles

Morphology of TCG-SLN was examined using scanning electron microscopy (SEM). The sample was put on a metal plate and coated with platinum using argon gas and examined at 10 kV magnification. Secondary electron digital pictures were acquired at a higher magnification [11].

Powder X-ray diffraction

Powder X-ray diffraction was used to evaluate the crystallinity of drugs and excipients. The analysis was performed using an X-ray diffractometer (X'Pert Pro, Holland), where the sample was exposed to nickel-filtered Cu K α radiation (40 kV, 30 mA) under standard experimental conditions

(anode material used was copper, scan range (start position ($^{\circ}$ 2Th), 2.0100; end position ($^{\circ}$ 2Th), 59.9900), step size of 0.0200, and 25 $^{\circ}$ C) [12].

Fourier transform infrared spectroscopy (FTIR) spectroscopy

The spectra were obtained for the pure drug, Compritol 888, physical mixtures of the drug and lipid, and lyophilized TCG-SLN, utilizing FTIR equipment (Shimadzu, Model No. 1800). Each sample was weighed and prepared in a KBr disk. The spectrum was analyzed across frequency range of 4000 – 400 cm^{-1} with a spectral resolution of 4 cm^{-1} [13].

In vitro release study

In vitro release studies of TCG-SLN and the pure drug were performed using a modified dialysis membrane diffusion technique [14]. Dialysis membrane (Himedia, Mumbai, India) with a molecular weight cut-off (MWCO) of 8,000–14,000 Da was previously soaked overnight in the dissolution media. The TCG-SLN formulation (5 mL of 0.1 %w/v) was placed in the dialysis bag, which was tied at both ends and placed in the dissolution apparatus II (Caleva, Germany) under standard laboratory conditions (dissolution medium of phosphate buffer, pH of 6.8, 100 rpm, and temperature maintained at 37 ± 5 $^{\circ}$ C). At predetermined intervals (0.1, 0.17, 0.25, 0.5, 1, 2, 8, 12, 15, 20, 22, and 24 h), a 5 mL aliquot of the sample was collected and replaced with an equivalent volume of new dissolving media to maintain sink conditions. The samples were filtered and analyzed spectrophotometrically using a UV-VIS (CECIL Aquarius, Germany) spectrophotometer at 255 nm.

Analytical validation of TCG-loaded SLNs

Instrumentation and chromatography conditions

The reversed high-performance liquid chromatography RP-HPLC was utilized as a validated technique with slight modification [15] under standard specifications (waters HPLC equipped with SPA- 20A detector, an isocratic chromatographic separation utilizing Symmetry® ODS-C18 (250 $\times$ 4.6mm; 5 μ m) column and Breeze software, flow rate maintained at 1.0 mL/min, column temperature at 25 $^{\circ}$ C, running time of 10 min, retention time of 5.7 min, injection volume of 0.1mL). Detection was carried out using a UV detector at 255 nm.

Validation of HPLC method

Although the proposed analytical method was validated previously, it was revalidated according to standard guidelines on the following parameters [16].

Linearity

Linearity was established by constructing calibration curves over a range of TCG concentrations (100-800 ng/mL). A calibration curve was constructed for TCG using the relative peak area (ratio of the peak area of the drug to the peak area of internal standard (IS)) against the corresponding concentrations using linear least squares regression analysis. Linearity of the proposed method was established using diazepam as the IS at 100 ng/ml [17].

Lower limit of detection (LOD) and lower limit of quantification (LOQ)

Limits of detection and quantification were determined using the signal-to-noise (S/N) approach. The lower limit of detection (LLOD) was determined by identifying the minimal concentration at which the signal-to-noise ratio ($S/N \approx 3$) was equal to or greater than three. Similarly, the limit of quantification (LOQ) was determined as the lowest concentration at which the signal-to-noise ratio ($S/N \approx 10$) was ≥ 10 (Eqs 2 and 3).

$$\text{Lower limit of detection (LLOD)} = 3.3 \delta/S \dots (2)$$

$$\text{Lower limit of quantification (LLOQ)} = 10 \delta/S \dots (3)$$

Where δ is standard deviation of y-intercept of calibration curves and S is mean of the slope of calibration curve.

Accuracy

Accuracy was evaluated as percent recovery (%) of three concentrations (100, 200, and 400 ng/mL) of spiked TCG solutions and blanks (using mobile phase for dilution), keeping the IS concentration constant (100 ng/mL of diazepam) in each sample. The samples were injected into the HPLC system in triplicate. Percent recovery for TCG was calculated using Eq. 4

$$\text{Recovery (\%)} = (A/B)100 \dots (4)$$

Where (A) is the net peak area of the drug in spiked plasma, and (B) is the peak area of the drug in a standard mixture (mobile phase).

Precision

Precision was quantified as relative standard deviation (RSD) of intra-day repeatability (analysis conducted on the same day ($n = 3$) with the same instrument) and inter-day reproducibility (three distinct days, $n = 3 \times 3$, utilizing the same device). Standard solutions (TCG at 100 ng/mL with a corresponding constant concentration of IS (100 ng/mL of diazepam) prepared in the mobile phase) were injected into the HPLC system three times daily for three consecutive days. Mean relative peak area (RPA), SD, and % RSD were recorded.

Ex vivo intestinal permeation study

The *ex vivo* permeation study of TCG-SLN was carried out using the non-everted gut sac method [18]. The small intestine was obtained from a fasted male sheep weighing about 18 kg. The small intestine was procured fresh, isolated, and washed and subsequently rinsed with cold normal saline solution to remove mesentery matter and extraneous tissue. After securing one end, the intestinal sac was filled with 1 mL of the formulation suspension containing approximately 0.8 mg of Ticagrelor. The other end of the sac was secured, and the sac was placed in a beaker containing Krebs-Ringer phosphate buffer saline (pH 7.4), maintained at 37 ± 1.0 °C in a magnetic stirrer (100 rpm) and continuously bubbled with oxygen (50 bubbles/min). The samples (5 mL) were withdrawn at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 15, 20, and 24 h and analyzed by HPLC. Apparent permeability coefficient was calculated using Eqs 5 and 6.

$$P_{app} = J / C^o \quad (2-4) \quad \dots\dots\dots (5)$$

$$\text{Where } J = (dC/dt)_{ss} * (V / SA)$$

$$SA = 2\pi rh \quad (2-5) \quad \dots\dots\dots (6)$$

Where (dc/dt) ss (µg/mL/h) was the rate of change of concentration under steady-state, SA is the effective intestinal permeation surface area, (r) is the intestinal radius, (h) is the segment length, V (mL) is the volume of the receiver section, and C^o (µg/mL) is the initial drug concentration [19].

Intestinal mucosal toxicity

Histopathological assessment was done to identify intestinal cilia toxicity *ex vivo*. The samples (P1, P2, and P3) were transferred into the intestinal cavity after they were washed with PBS (pH 6.8). Sample P1 served as control and was subjected to 100 µL PBS, sample P2 was treated with 100 µL isopropyl alcohol, and sample P3 was filled with the optimized TCG-SLN for three days. After 3 days, the intestinal wall was examined histologically and photographed using a polarizing microscope.

Short-term stability study of TCG-SLNs

The optimized formulation was separated into two firmly sealed amber glass containers to examine the short-term stability of liquid TCG-SLNs under different storage conditions. Each container was stored at 2 – 8 °C (refrigerator) and 25 °C (room temperature) for 90 days. Samples were taken at the beginning of the study (day 1), middle period (day 45) and at the end of the study (day 90). Mean particle size, polydispersity index (PDI), zeta potential (ZP), and entrapment efficiency (% EE) of the nanoparticles were recorded in triplicate [20].

Statistical analysis

The PS, PDI, and EE data were analyzed using one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Optimization of TCG - SLN

The effect of each independent variable (A, B, and C) on the responses (Y1, Y2, and Y3) was studied and designed using the software Design Model. Results showed that the design matrix was significant ($p < 0.05$, F-value of 553.34).

Preliminary experiment

Three-dimensional plots and coefficient equations were used to investigate the relationships among the factors. The results generated 39 runs in the design (Table 3).

Table 2: Reduced regression results of the measured responses for the quadratic model

Response	Sequential <i>P</i> -value	R ²	Adjusted R ²	Predicted R ²
Y1	< 0.0001	0.9928	0.991	0.9861
Y2	< 0.0001	0.9209	0.9011	0.8612
Y3	< 0.0001	0.9754	0.9722	0.9679

Table 3: Results of preliminary experiments

Run	Factor 1	Factor 2	Factor 3
	<i>Lipid: Surfactant (mg)</i>	<i>ST (Minute)</i>	<i>Surfactant type</i>
1	1	180:180	0
2	1	180:180	5
3	1	180:180	0
4	1	180:180	10
5	1	180:180	5
6	1	180:180	10
7	1	180:180	0
8	1	180:180	5
9	1	180:180	10
10	0	90:180	5
11	0	90:180	0
12	0	90:180	10
13	0	90:180	5
14	0	90:180	5
15	0	90:180	5
16	0	90:180	5
17	0	90:180	5
18	0	90:180	5
19	0	90:180	5
20	0	90:180	10
21	0	90:180	5
22	0	90:180	5
23	0	90:180	5
24	0	90:180	5
25	0	90:180	0
26	0	90:180	5
27	0	90:180	10
28	0	90:180	0
29	0	90:180	5
30	0	90:180	5
31	-1	90:270	10
32	-1	90:270	10
33	-1	90:270	0
34	-1	90:270	0
35	-1	90:270	5
36	-1	90:270	5
37	-1	90:270	0
38	-1	90:270	5
39	-1	90:270	10

Note: Chremophor (Chr), Tween (TW), Sonication time (ST)

Effect of independent variables on particle size

Statistical analysis of the design model and the examined factors was presented using statistical expressions (Table 4). Quadratic equation generated from the software of A, B, and C for the particle size is:

$$\sqrt{PS} = 7.07 + 6.02A - 2.63B + 0.27C1 - 0.74C2 + 1.02AB + 0.097AC1 + 0.45AC2 - 0.11BC1 - 0.12BC2 + 9.91A^2 + 3.74B^2 \dots (7)$$

Inclusion of a quadratic term for (A) in Eq. 7 suggested that the relationship is not consistently linear (curvature). The surface response

exhibited a U-shaped configuration (Figure 1). The change in ratio by increasing surfactant amount to 270 led to minimal increase in particle sizes (Table 3).

Effect of independent variables on PDI

The statistical analysis of the design model and the examined factors was presented using statistical expressions (Table 5). The quadratic equation obtained for PDI was:

$$Y2 = 0.366 + 0.1349A - 0.1216B - 0.037C1 + 0.0313C2 + 0.0388AC1 + 0.001AC2 + 0.1244A^2 \dots (8)$$

Table 4: Analysis of variance (ANOVA) for 3-level factorial design model on PS

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	1908.20	11	173.47	402.86	< 0.0001	significant
A-ratio	653.48	1	653.48	1517.62	< 0.0001	
B-st	87.19	1	87.19	202.50	< 0.0001	
C-surfactant type	9.06	2	4.53	10.52	0.0005	
AB	10.14	1	10.14	23.54	< 0.0001	
AC	2.75	2	1.38	3.20	0.0588	
BC	0.4355	2	0.2177	0.5057	0.6094	
A ²	680.10	1	680.10	1579.44	< 0.0001	
B ²	87.40	1	87.40	202.98	< 0.0001	
Residual	10.33	24	0.4306			
Lack of Fit	9.94	12	0.8286	25.40	< 0.0001	significant
Pure Error	0.3914	12	0.0326			
Cor Total	1918.53	35				

Factor Coding: Actual

ps

● Design Points

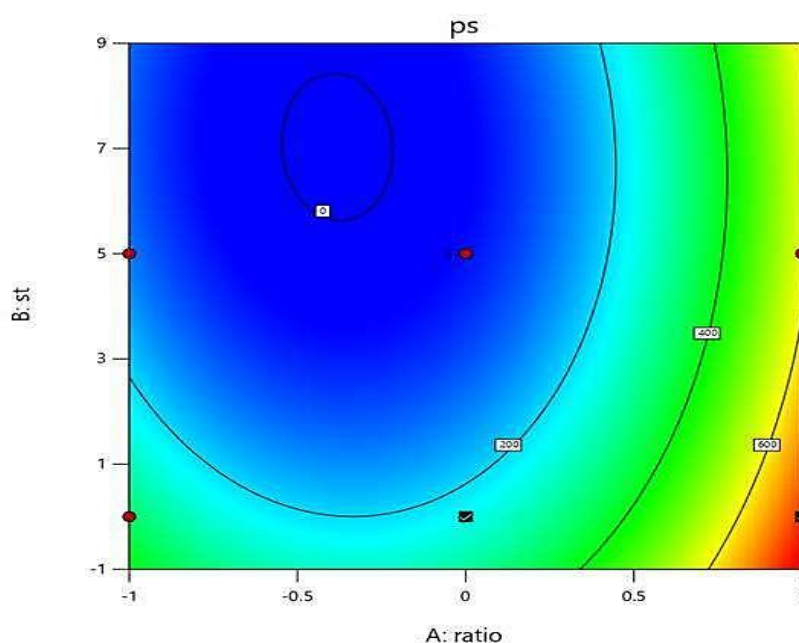
41.54 777

X1 = A

X2 = B

Actual Factor

C = chr

**Figure 1:** Contour plot expressing the effect of independent variables on PS**Table 5:** Analysis of variance (ANOVA) for 3-level factorial design model on PDI

Source	Sum of Squares	df	Mean Square	F-value	P-value
Model	0.6825	7	0.0975	46.57	< 0.0001
A-ratio	0.2751	1	0.2751	131.4	< 0.0001
B-st	0.2269	1	0.2269	108.37	< 0.0001
C-st	0.0303	2	0.0151	7.23	0.0029
AC	0.0166	2	0.0083	3.95	0.0307
A ²	0.1353	1	0.1353	64.62	< 0.0001
Residual	0.0586	28	0.0021		
Lack of Fit	0.0452	16	0.0028	2.54	0.0544
Pure Error	0.0134	12	0.0011		
Cor Total	0.7411	35			

Effect of independent variables on EE

There was no significant effect of surfactant type on % EE ($p > 0.05$). The Optimized TCG-SLN showed a particle size of 67 nm, PDI of 0.165, and EE of 87.5 %. Statistical analysis of the

design model and the examined factors was presented using statistical expressions (Table 6). The quadratic equation of EE was presented as:

$$Y_3 = 84.50 - 6.81A + 2.42B - 4.94A^2 - 4.32B^2 \dots \dots (9)$$

Table 6: Analysis of variance (ANOVA) for 3-level factorial design model on EE

Source	Sum of Squares	df	Mean Square	F-value	P-value
Model	1265.74	4	316.43	335.28	< 0.0001
A-ratio	731.45	1	731.45	775	< 0.0001
B-st	91.67	1	91.67	97.13	< 0.0001
A ²	174.59	1	174.59	184.99	< 0.0001
B ²	131.61	1	131.61	139.45	< 0.0001
Residual	29.26	31	0.9438		
Lack of Fit	21.1	19	1.11	1.63	0.1931
Pure Error	8.15	12	0.6796		
Cor Total	1294.99	35			

Characterization of TCG-SLN

Morphology of SLNs

The obtained SEM images of TCG-SLN appeared as non-aggregated and spherical-shaped SLNs with various sizes, most of which were within the nanometric range (Figure 2).

Powder X-ray diffraction (PXRD)

The PXRD pattern of pure TCG showed a highly crystalline nature (Figure 3), evidenced by the numerous distinctive and intense peaks. The PXRD of Compritol 888 displayed sharp and distinctive peaks different from TCG-SLN spectrum.

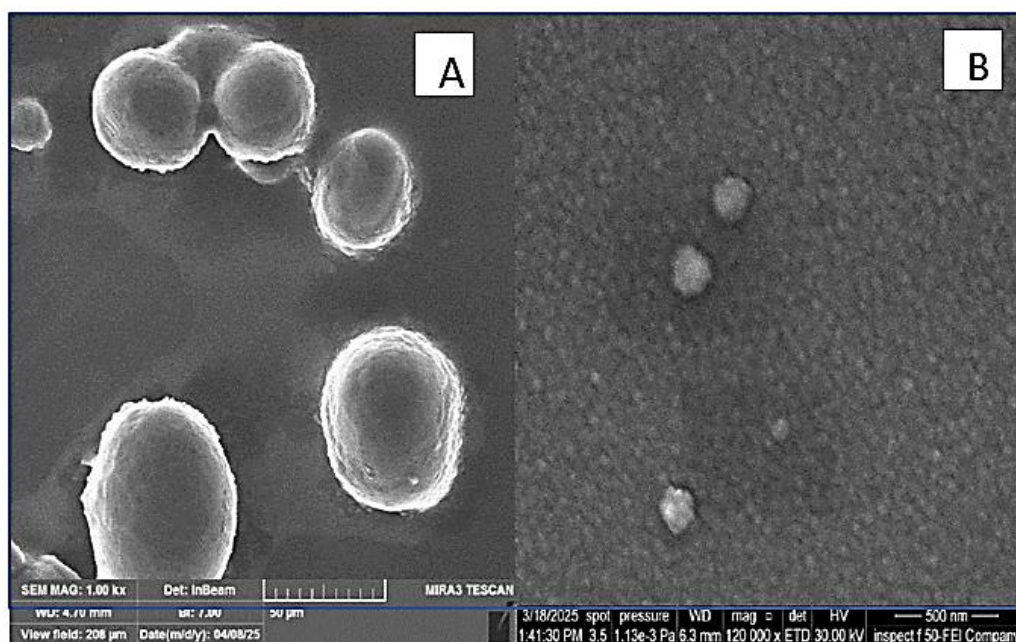
Fourier transform infrared (FTIR) spectroscopy

The main absorption bands of ticagrelor are represented in Figure 4. Absorption bands of 3408, 3292 cm⁻¹ represented N-H, O-H

stretching respectively, 2916 for C-H stretching, 1735, 1703 cm⁻¹ represented C=O in the carbonyl group, 1624, 1517 cm⁻¹ for benzyl ring, and other bands between 1400- 1000 cm⁻¹ represented C-O, and C-F respectively, characteristic of TCG confirming drug purity. Spectra were taken at 3415 cm represented vibrational band for O-H stretching, 2916, 2848 cm⁻¹ for C-H stretching of long alkyl chain, 1735 cm for a strong ester C=O, 1174, 1060 cm⁻¹ for C-C, C-O, and 721 cm⁻¹ for C-H bending vibration characteristic of Compritol 888.

In vitro release of ticagrelor

The *in vitro* drug release of TCG-SLNs formulation was conducted over 24 h and compared with pure TCG suspension. The SLNs showed a biphasic release profile with initial burst release of ≈32 % in the first hour, followed by a prolonged release phase (≈88 % in 24 h; Figure 5).

**Figure 2:** SEM images of optimum formula, scale bar (A) 50µm and (B) 500 nm

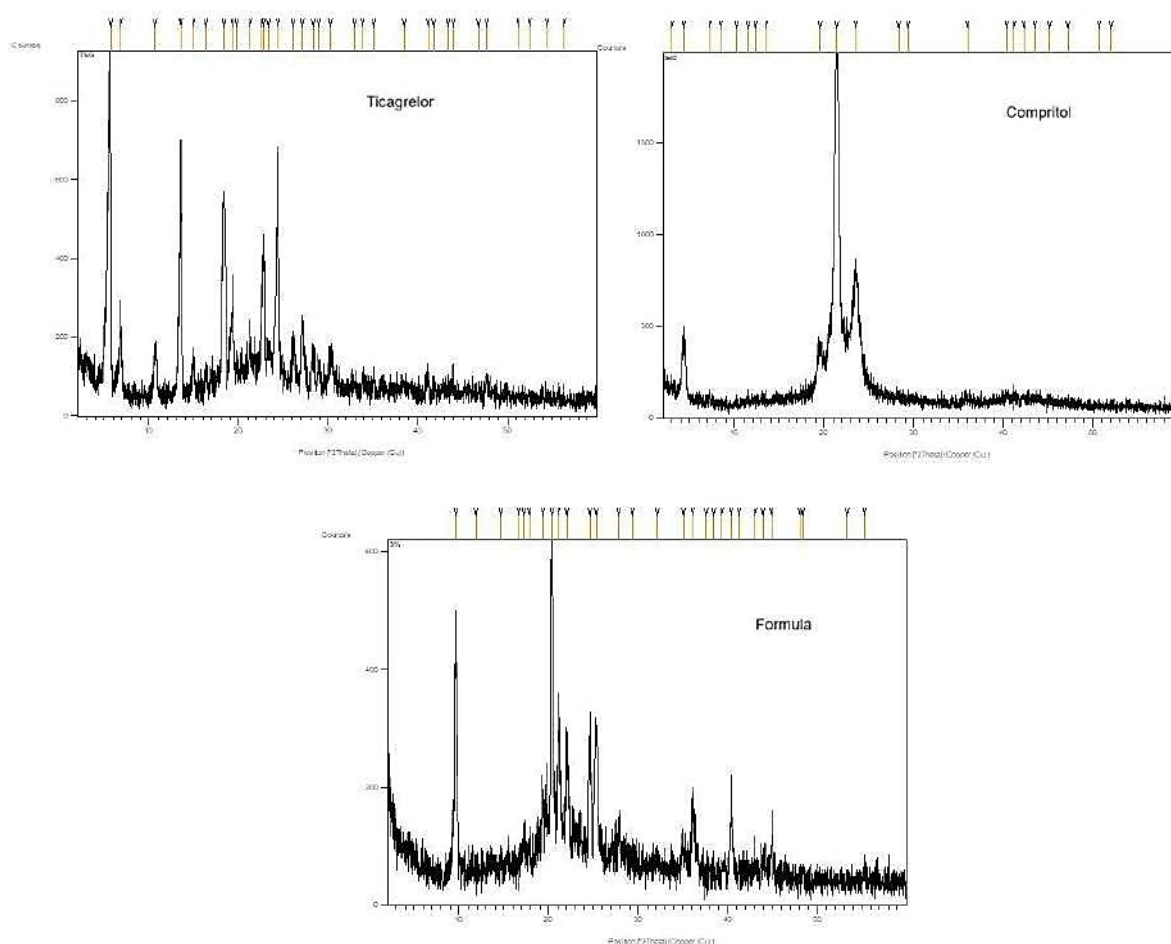


Figure 3: PXRD analysis of Ticagrelor, Compritol 888, and TCG-SLN (Formula)

Validation of HPLC

Calibration curve of TCG by HPLC method

The calibration was established by plotting a graph of concentration of TCG (x-axis) against the peak area (y-axis), and the equation obtained was $y = 149.68x - 8273.4$ (Figure 6).

Limit of detection (LOD) and limit of quantification (LOQ)

The calculated LOD and LOQ were found to be 6 and 18.18 ng/mL in mobile phase, respectively, which represented the least squares regression data of the method.

Accuracy

Average percent recovery for TCG for spiked sample was 99 % at 100, 200, and 300 ng/mL (Table 7).

Precision

Intra-day and inter-day % RSD values for the standard solutions of TCG in the mobile phase were 0.165 and 0.164, respectively. Therefore, the proposed analysis method was precise since the % RSD was less than 2.0 %.

Table 7: Recovery of NMD in spiked sample and mobile phase

Concentration (ng/mL)	Relative peak area in mobile phase	Recovery in mobile phase (%)
100	0.203	98.5
200	0.336	99.7
300	0.87	98.8
Average		99

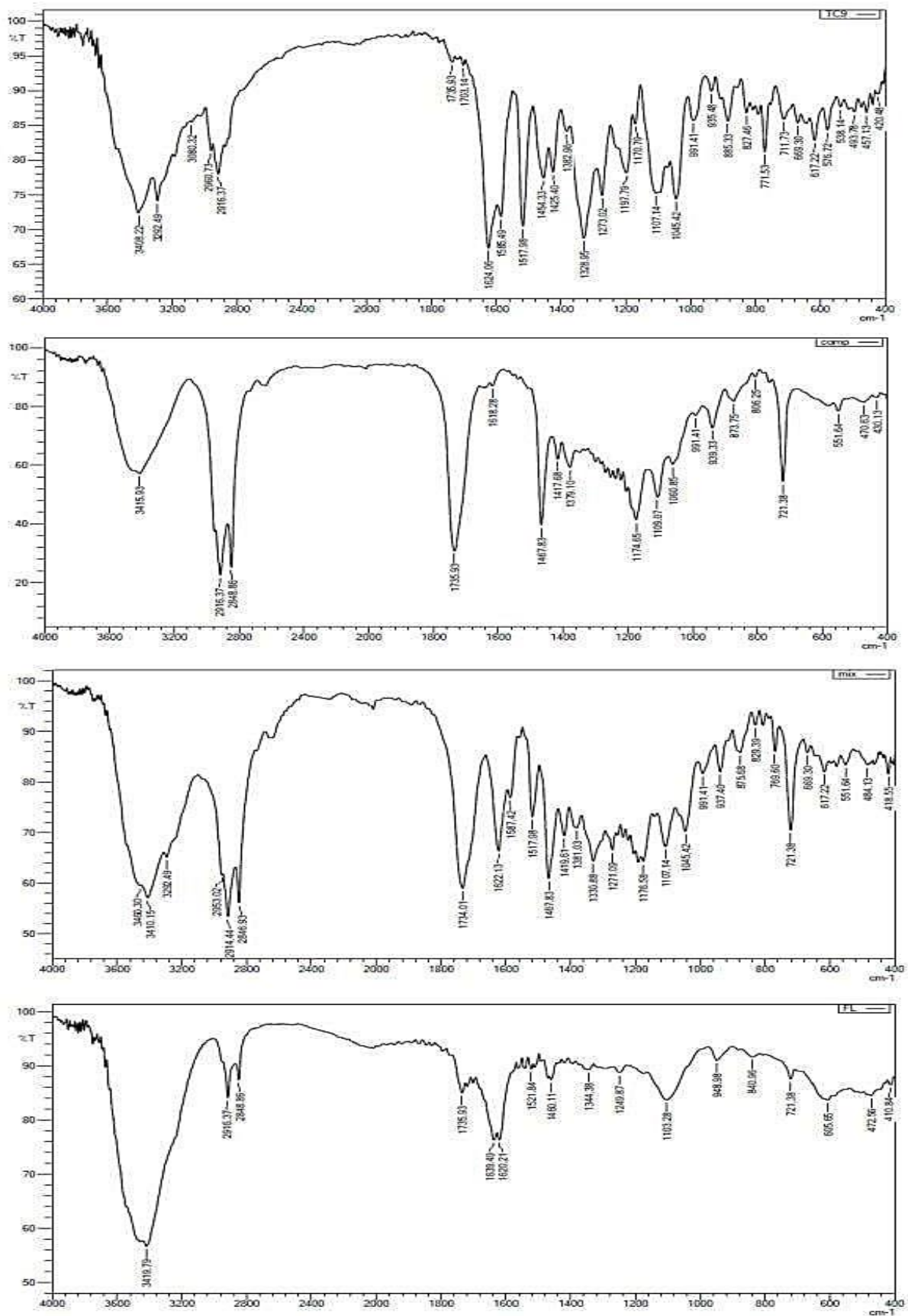


Figure 4: FTIR for A: ticagrelor, B: Compritol 888, C: physical mixture, and D: TCG-SLN

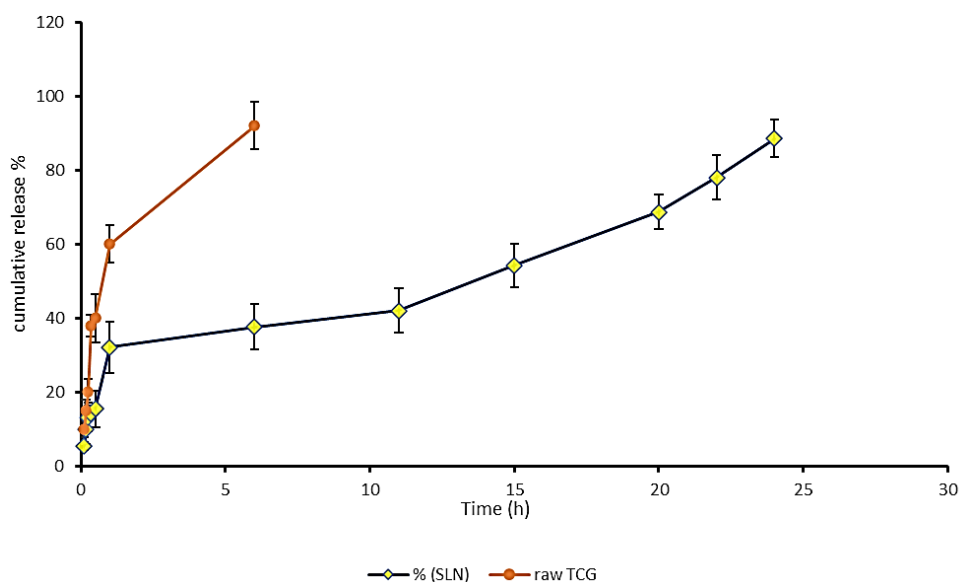


Figure 5: Cumulative release profile of pure TCG and TCG-SLNs

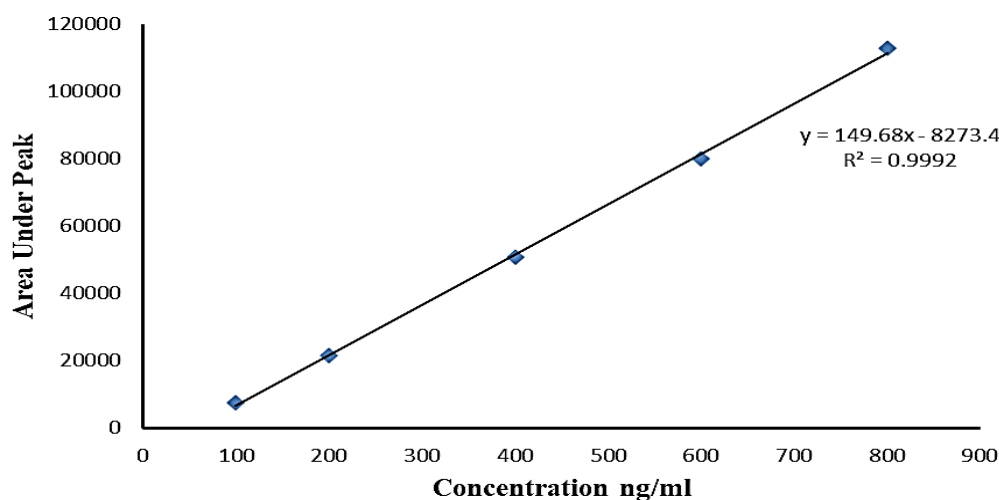


Figure 6: Calibration curve of TCG with a constant concentration of internal standard (IS) in the mobile phase

Table 8: Precision of TCG in mobile phase

Precision	Mean RPA±SD	RSD %	Sample type
Intra-day	0.165±0.0023	1.393	RPA in the mobile phase
Inter-day	0.164±0.0015	0.914	

Relative peak area (RPA), relative standard deviation (RSD), and standard deviation (SD)

Ex vivo intestinal permeability

The flux (F , $\mu\text{g/ml/h}$) was obtained from the slope of the linear regression equations, and the apparent permeability coefficient (P_{app} , cm/h) of the pure drug was 0.285 and 0.942, respectively (Table 9).

Intestinal mucosal toxicity study

The intestinal mucosa treated with PBS (negative control) showed no inflammation or damage,

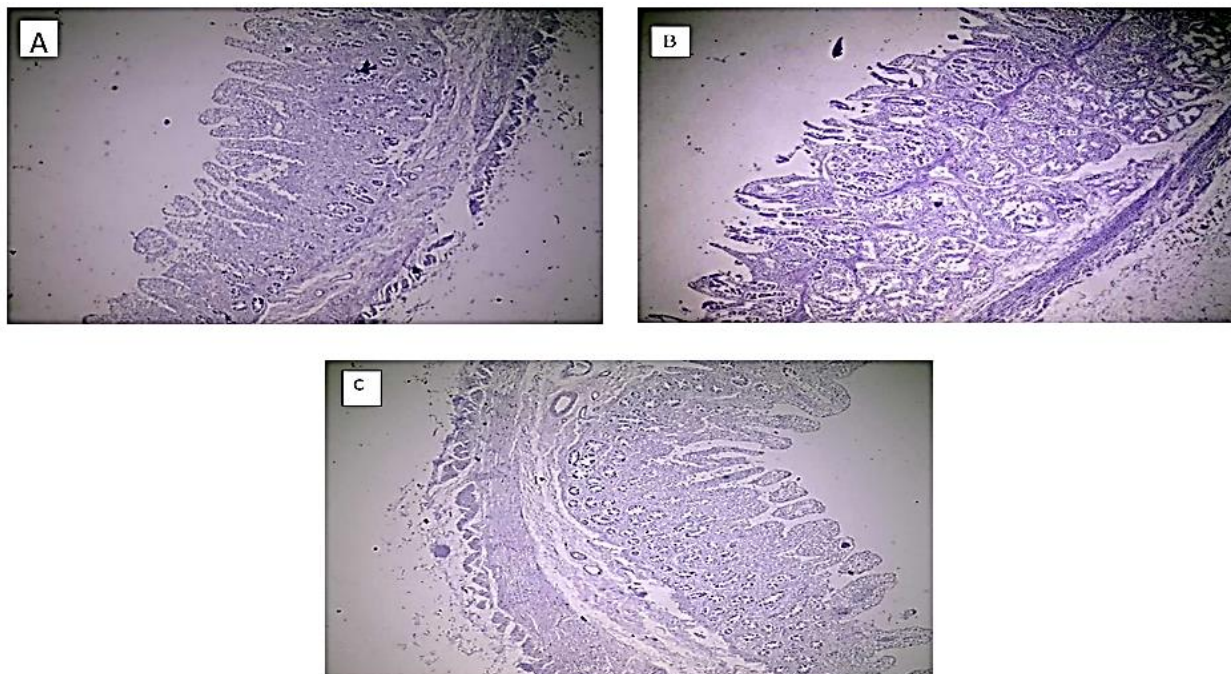
whereas the positive control exhibited significant villus degeneration and damage. The TCG-SLN restored histological structure, resembling normal morphology, despite a slight reduction in villus length (Figure 7).

Short-term stability study

There was no significant difference in physicochemical properties of TCG-SLNs after 90 days (Table 10).

Table 9: The *ex vivo* absorption parameters of ticagrelor from optimized formula and pure drug (mean $\pm$ SD, n = 3)

Parameter	Pure drug	SLN Formulation
Slope ($\mu\text{g/ml/h}$)	11.748	38.839
Flux J ($\mu\text{g/cm}^2/\text{h}$)	178.1	588.7
Papp(cm/h)	0.285	0.942
Permeability enhancement fold	-	3.3x

**Figure 7:** Histopathological examinations of intestinal sections (A) Control, (B) positive, (C) TCG-SLN**Table 10:** Short-term stability of TCG-SLNs (mean $\pm$ SD, n = 3)

Temp	Day 1 2-8 C	25C	Day 45 2-8C	25C	Day 90 2-8C	25C
PS (nm)	67 $\pm$ 3.75	67 $\pm$ 3.75	105.7	165.6	121.4	422.1
PDI	0.165 $\pm$ 0.041	0.165 $\pm$ 0.041	0.205	0.307	0.308	0.547
EE (%)	87.5 $\pm$ 3	87.5 $\pm$ 3	86 $\pm$ 3.6	84 $\pm$ 9.9	80 $\pm$ 5.5	76 $\pm$ 5.88

Particle size (PS), polydispersity index (PDI), entrapment efficiency (EE)

DISCUSSION

The TCG-SLN was optimized by fitting the responses into Design Expert program (version 11). The 3-level factorial design was considered most suitable due to its ability to detect nonlinear relationships and interaction effects among the studied variables. The results revealed that the quadratic model was the most significant and best-fitting model for all responses ($p < 0.05$), indicating the presence of curvature in the response surfaces. This suggests that variations in formulation and processing parameters exert a complex, non-linear influence on PS, PDI, and EE, consistent with a previous study [21]. This was based on several criteria such as elevated R^2 , adjusted R^2 , and predicted R^2 values from the

quadratic model, indicating exceptional fit and predictive efficacy. Adequate precision represents the signal-to-noise ratio. Values > 4 are desirable because it enables the design to navigate the entire space of the experiment. The results showed an adequate precision > 28 , suggesting an adequate signal that is used to navigate a design space [22]. The model further showed that among the three variables, (A) has the most significant synergistic linear effect on PS (positive coefficient of +6.02). This showed that an increase in lipid amount relative to surfactant led to a significant increase in particle size. This may be due to higher viscosity and surface tension. High viscosity may impact emulsion homogenization by hindering lipid spread, resulting in aggregation and higher PS. Appearance of a possible interaction between A

and B at their respective levels was indicated by the presence of AB term in the equation. Sonication time (ST) has a significant antagonistic linear effect on PS. This reduction may be attributed to the ability of the process to decompose the emulsion droplets into nanoemulsions with a uniform distribution, resulting in lower particle size. The presence of the quadratic term of (B^2) with a relatively large coefficient suggested a significant nonlinear effect. An increase in ST caused a complete breakdown of pre-emulsion particles as a result of high temperature and pressure, leading to higher PS. The rise in PS at 10 min sonication time indicated that further increase may produce large aggregates and higher PS. With no sonication, the pre-emulsion mixture underwent phase separation, and upon cooling, this led to the formation of larger particles due to the uncontrolled crystallization of the lipid [23]. Surfactant type is a categorical factor with three levels and has less effect on PS compared to other factors. In order of their effect on PS, tween 60 < chremophor < tween 60 and chremophor. Chremophor slightly increased PS compared to C2 (Tween 60). Chremophor is considered a P-gp inhibitor (decreases the absorption of Ticagrelor) despite its good result in the preliminary experiment [24]. Furthermore, (A) has a synergistic linear effect with coefficient on PDI. Therefore, increasing the lipid-to-surfactant ratio led to higher PDI due to insufficient availability of surfactant molecules to coat the surface of all lipid droplets, leading to particle aggregation and dispersity [25]. Formulation F31-F39 with higher lipid and surfactant ratio produced particles of smaller PDI. The surfactant type significantly affected PDI, indicating that the Cremophor yielded smaller PDI compared to Tween 60. The most significant factor that affected EE was A, where there was an antagonistic linear relationship (coefficient of -6.81A). This effect may have resulted from rising viscosity in the aqueous medium with increasing concentration. This action led to fewer drugs being incorporated into the lipid and less lipid being able to disperse in water. Reduction in entrapment efficiency of TCG may have resulted from the decrease in lipid surface area, consistent with an earlier study [26]. Also, an increase in surfactant concentration led to increase in EE. This effect is attributed to the observation that solubility and incorporation of TCG in Compritol were enhanced as concentration of surfactant increased. An earlier study found that incorporation of more TCG in the lipid results in higher entrapment efficiency [27]. Sonication time has a significant synergistic linear relationship with EE (+3.42B), which implies that increasing ST increases EE. This is

because higher ST led to rearrangement and reassembly of the particles under suitable probe-sonication conditions, leading to formation of smaller particles. The wrinkled surface and absence of drug crystals were due to drug entrapment within the lipid matrix, consistent with a previous study [28].

Results of the PXRD study showed that Compritol 888 appeared as random crystalline structure. However, the diffractogram of optimized SLN showed the absence of TCG characteristic peaks in the amorphous state or molecularly dispersed structure due to drug entrapment. The spectrum of the physical mixture showed distinct peaks of different components. This suggests that there has been no interaction between TCG and the excipients used in the formulation. The optimized spectrum was dominated by the principal absorption bands of Compritol 888, although the O-H band shifted from 3415 to 3419 cm^{-1} . There were also fewer peaks of TCG (1521, 1620 cm^{-1}) with slight shifts and reduced intensity, confirming physical encapsulation of the drug within the lipid matrix and no chemical alteration. As a result, the molecular structure and chemical integrity of TCG did not change during the formulation process. Thus, FTIR spectroscopy further validated the compatibility between TCG and other excipients before and after formulation. The initial burst release may be due to the presence of the drug on the surfaces of the solid lipid nanoparticles (SLNs) and external phase. Sustained release profile is attributed to highly organized crystalline structure of the solid lipid matrix, which limits drug diffusion rate to the lipid interface prior to partitioning between the lipid and aqueous phases. Inclusion of Compritol 888 (a key component of the lipid matrix) is widely recognized as a sustained-release agent [29]. Percentage cumulative release from pure TCG was $\approx 92\%$ within 6 h.

The release profile of TCG-SLN is advantageous for preventing drug degradation in acidic environments and enhancing intestinal absorption. This approach would facilitate lymphatic uptake and circumvent CYP-mediated hepatic first-pass metabolism [30]. The linearity was evaluated from calibration curves of TCG (constructed once in the mobile phase and in the spiked plasma samples), which yielded a linear correlation over the predicted concentration range. Intestinal permeability showed that the optimized formulation of TCG-SLN exhibited significantly higher permeability compared to the pure drug suspension ($p < 0.05$), consistent with a previous study on artesunate-loaded SLNs [31]. This effect may be attributed to various

factors, like diminutive particle size and significant adhesion resulting from the very high surface area, which facilitates drug diffusion and dissolution [32], and the incorporation of permeability-enhancing excipients such as Cremophor EL and Tween (which reduce intestinal P-glycoprotein efflux pump activity, likely enhancing drug permeability) [30].

The short-term stability test results showed a significant increase in PS and PDI at 25 °C ($p < 0.05$) at day 90 compared to day 1. Also, the rate of particle growth was greater at 25 °C compared to 2 – 8 °C. This is due to the correlation between elevated temperature and increased energy, resulting in a higher rate of particle collisions and, subsequently, particle aggregation [33].

CONCLUSION

The TCG-SLN optimum formula, consisting of lipid: surfactant (90:247 w/w), exhibited small particle size, narrow size distribution, high encapsulation efficiency, sustained-release profile, and improved permeability compared to the pure drug. Future studies should investigate the safety and clinical feasibility of the formulation.

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Use of Artificial intelligence / large language models

The authors confirm no AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Conflict of interest

The authors declare no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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