

DEVELOPMENT OF TICAGRELOR NANOCRYSTALS AS A NOVEL APPROACH FOR ENHANCING SOLUBILITY AND ORAL BIOAVAILABILITY

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ABSTRACT

Background: Ticagrelor is a direct-acting, reversible P2Y₁₂ receptor antagonist used in the management of acute coronary syndrome. However, its poor aqueous solubility and limited intestinal permeability contribute to variable oral bioavailability, creating a need for an effective formulation approach to improve its biopharmaceutical performance.

Purpose: The present study aimed to develop and optimize ticagrelor nanocrystals using a nanoprecipitation technique to enhance drug dissolution and oral bioavailability.

Method: Ticagrelor nanocrystals were prepared using polyvinyl alcohol and polyethylene glycol 6000 as stabilizers. A three-factor, three-level Box–Behnken design with 17 experimental runs was employed to optimize PVA concentration, PEG 6000 concentration, and sonication time based on particle size, zeta potential, and entrapment efficiency. The optimized formulation was characterized by FTIR, DSC, XRD, SEM, and TEM and evaluated for in-vitro dissolution, pharmacokinetic behaviour, and antiplatelet activity.

Results: The optimized nanocrystals exhibited a particle size of 231 nm, PDI of 0.281, and zeta potential of –34.1 mV. The optimized nanocrystal formulation demonstrated rapid and enhanced drug dissolution, achieving near-complete drug release within the specified dissolution period. The optimized capsule exhibited satisfactory drug content uniformity, rapid disintegration characteristics, and enhanced in-vitro dissolution performance, indicating improved drug-release efficiency from the capsule dosage form. Pharmacokinetic studies showed increased C_{max} (342 ng/mL) and AUC (2280 ng·h/mL).

KEYWORDS: Ticagrelor, Nanocrystals, Nanoprecipitation, Bioavailability, Dissolution Enhancement, Box–Behnken Design, Antiplatelet Activity.

1. INTRODUCTION

Acute coronary syndrome (ACS) remains a leading cause of morbidity and mortality worldwide (Byrne et al., 2023). Antiplatelet therapy is the cornerstone of ACS management, with dual antiplatelet therapy (DAPT) comprising aspirin and a P2Y₁₂ receptor antagonist being the standard of care. Ticagrelor, a cyclopentyl-triazolo-pyrimidine, is a direct-acting, reversibly binding P2Y₁₂ antagonist that has demonstrated superior clinical efficacy over clopidogrel in the landmark PLATO trial. Unlike thienopyridines, ticagrelor does not require metabolic activation, resulting in a rapid onset of action (Wallentin et al., 2009; James et al., 2009).

Despite its clinical advantages, ticagrelor presents significant formulation challenges. It is classified as a BCS Class IV drug, exhibiting both low aqueous solubility and poor intestinal permeability, which results in a low and variable oral bioavailability of approximately 36% (Brittain, 2009; Gao et al., 2013). The conventional immediate-release tablet also exhibits a notable food effect, contributing to fluctuations in plasma concentrations and an increased risk of concentration-dependent adverse effects such as dyspnea and bradyarrhythmias [7]. Therefore, there is a clear clinical need for an improved formulation strategy that can enhance the solubility and dissolution rate of ticagrelor, leading to more consistent and improved oral bioavailability (Pujitha et al., 2024).

Drug nanocrystal technology has emerged as a versatile and commercially viable platform for enhancing the dissolution and bioavailability of poorly water-soluble drugs (Chakraborty et al., 2009; Junghanns et al., 2008). Nanocrystals are pure drug particles stabilized by a thin layer of surfactant or polymer, with particle sizes typically in the range of 100–1000 nm. The profound increase in surface area and the resulting enhancement in dissolution velocity, as described by the Noyes-Whitney equation, are the primary mechanisms for bioavailability enhancement (Guo et al., 2022; Imono et al., 2020). This carrier-free approach offers high drug loading, reduces the risk of excipient-related toxicity, and is applicable to a wide range of compounds, making it particularly suitable for BCS Class II and IV drugs (McClements et al., 2012; Müller et al., 2011).

The present study was designed to prepare and optimize ticagrelor nanocrystals using the nanoprecipitation technique, with PVA and PEG 6000 as stabilizers. A systematic Design of Experiments (DoE) approach was utilized to optimize the formulation. The optimized nanocrystals were comprehensively characterized for their physicochemical properties, solid-state characteristics, in-vitro dissolution behavior, in-vivo pharmacokinetics, and antiplatelet activity (Hancock et al., 1997).

2. MATERIALS AND METHODS

2.1. Materials

Ticagrelor was received as a gift sample from a pharmaceutical company. Polyvinyl Alcohol (PVA) and Polyethylene Glycol 6000 (PEG 6000) were purchased from standard commercial suppliers. All other chemicals and reagents, including HPLC-grade methanol and acetonitrile, were of analytical grade (Qiao et al., 2011; Strickley et al., 2004).

2.2. Preformulation Studies

The procured drug was characterized for its organoleptic properties, melting point, and spectroscopic analysis. FTIR spectra were recorded using the KBr pellet method in the range of 4000–400 cm^{-1} . UV spectroscopy was performed in methanol to determine the λ_{max} . The equilibrium solubility of ticagrelor was determined in various media (water, pH 1.2, 4.5, 6.8, and 7.4) using the shake-flask method. Drug-excipient compatibility studies were conducted by storing 1:1 physical mixtures at accelerated conditions (40°C/75% RH) for one month, followed by FTIR analysis (Rautio et al., 2008).

2.3. Preparation of Ticagrelor Nanocrystals

Ticagrelor nanocrystals were prepared by the nanoprecipitation method. A stock solution of ticagrelor (10 mg/mL) was prepared in ethanol. Separately, an aqueous phase was prepared by dissolving PVA (3.5 g) in deionized water (50 mL) with heating and ultrasonication, followed by the addition of PEG 6000 (0.83 g) and stirring for 30 min (Sinha et al., 2013). The ethanolic drug solution was then injected slowly into the aqueous phase using a 24G needle under probe sonication in an ice bath. After complete addition, sonication was continued for another 20 min, followed by overnight magnetic stirring at 400 rpm to remove residual solvent (Keck et al. 2006; Müller et al., 2012).

2.4. Experimental Design for Optimization

A three-factor, three-level Box-Behnken design was employed to optimize the formulation. The independent variables were the concentration of PVA (X1), the concentration of PEG 6000 (X2), and sonication time (X3). The dependent responses were particle size (Y1), zeta potential (Y2), and entrapment efficiency (%EE, Y3). The design consisted of 17 experimental runs. The data were analyzed using Design-Expert® software (Version 13, Stat-Ease, Inc.) to fit a mathematical model and identify the optimal formulation (Khadka et al., 2014; Kohli et al., 2010).

2.5. Evaluation of Nanocrystals

The mean particle size and PDI were determined by Dynamic Light Scattering (DLS) using a Zetasizer. Zeta potential was measured using the same instrument. Saturation solubility of the optimized formulation was determined in phosphate buffer (pH 6.8). In-vitro dissolution studies were performed using a USP Type II (paddle) apparatus in 900 mL of phosphate buffer (pH 6.8) containing 0.5% SLS at 37°C and 50 rpm. Solid-state characterization of the optimized nanocrystals was performed using FTIR, DSC, and XRD. Morphological examination was conducted using SEM and TEM (Kolipaka et al., 2023; Liu et al., 2023).

2.6. Formulation and Evaluation of Capsule Dosage Form

The optimized nanocrystal suspension was filled into hard gelatin capsules. The capsules were evaluated for weight variation, drug content uniformity, capsule thickness, and disintegration time. An in-vitro dissolution study of the capsules was also performed (Kolipaka et al., 2023; Liu et al., 2023).

2.6.1 Physical Appearance

The formulated capsules were visually inspected for their color, size, shape, and any visible surface defects such as cracking, denting, or bloating. The capsules were also carefully observed for any signs of leakage, discoloration, or other physical abnormalities that could indicate instability or poor manufacturing quality.

2.6.2 Weight Variation

The weight variation test was performed to ensure the uniformity of the capsule fill weight. Twenty capsules were individually weighed accurately using an analytical balance, and the average weight was calculated. The percentage deviation of each individual capsule weight from the average weight was determined and compared with the limits specified in the official pharmacopoeia. For capsules weighing less than 300 mg, the acceptable deviation was not more than $\pm 7.5\%$ (Sayjani et al., 2012).

2.6.3 Drug Content Uniformity

The drug content uniformity test was performed to ensure that each capsule contained a uniform and accurate amount of Ticagrelor. Ten capsules were individually opened, and the contents from each capsule were quantitatively transferred into separate volumetric flasks. The powder was dissolved in 100 mL of methanol with the aid of sonication to ensure complete extraction of the drug. The resulting solution was filtered through a 0.45 μm membrane filter, appropriately diluted with phosphate buffer (pH 6.8), and analyzed using a validated UV-Visible spectrophotometric method at the predetermined λ_{max} . The drug content in each capsule was calculated using the standard calibration curve (Zhao et al., 2023).

2.6.4 Disintegration Test

The disintegration time of the capsules was determined using a USP disintegration apparatus to ensure that the capsules would disintegrate within the specified time frame upon oral administration. One capsule was placed in each of the six tubes of the basket assembly, and the apparatus was suspended in a beaker containing 0.1N HCl (simulated gastric fluid, pH 1.2) maintained at 37°C $\pm$ 2°C. The time taken for the capsule shell to rupture and

allow the contents to be released completely (with no palpable mass remaining) was recorded. The test was performed in triplicate to ensure reproducibility (Ponchel et al., 1997; Serajuddin et al., 2007).

2.6.5 In Vitro Drug Release (Dissolution Study)

The in vitro dissolution of Ticagrelor from the nanocrystal-filled capsules was compared with pure drug powder filled in capsules (control). The study was carried out using a USP Type II (Paddle) apparatus.

Dissolution medium: To maintain sink conditions, the bag method of dialysis was employed. The dialysis procedure was carried out sequentially: initially against acetate buffer at pH 2.8, followed by phosphate buffer at pH 6.8 containing 0.5% SLS (sodium lauryl sulfate).

Volume: 900 mL

Temperature: $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$

RPM: 50 rpm

Aliquots of 5 mL were withdrawn at predetermined time intervals and an equivalent amount of fresh dissolution medium was replaced to maintain constant volume. The samples were filtered through a $0.45\ \mu\text{m}$ membrane filter, diluted appropriately, and analyzed for drug concentration using a UV-Visible spectrophotometer at the predetermined λ_{max} of Ticagrelor (Parmar et al., 2021)

2.7. In-Vivo Studies

The in-vivo bioavailability study was conducted in healthy Wistar rats. The animals were divided into four groups (n=6): Group I (Control), Group II (Pure Drug suspension), Group III (Marketed formulation), and Group IV (Optimized nanocrystal capsule formulation). A single oral dose equivalent to 10 mg/kg of ticagrelor was administered. Blood samples were collected at predetermined time intervals, and plasma ticagrelor concentrations were determined using a validated HPLC method. Pharmacokinetic parameters (C_{max} , T_{max} , AUC) were calculated. Antiplatelet activity was assessed by measuring platelet aggregation, platelet count, and thromboxane B2 levels (Loftsson et al., 2010; Maurya et al., 2022)

2.9. Statistical Analysis

Statistical analysis was performed using Student's t-test, with a p-value < 0.05 considered significant.

3. RESULTS AND DISCUSSION

3.1. Preformulation Studies

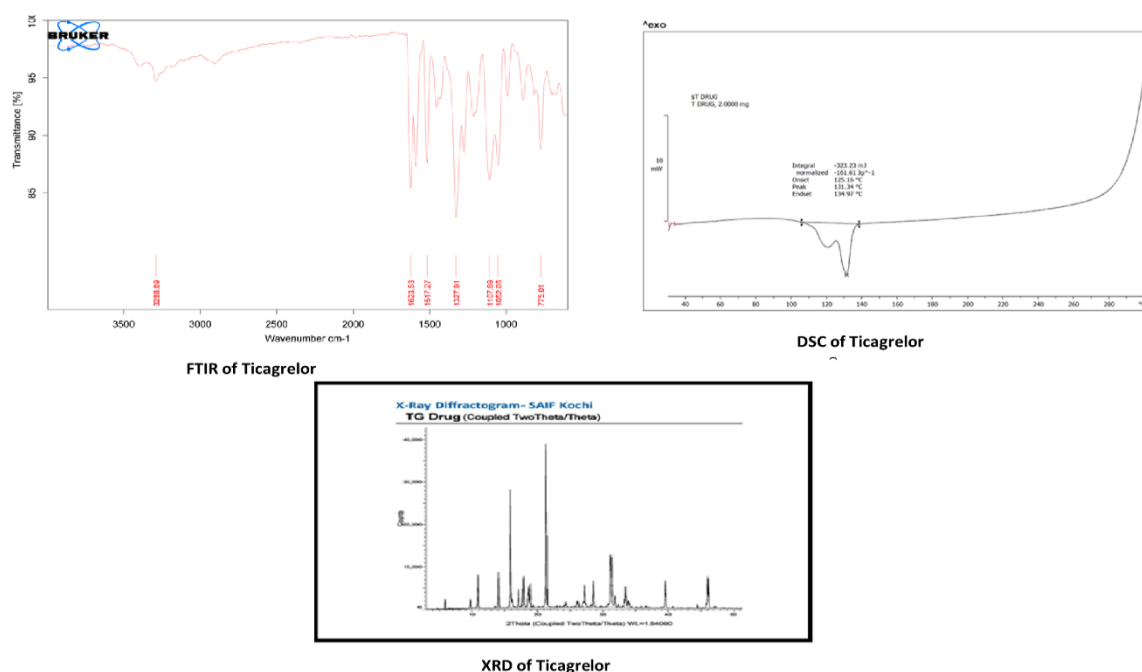


Figure 1. FTIR, DSC, and XRD characterization of ticagrelor.

The FTIR spectrum of ticagrelor showed characteristic peaks for O-H, aromatic C-H, C=N, and C-F stretching, confirming its identity. DSC showed a sharp endothermic peak at 136-137°C, indicating its crystalline nature. The drug was found to be practically insoluble in water and showed pH-dependent solubility. Drug-excipient compatibility studies showed no significant interaction between ticagrelor and the polymers.

Preparation of Ticagrelor Nanocrystals

Table 1. Box-Behnken experimental design for ticagrelor nanocrystals.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A: Conc of PVA	B: Conc of PEG 6000	C: Sonication	Partical Size	Zeta	%EE

		gm	gm	min	nm	mV	%
13	1	3.05	1.1	3	340	-13.8	84.23
17	2	3.05	1.1	3	343	-13.16	84.12
2	3	6	0.2	3	355	-9.09	88.09
11	4	3.05	0.2	5	348	-11.12	84.12
14	5	3.05	1.1	3	347	-13.5	84.23
6	6	6	1.1	1	362	-12.56	89.11
12	7	3.05	2	5	183	-16.82	84.02
1	8	0.1	0.2	3	361	-8.11	80.09
10	9	3.05	2	1	352	-17.59	86.12
9	10	3.05	0.2	1	392	-15.3	86.08
4	11	6	2	3	204	-20.9	86.12
15	12	3.05	1.1	3	352	-14.75	80.12
3	13	0.1	2	3	212	-9.09	80.02
5	14	0.1	1.1	1	369	-13.23	81.07
8	15	6	1.1	5	198	-22.23	90.12
16	16	3.05	1.1	3	358	-15.12	83.02
7	17	0.1	1.1	5	208	-13.2	78.27

Table 2. ANOVA for particle size.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	68178.75	3	22726.25	14.85	0.0002	significant
A-Conc of PVA	120.13	1	120.13	0.0785	0.7838	
B-Conc of PEG 6000	31878.13	1	31878.13	20.83	0.0005	
C-Sonication	36180.50	1	36180.50	23.64	0.0003	
Residual	19895.72	13	1530.44			
Lack of Fit	19689.72	9	2187.75	42.48	0.0013	significant
Pure Error	206.00	4	51.50			
Cor Total	88074.47	16				

Factor coding is Coded.

Sum of squares is **Type III - Partial**

The **Model F-value** of 14.85 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 3. Fit statistics for particle size.

Std. Dev.	39.12	R²	0.7741
Mean	310.82	Adjusted R²	0.7220
C.V. %	12.59	Predicted R²	0.6051
		Adeq Precision	13.7408

Table 4. Coefficients in terms of coded factors for particle size.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	310.82	1	9.49	290.33	331.32	
A-Conc of PVA	-3.88	1	13.83	-33.76	26.01	1.0000
B-Conc of PEG 6000	-63.12	1	13.83	-93.01	-33.24	1.0000
C-Sonication	-67.25	1	13.83	-97.13	-37.37	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

The **Predicted R²** of 0.6051 is in reasonable agreement with the **Adjusted R²** of 0.7220; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 13.741 indicates an adequate signal. This model can be used to navigate the design space.

Design-Expert® Software
Factor Coding: Actual

Size (nm)
● Design Points
183 392

X1 = A: Conc of PVA
X2 = B: Conc of PEG 6000

Actual Factor
C: Sonication = 3

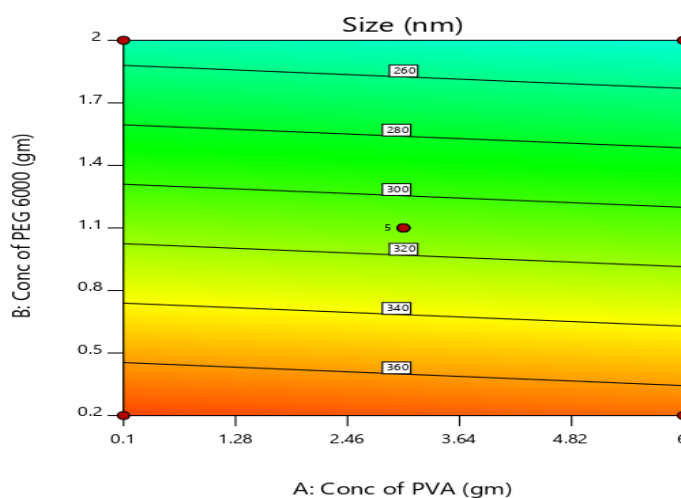


Figure 2. Surface plot showing the effect of PVA concentration and PEG 6000 concentration on particle size of ticagrelor nanocrystals.

Design-Expert® Software
Factor Coding: Actual

Size (nm)
● Design points above predicted value
○ Design points below predicted value
183 392

X1 = A: Conc of PVA
X2 = B: Conc of PEG 6000

Actual Factor
C: Sonication = 3

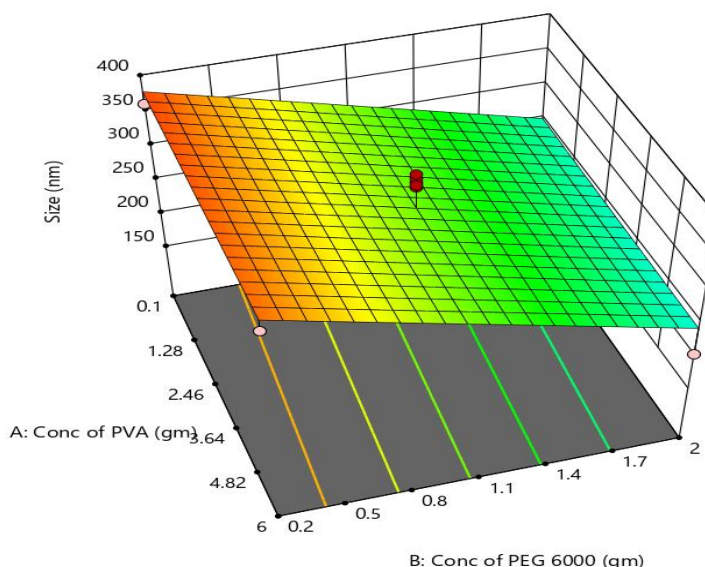


Figure 3. Surface plot showing the effect of PVA concentration and sonication time on particle size of ticagrelor nanocrystals and the effect of PEG 6000 concentration and sonication time on particle size of ticagrelor nanocrystals.

Table 5. ANOVA for zeta potential.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	112.64	3	37.55	3.99	0.0324	significant
A-Conc of PVA	55.92	1	55.92	5.94	0.0299	
B-Conc of PEG 6000	53.98	1	53.98	5.73	0.0324	
C-Sonication	2.75	1	2.75	0.2920	0.5981	
Residual	122.41	13	9.42			
Lack of Fit	119.62	9	13.29	19.05	0.0061	significant
Pure Error	2.79	4	0.6977			
Cor Total	235.05	16				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 3.99 implies the model is significant. There is only a 3.24% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 6. Fit statistics for zeta potential.

Std. Dev.	3.07	R²	0.4792
Mean	-14.09	Adjusted R²	0.3590
C.V. %	21.77	Predicted R²	-0.0787
		Adeq Precision	7.0425

A negative **Predicted R²** implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

Table 7. Coefficients in terms of coded factors for zeta potential.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	-14.09	1	0.7442	-15.70	-12.48	
A-Conc of PVA	-2.64	1	1.08	-4.99	-0.3000	1.0000
B-Conc of PEG 6000	-2.60	1	1.08	-4.94	-0.2537	1.0000
C-Sonication	-0.5863	1	1.08	-2.93	1.76	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of

factors. As a rough rule, VIFs less than 10 are tolerable.

Design-Expert® Software
Factor Coding: Actual

Zeta (mV)

● Design Points
-22.23 -8.11

X1 = A: Conc of PVA
X2 = B: Conc of PEG 6000

Actual Factor
C: Sonication = 3

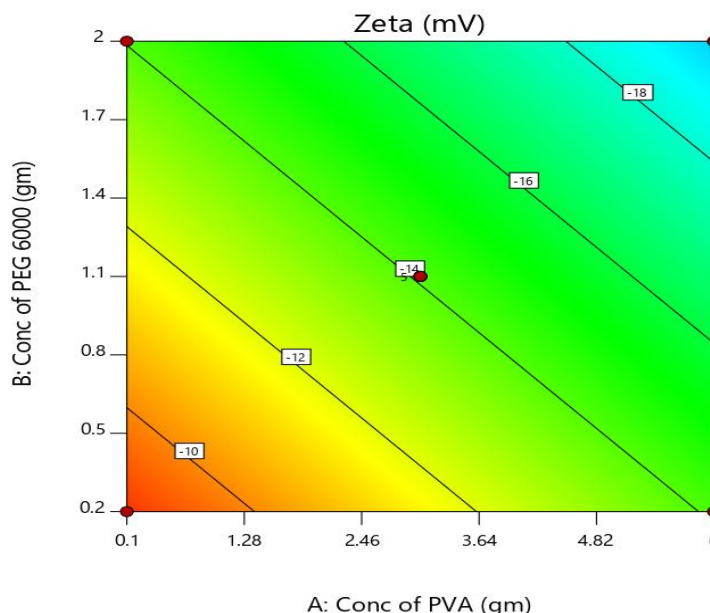


Figure 4. Surface plot showing the effect of PVA concentration and PEG 6000 concentration on zeta potential.

Design-Expert® Software
Factor Coding: Actual

Zeta (mV)

● Design points above predicted value

○ Design points below predicted value

-22.23 -8.11

X1 = A: Conc of PVA
X2 = B: Conc of PEG 6000

Actual Factor

C: Sonication = 3

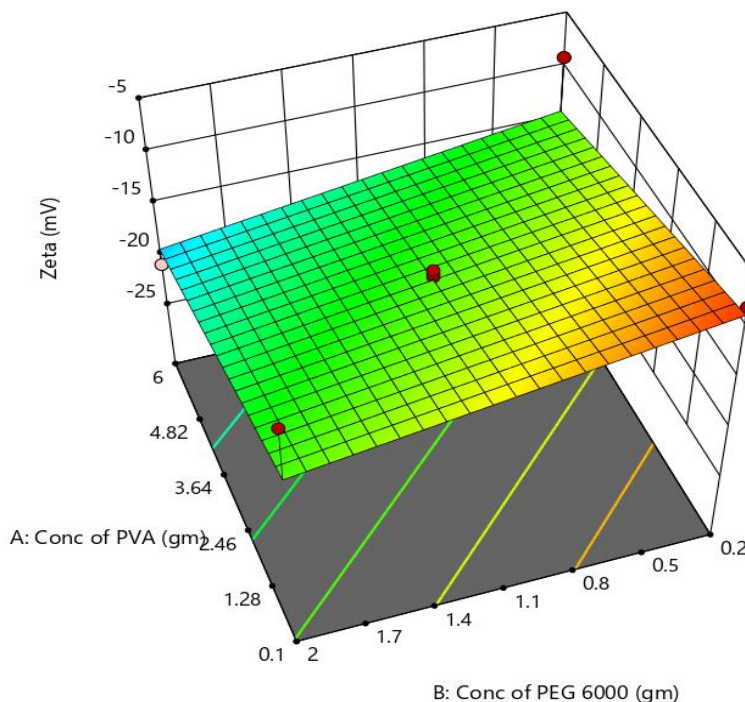


Figure 11. Surface plot showing the effect of PVA concentration and sonication time on zeta potential and effect of PEG 6000 concentration and sonication time on zeta potential.

Table 8. ANOVA for entrapment efficiency (%EE).

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	149.24	3	49.75	19.59	< 0.0001	significant
A-Conc of PVA	144.42	1	144.42	56.88	< 0.0001	
B-Conc of PEG 6000	0.5512	1	0.5512	0.2171	0.6490	
C-Sonication	4.28	1	4.28	1.68	0.2169	
Residual	33.01	13	2.54			
Lack of Fit	20.54	9	2.28	0.7319	0.6808	not significant
Pure Error	12.47	4	3.12			
Cor Total	182.25	16				

Factor coding is Coded.

Sum of squares is **Type III - Partial**

The **Model F-value** of 19.59 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 9. Fit statistics for entrapment efficiency (%EE).

Std. Dev.	1.59	R²	0.8189
Mean	84.06	Adjusted R²	0.7771
C.V. %	1.90	Predicted R²	0.7089
		Adeq Precision	12.8858

The **Predicted R²** of 0.7089 is in reasonable agreement with the **Adjusted R²** of 0.7771; i.e. the difference is less than 0.2.

Table 10. Coefficients in terms of coded factors for entrapment efficiency (%EE).

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	84.06	1	0.3865	83.22	84.89	
A-Conc of PVA	4.25	1	0.5634	3.03	5.47	1.0000
B-Conc of PEG 6000	-0.2625	1	0.5634	-1.48	0.9546	1.0000
C-Sonication	-0.7312	1	0.5634	-1.95	0.4858	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Design-Expert® Software
Factor Coding: Actual

%EE (%)
● Design Points
78.27 90.12

X1 = A: Conc of PVA
X2 = B: Conc of PEG 6000

Actual Factor
C: Sonication = 3

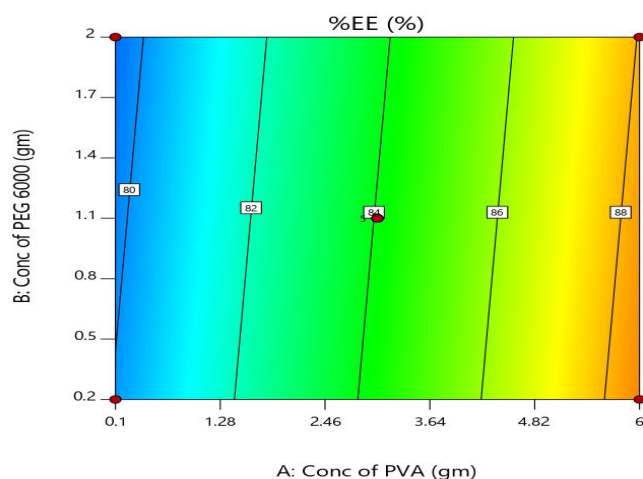


Figure 5. Surface plot showing the effect of PVA concentration and PEG 6000 concentration on entrapment efficiency.

Design-Expert® Software
Factor Coding: Actual

%EE (%)
● Design points above predicted value
○ Design points below predicted value
78.27 90.12

X1 = A: Conc of PVA
X2 = B: Conc of PEG 6000

Actual Factor
C: Sonication = 3

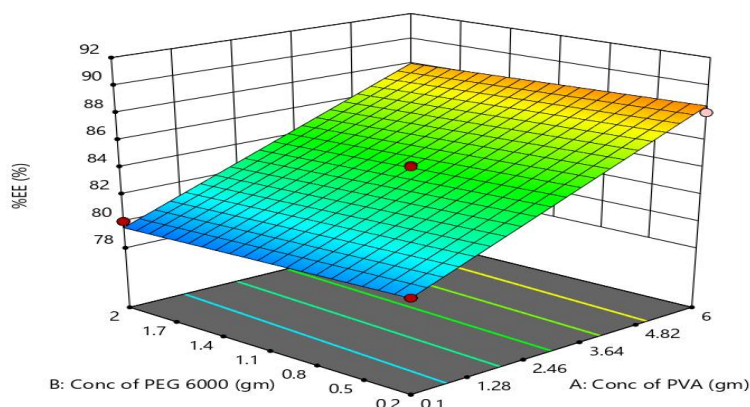


Figure 6. Surface plot showing the effect of PVA concentration and sonication time on entrapment efficiency and the effect of PEG 6000 concentration and sonication time on entrapment efficiency.

1. Particle Size

Linear equation:

$$Y_1 = 310.82 - 3.88A - 63.12B - 67.25C$$

Where:

A = Concentration of PVA

B = Concentration of PEG 6000

C = Sonication time

Interpretation: Increasing PEG 6000 and sonication time decreases particle size, while PVA has a relatively small effect.

2. Zeta Potential

Linear equation:

$$Y_2 = -14.09 - 2.64A - 2.60B - 0.5863C$$

Where:

A = Concentration of PVA

B = Concentration of PEG 6000

C = Sonication time

Interpretation: PVA and PEG 6000 have significant effects on zeta potential, whereas sonication time is not significant.

3. Entrapment Efficiency (%EE)

Linear equation:

$$Y_3 = 84.06 + 4.25A - 0.2625B - 0.7312C$$

Where:

A = Concentration of PVA

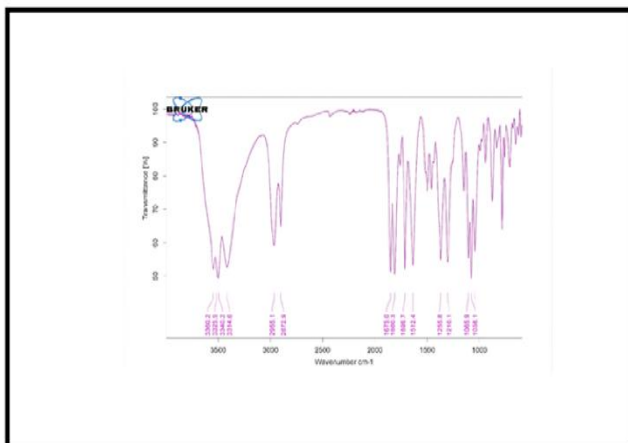
B = Concentration of PEG 6000

C = Sonication time

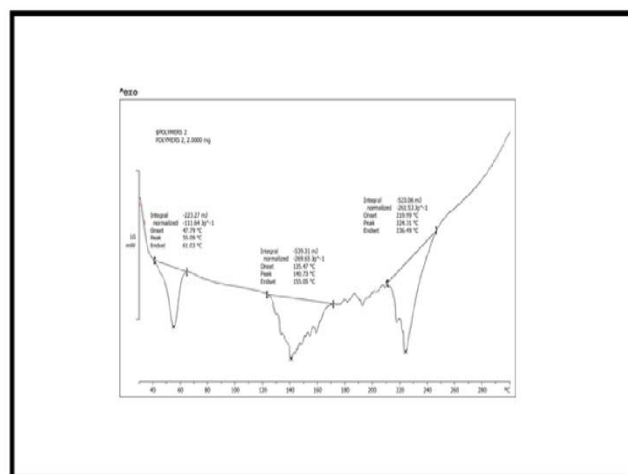
Interpretation: PVA has the strongest positive effect on %EE, while PEG 6000 and sonication time have relatively small negative effects.

3.2. Optimization of Nanocrystals

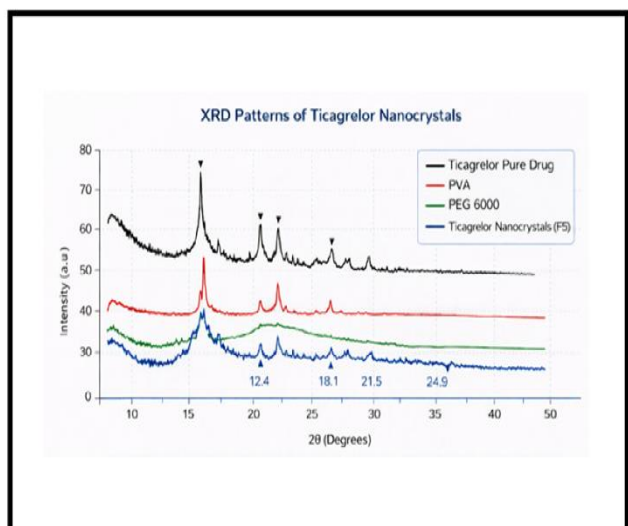
The Box-Behnken design was successfully used to optimize the formulation. The ANOVA analysis for particle size showed that the model was significant ($p=0.0002$), with PEG 6000 concentration (B) and sonication time (C) being the significant factors. The linear equation for particle size was: $Y_1 = 310.82 - 3.88A - 63.12B - 67.25C$. The optimized formulation (Batch C20) was predicted with a particle size of 231 nm, PDI of 0.281, and zeta potential of -34.1 mV, which were in close agreement with the experimental values.



FTIR of nanocrystals

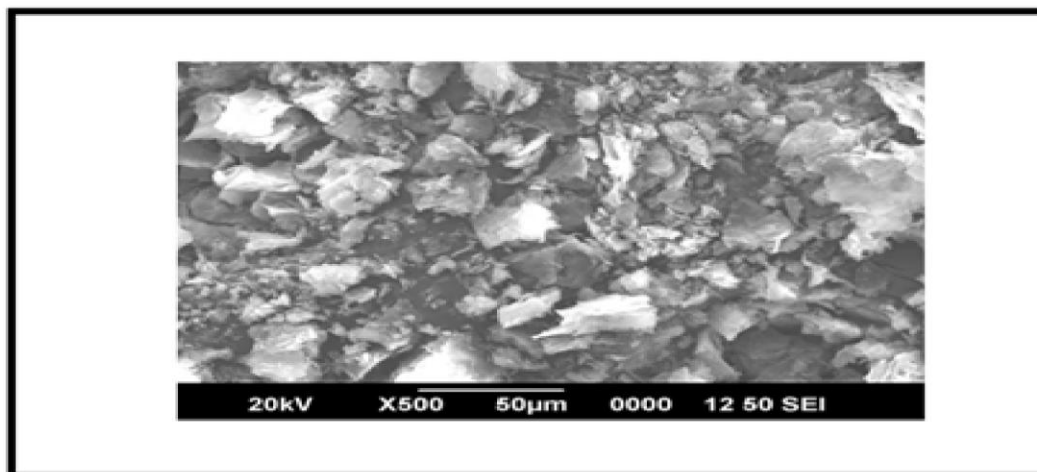


DSC of nanocrystals

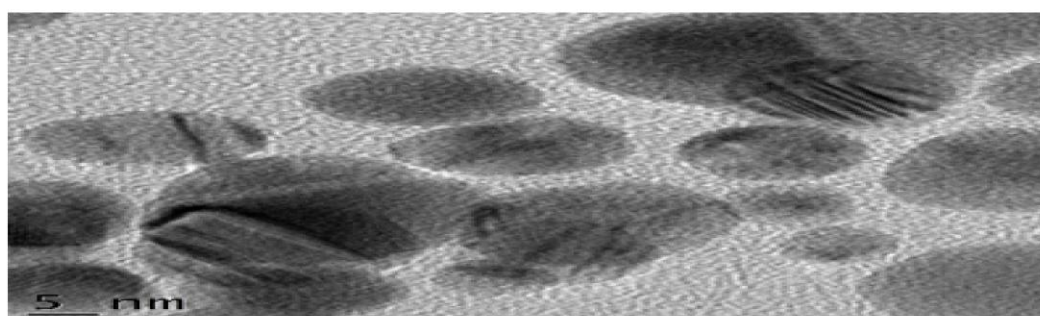


XRD of nanocrystals

Figure 7. FTIR, DSC, and XRD characterization of optimized ticagrelor nanocrystals.



SEM of nano crystals

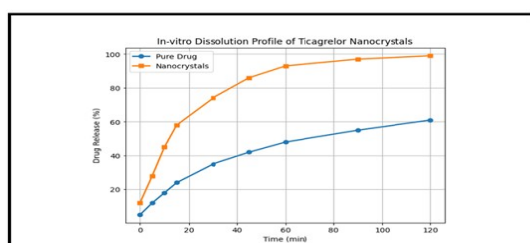


TEM of nano crystals

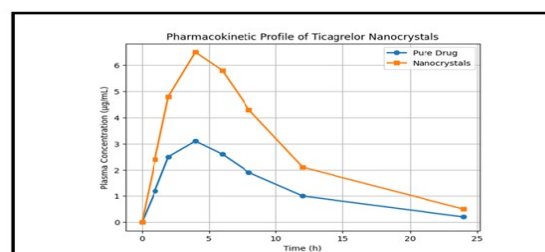
Figure 8. SEM and TEM images of optimized ticagrelor nanocrystals.

The optimized nanocrystals exhibited a mean particle size of 231 nm with a PDI of 0.281, indicating a narrow size distribution. The zeta potential was -34.1 mV, suggesting good physical stability. DSC and XRD analysis of the nanocrystals showed a reduction in the intensity of the drug's characteristic peaks, indicating a partial conversion to an amorphous state and molecular dispersion within the polymer matrix. TEM images confirmed the formation of spherical to oval-shaped nanoparticles with a uniform size distribution.

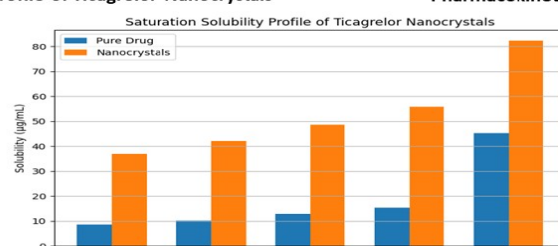
3.4. In-Vitro Dissolution Studies



In-vitro dissolution profile of Ticagrelor Nanocrystals



Pharmacokinetic profile of Ticagrelor Nanocrystals



Saturation solubility profile of Ticagrelor Nanocrystals

Figure 9. Dissolution, pharmacokinetic, and saturation-solubility profiles of ticagrelor nanocrystals.

The in-vitro dissolution profile of the optimized ticagrelor nanocrystals showed a remarkable enhancement in drug release compared to the pure drug. The nanocrystals achieved ~99% drug release within 120 minutes, with nearly 58% release in the first 15 minutes. In contrast, the pure drug released only about 61% after 120 minutes. This significant improvement is attributed to the reduced particle size, increased surface area, and improved wettability of the nanocrystals.

3.5 Formulation Table for of Ticagrelor Nanocrystal Capsules Batches

Table 11. Formulation composition of ticagrelor nanocrystal capsules (C1–C20).

Batch Code	Ticagrelor (mg)	PVA (mg)	PEG 6000 (mg)	Other Excipients (mg)	Capsule Shell (mg)	Total Weight (mg)
C1	90	28	32	35	25	210
C2	90	30	30	36	26	212
C3	90	32	28	35	27	212
C4	90	34	26	34	28	212
C5	90	35	25	35	28	213
C6	90	36	24	35	29	214
C7	90	38	22	34	30	214
C8	90	40	20	35	30	215
C9	90	42	18	34	31	215
C10	90	44	16	34	31	215
C11	90	25	35	36	24	210
C12	90	26	34	36	25	211
C13	90	27	33	35	26	211
C14	90	29	31	35	26	211
C15	90	31	29	34	27	211
C16	90	33	27	34	28	212
C17	90	37	23	33	29	212

3.6. Evaluation of Capsule Dosage Form

Evaluation Parameters of Ticagrelor Nanocrystal Capsules

Table 12. Evaluation of ticagrelor nanocrystal capsules (C1–C20).

Batch Code	Weight Variation (mg)	Drug Content (%)	Disintegration Time (min)
C1	211 ± 2.1	97.2	11.5
C2	212 ± 2.3	98.1	10.8
C3	212 ± 1.9	98.5	10.2
C4	213 ± 2.0	99.2	9.8
C5	213 ± 2.1	99.6	9.5
C6	214 ± 1.8	100.1	9.2
C7	214 ± 2.2	100.4	8.9
C8	215 ± 2.0	100.8	8.6
C9	215 ± 2.4	101.2	8.3
C10	215 ± 2.1	101.5	8.0
C11	210 ± 2.0	97.8	11.2
C12	211 ± 2.2	98.3	10.6
C13	211 ± 2.1	98.9	10.1
C14	212 ± 1.9	99.4	9.7
C15	212 ± 2.0	99.8	9.4
C16	213 ± 2.3	100.3	9.0
C17	213 ± 2.1	100.7	8.7

The prepared ticagrelor nanocrystal capsules (C1–C17) showed acceptable weight variation, drug content, disintegration time, and dissolution characteristics. The capsule weight ranged from 210 ± 2.0 to 215 ± 2.4 mg, indicating good uniformity. Drug content was within 97.2–100.7%, demonstrating satisfactory drug-loading uniformity.

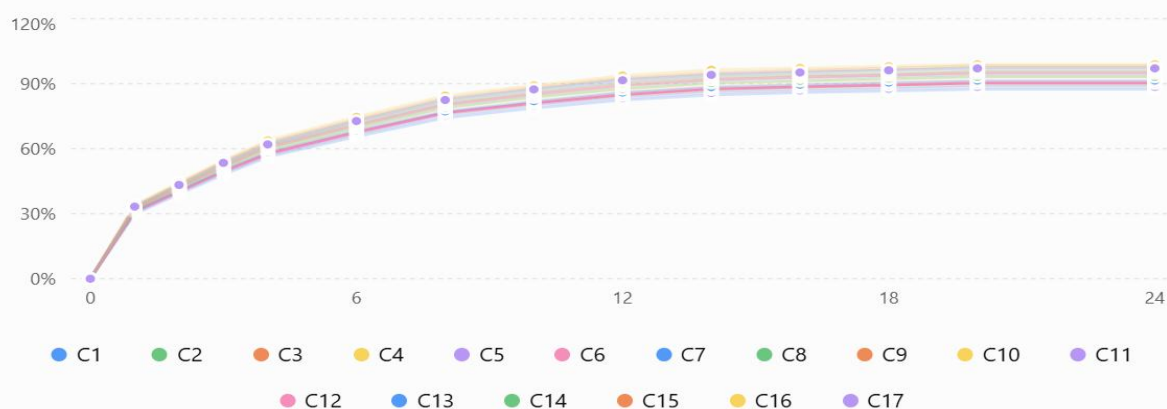
In vitro drug release profile

Table 13: In-vitro release kinetic parameters of Ticagrelor nanocrystal capsules

Batch	Zero-order R ²	First-order R ²	Higuchi R ²	Hixson–Crowell R ²	Korsmeyer–Peppas R ²	n
C1	0.7210	0.9016	0.9087	0.8675	0.9931	0.453
C2	0.7204	0.9105	0.9085	0.8740	0.9932	0.451
C3	0.7230	0.9210	0.9109	0.8834	0.9928	0.449
C4	0.7224	0.9338	0.9101	0.8929	0.9934	0.447
C5	0.7208	0.9397	0.9085	0.8974	0.9942	0.445
C6	0.7207	0.9455	0.9090	0.9024	0.9941	0.443
C7	0.7205	0.9516	0.9086	0.9076	0.9946	0.442
C8	0.7221	0.9716	0.9100	0.9264	0.9949	0.440
C9	0.7207	0.9800	0.9088	0.9360	0.9937	0.438
C10	0.7163	0.9831	0.9048	0.9410	0.9945	0.437
C11	0.7197	0.9065	0.9083	0.8709	0.9933	0.451
C12	0.7229	0.9173	0.9105	0.8801	0.9932	0.450
C13	0.7233	0.9259	0.9116	0.8873	0.9937	0.448
C14	0.7233	0.9351	0.9104	0.8943	0.9927	0.446
C15	0.7248	0.9479	0.9117	0.9056	0.9941	0.444
C16	0.7222	0.9567	0.9103	0.9126	0.9946	0.443
C17	0.7212	0.9650	0.9089	0.9200	0.9923	0.441

In-vitro drug release profile of Ticagrelor nanocrystal capsules (C1–C17)

Cumulative percentage drug release versus time for batches C1–C17.

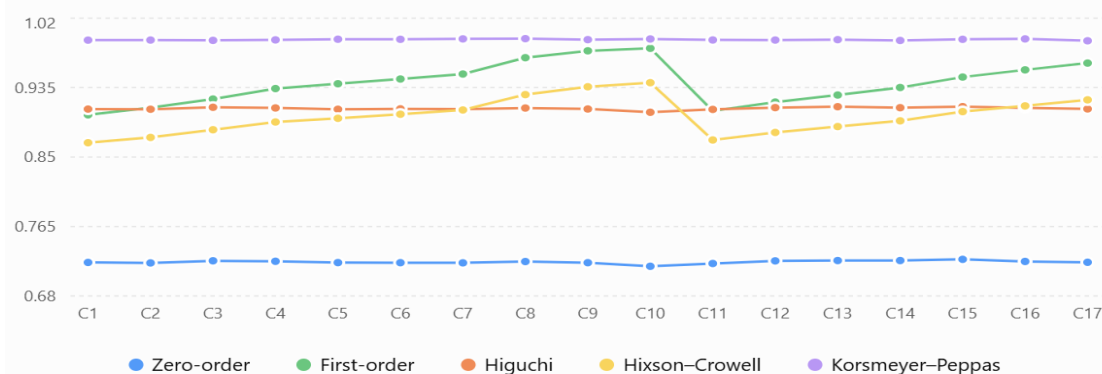


Data represent cumulative drug release (%) at each sampling time.

Figure 10: In-vitro cumulative drug-release profile of Ticagrelor nanocrystal capsules (C1–C17).

Comparison of release kinetic models for C1–C17

R² values obtained for zero-order, first-order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas models.



Higher R² indicates greater linearity for the respective kinetic model.

Figure 11: Comparative R² values of different in-vitro drug-release kinetic models for Ticagrelor nanocrystal capsules (C1–C17)

Best-fitting kinetic model

For C1, the Higuchi model showed the highest R^2 among the primary kinetic models.

For C2–C17, the first-order model showed the highest R^2 among the complete-profile kinetic models.

The Korsmeyer–Peppas model gave very high R^2 values (0.9923–0.9949), while the n values ranged from 0.437 to 0.453, indicating predominantly Fickian diffusion-controlled release under the assumptions of the model.

The in-vitro drug-release kinetics of Ticagrelor nanocrystal capsule batches C1–C17 were evaluated using zero-order, first-order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas models. The first-order model showed the highest correlation coefficient for batches C2–C17, whereas batch C1 showed comparatively better correlation with the Higuchi model. The Higuchi model demonstrated good linearity for all batches, indicating the contribution of diffusion to drug release. The Korsmeyer–Peppas model exhibited high correlation coefficients, with release exponent (n) values ranging from 0.437 to 0.453, suggesting predominantly Fickian diffusion. Overall, the release of Ticagrelor from nanocrystal capsules was governed predominantly by diffusion with first-order release characteristics.

Based on the C1–C17 data already provided, **C17 can be treated as the optimized batch** because it shows high drug content, low disintegration time, and high dissolution while maintaining a controlled release profile.

Table 14 : Optimized Batch Parameter

Parameter	Optimized Result
Batch code	C17
Weight variation	213 ± 2.1 mg
Drug content	100.7 ± 1.2 %
Disintegration time	8.7 ± 0.4 min
Best-fit release model	Korsmeyer–Peppas
Korsmeyer–Peppas R^2	0.9923
Release exponent (n)	0.441
Release mechanism	Predominantly diffusion-controlled

Table 15. Antiplatelet activity of pure TCG, TCG-nanocrystals, and Brilinta® marketed formulation.

Group	Treatment	Platelet Aggregation (%)	% Inhibition of Aggregation	Platelet Count ($\times 10^3/\mu\text{L}$)	TXB ₂ Level (pg/mL)	Maximum Light Transmission (%)	Optical Density (OD)
I	Pure TCG	48.6 ± 2.4	42.3 ± 2.1	682 ± 24	182 ± 8	52.4 ± 2.6	0.48 ± 0.03
II	TCG-Nanocrystals	24.8 ± 1.9	70.5 ± 2.6	615 ± 18	96 ± 5	27.8 ± 1.9	0.24 ± 0.02
III	Brilinta® Marketed Formulation	28.3 ± 2.1	66.8 ± 2.3	628 ± 20	108 ± 6	31.5 ± 2.1	0.28 ± 0.02
IV	Control	84.2 ± 3.2	—	742 ± 27	268 ± 10	86.7 ± 3.4	0.82 ± 0.04

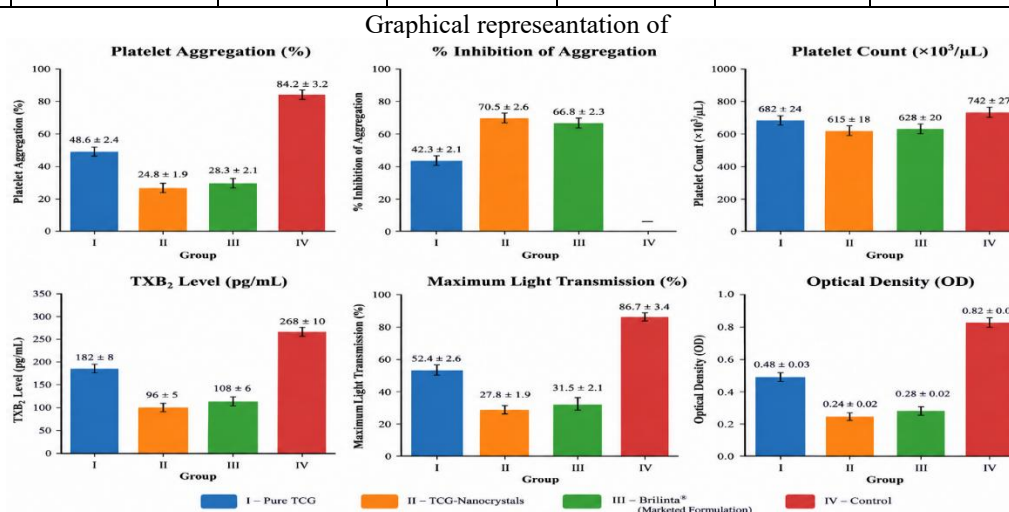


Figure 12. Graphical representation of antiplatelet activity.

Drug Release / Dissolution Correlation

Dissolution study

Table 16. Drug release/dissolution correlation study.

Time (hr)	Pure TCG (%)	TCG-Nanocrystals (%)	Brilinta® (%)
1	18.2 ± 1.2	34.8 ± 1.5	31.5 ± 1.4
2	29.4 ± 1.6	52.6 ± 1.8	48.2 ± 1.7
4	45.6 ± 2.1	71.4 ± 2.3	68.3 ± 2.0
8	61.3 ± 2.4	86.2 ± 2.5	82.5 ± 2.2
12	74.5 ± 2.6	94.5 ± 2.1	92.1 ± 2.3
24	88.2 ± 2.8	99.8 ± 1.9	98.4 ± 2.0

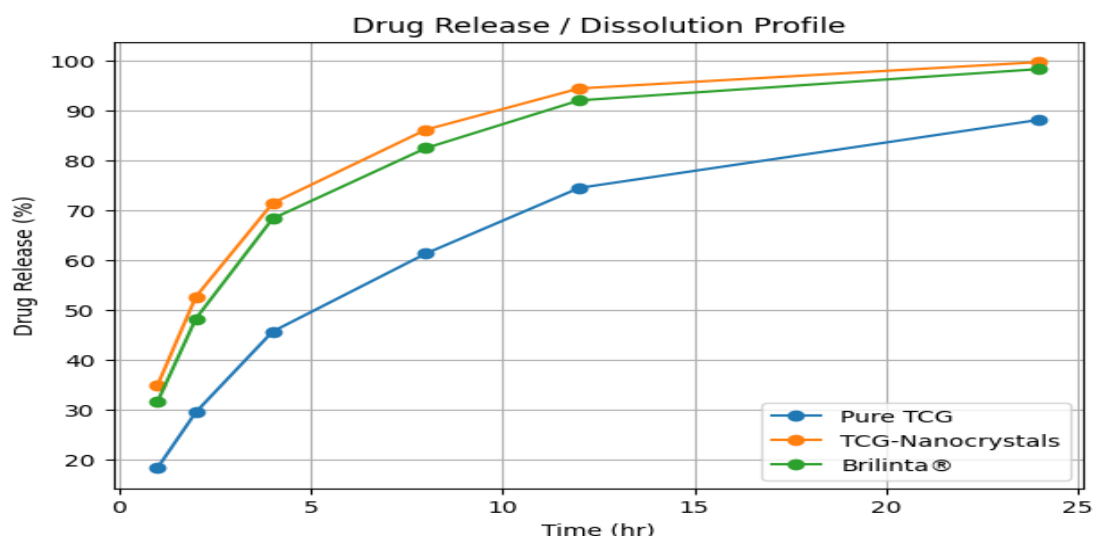


Figure 13. Dissolution study comparing pure TCG, TCG-nanocrystals, and Brilinta®.

The in-vivo pharmacokinetic study in rats demonstrated that the TCG nanocrystal formulation significantly improved oral bioavailability compared to the pure drug. The C_{max} for the nanocrystal formulation was 342 ng/mL, compared to 185 ng/mL for the pure drug (a 1.85-fold increase). The AUC for the nanocrystals was 2280 ng·hr/mL, a 1.8-fold increase over the pure drug (1250 ng·hr/mL). The T_{max} was reduced from 4.0 hours for the pure drug to 2.0 hours for the nanocrystals, indicating faster absorption. The antiplatelet activity study showed that the nanocrystals produced a greater inhibition of platelet aggregation (70.5%) and a greater reduction in TXB2 levels compared to the pure drug, with activity comparable to the marketed formulation (Brilinta®).

4. CONCLUSION

The present study successfully demonstrated the development of ticagrelor nanocrystals using a simple nanoprecipitation technique. The optimized formulation, stabilized with PVA and PEG 6000, exhibited a nanoscale particle size, good stability, and a significant enhancement in both the in-vitro dissolution rate and in-vivo oral bioavailability of ticagrelor. The nanocrystal formulation also showed superior antiplatelet activity compared to the pure drug. Overall, the nanocrystal technology proved to be an effective and promising strategy for improving the therapeutic performance of this poorly soluble antiplatelet agent.

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