International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 6, 2023

Original Article

# DESIGN OF EXPERIMENTS AND OPTIMIZATION OF AMORPHOUS SOLID DISPERSION OF A BCS CLASS IV ANTI-PLATELET DRUG THROUGH FACTORIAL DESIGN

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***Received: 15 Apr 2023, Revised and Accepted: 16 Aug 2023***

**ABSTRACT**

**Objective:** The aim of this study was to evaluate and optimize the amorphous solid dispersion of a low-soluble BCS Class IV anti-platelet drug using factorial design in line with the Quality by Design (QbD) principle.

**Methods:** Ticagrelor, a low-soluble anti-platelet agent, was used as the model drug for the current study. A solid dispersion technique was explored to improve the dissolution of ticagrelor. The extent of amorphization of ticagrelor with the solid dispersion approach was evaluated with powder X-Ray diffraction (p-XRD) and differential scanning calorimetry (DSC). The principle of factorial design (FD) was adopted to optimize the formulation of ticagrelor solid dispersion. Design Expert® 13 (Stat-Ease Inc., Minneapolis, MN, USA) was explored for the Design of experiments (DoE) and Statistical evaluation. The experiments were designed with three factors at two levels (a $2^3$-factor design) and two responses. The significance of the model was evaluated by analysis of variance (ANOVA) and fit statistics. Various statistical parameters such as sequential p-values, lack of fit, squared correlation coefficient ($R^2$), adjusted $R^2$, and adequate precision were considered in fit statistics.

**Results:** The crystalline ticagrelor has completely amorphized, as indicated by the powder x-ray diffraction (p-XRD) and differential scanning calorimetry (DSC) of the solid dispersion of ticagrelor prepared with copovidone VA 64 and vitamin E TPGS through solvent evaporation technique. An increase in ticagrelor dissolution by 10.7 fold was possible through solid dispersion technology. The lack of fit F-values of 0.11 and 0.00 in the factorial model for response dissolution at 10 min and disintegration time, respectively, are indicative of a good fit. The ANOVA and the fit statistics for the selected factorial model were found to be significant.

**Conclusion:** A solid dispersion technique with carrier copovidone VA 64 and vitamin E TPGS could enhance the dissolution of ticagrelor significantly, to an extent of 10.7 fold. Factorial design is an important tool in optimizing the amorphous solid dispersion of ticagrelor and establishing the design space.

**Keywords:** Amorphous solid dispersion, Dissolution enhancement, Quality by design, Design of experiments, Factorial design, Design expert (Stat-Ease)


DOI: https://dx.doi.org/10.22159/ijap.2023v15i6.48767. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

## INTRODUCTION

Quality by Design (QbD) has been an integral part of pharmaceutical product development and has been adopted into the regulatory framework of the USFDA, EMA, and other stringent global regulatory agencies for the 21st century "Quality Systems". With the joint initiative of USFDA, EMA, and PMDA, the concept of quality by design has been included in the ICH Q8, Q9, and Q10 guidelines in support of innovation and the global development of high-quality medicines for the benefit of patients [1].

Design of Experiment (DoE) is an important tool of Quality by Design (QbD), which enables a diverse range of experimental designs to enhance product and process understanding through statistical, analytical, and risk-management methodologies [2].

Drug dissolution and permeability play a very critical role in achieving the desired bioavailability and pharmacological response, which in turn affect the clinical safety and efficacy of a drug significantly.

Ticagrelor is a low-soluble, low-permeable BCS Class IV drug substance (not ionized in the physiological pH range), exhibiting a very low solubility (less than 10 g/ml) and moderate intrinsic permeability. The mean absolute bioavailability of ticagrelor is about 36% (range: 30%–42%). Absorption of ticagrelor occurs with a median Tmax of 1.5 h (range 1.0–4.0). The formation of the major circulating metabolite AR-C124910XX (active) from ticagrelor occurs with a median t max of 2.5 h (range 1.5–5.0) [3]. Therefore, there is potentially a higher risk that changes in formulation and processing parameters can affect the dissolution and clinical performance of drug products.

Solid dispersion is one of the promising techniques to enhance the dissolution rate of poorly soluble BCS Class IV drugs. Various polymers, e.g., Soluplus, Hydroxypropylmethylcellulose acetate succinate (HPMCAS), and copovidone VA 64, are known to be very potent carriers in the preparation of amorphous solid dispersion. Kolidone VA 64 (BASF) and Plasdone S 630 (ASHALAND) are explored as carriers for solid dispersion both by solvent evaporation and hot melt extrusion technology [4, 5]. Carbopol and acacia gum are reported as carriers for the preparation of solid dispersion of low-soluble drugs by the kneading method [6].

Apart from solubility, Permeability plays an important role in bioavailability. P-glycoprotein (P-GP), an efflux transporter, plays an important role in drug transport. It pumps drugs into the lumen, reducing their absorption. Many drugs that are transported by P-glycoprotein (P-GP) are also metabolized by cytochrome P450 3A4. There are examples of drugs and excipients that can induce or inhibit P-glycoprotein (P-GP) efflux and have a significant impact on bioavailability. Surfactants like vitamin E, TPGS, and polysorbate have been reported to play an important role as intestinal permeation enhancers by inhibiting the P-glycoprotein (P-GP) pump [7–10].

As per the USFDA dissolution method database, the recommended dissolution method by the Office of Generic Drugs (OGD) for ticagrelor tablets is 0.2% w/v polysorbate 80 in water, 900 ml, Paddle at 75 RPM, with time points at 10, 20, 30, 45, and 75 min [11]. However, as water lacks the buffer capacity and the use of surfactant would lead to a lower discriminatory power of the dissolution medium, Phosphate buffer pH 6.8 without any surfactant was considered as the discriminatory medium for the evaluation of the *in vitro* dissolution performance of the formulations.

In the current study, a solid dispersion of ticagrelor was prepared with various polymers by solvent evaporation technique, and the solid dispersion (SD) thus obtained was characterized through powder x-ray diffraction (p-XRD) to evaluate the extent of amorphization of crystalline ticagrelor. Further, film-coated tablets

were prepared with the ticagrelor solid dispersion obtained after solvent evaporation and evaluated dissolution rate.

Based on the polymer screening study with different polymers, the solid dispersion formulation with copovidone VA 64 and vitamin E TPGS was found to be a superior formulation with respect to *in vitro* dissolution performance. Hence, the formulation was taken further in an optimization study through the design of experiments (DoE) and factorial design (FD).

The experimental design and statistical analysis were performed using Design Expert® 13 (Stat-Ease Inc., Minneapolis, MN, USA). The experiments were designed with three factors at two levels (a $2^3$-factorial design) and two responses. The factors, copovidone VA 64, Vitamin E TPGS, and crospovidone XL10 have a definite impact on the responses, i.e., the dissolution rate and disintegration time. The above factors and responses are crucial for the clinical performance of the ticagrelor tablet dosage form, hence were thoroughly assessed.

## MATERIALS AND METHODS

### Materials

Ticagrelor was acquired as a gift sample from Mankind Research Centre (A division of Mankind Pharma Ltd., India). Kovidone VA 64 was supplied by Boai NKY Pharmaceuticals Ltd. (Henan Province, China), Kollidon® VA 64 was supplied by BASF and Pearlitol SD 200 (Roquette) was supplied by Signet Chemical Corporation (Mumbai, India). Anhydrous calcium hydrogen phosphate was supplied by Sudeep Pharma (India), Sodium starch glycollate was supplied by Amit Hydrocolloid (India), and polyplasdone XL was supplied by Ashland (New Milford, CT, USA). A gift sample of vitamin E TPGS was taken from Seqens GmbH (Germany), Opadry YS-1-7040 white from Colorcon Asia Pvt. Ltd. (India), and Polysorbate 80 was purchased from Croda Singapore PTE Ltd.

High-performance liquid chromatography (HPLC)-grade acetonitrile (ACN) and methanol were obtained from Merck Chemicals (Germany). Ammonium acetate, Ammonium hydroxide, and potassium dihydrogen orthophosphate (KH2PO4) were collected from Qualigen (Thermo Fisher Scientific, Mumbai, India). Analytical column Chromosil, 250 × 4.6 mm, 5.0 m, was purchased from Chrom Separations, Inc. US.

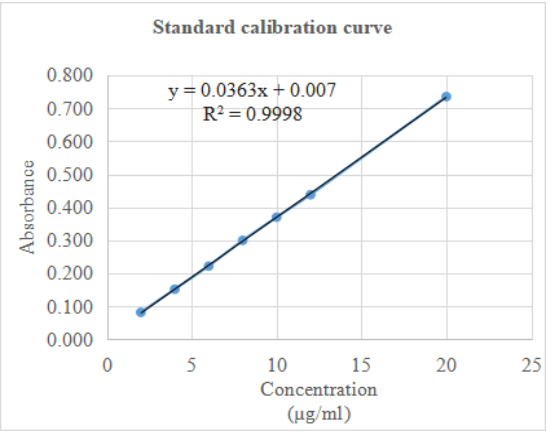
### Methods

#### HPLC analysis

A Shimadzu LC-10 AT VP HPLC system (Shimadzu Corporation, Japan) equipped with a PDA detector was used for ticagrelor analysis. The quantification of ticagrelor was carried out by reverse-phase HPLC with modifications to the previously described method by Bueno *et al.* (2017). The Analytical column was a C8 reverse-phase column (Chromosil, 250 × 4.6 mm, 5.0 m). The mobile phase was composed of acetonitrile: ammonium acetate, 50 mmol (57:43, v/v), with pH adjusted to 8.2 with ammonium hydroxide 6 M. The column temperature was maintained at 25 °C, and the injection volume was 20 L with a flow rate of 0.7 ml/min. The absorbance of ticagrelor was set at 270 nm [12].

#### UV analysis

A UV-Visible spectrophotometer (Shimadzu 1900 series with lab solution software) was used for the dissolution of ticagrelor formulations. For standard preparation, Phosphate buffer pH 6.8 and methanol were prepared in a 70:30 ratio. For the standard calibration curve, different concentrations of ticagrelor standard solution were prepared, ranging from 2 mcg/ml to 20 mcg/ml, and absorbance was measured at 222 nm. Similarly, dissolution samples of ticagrelor were taken at 5, 10, 10, 15, 30, 45, 60, and 75 min and were diluted with the dissolution medium. The absorbance of the diluted samples was measured at 222 nm) [13].



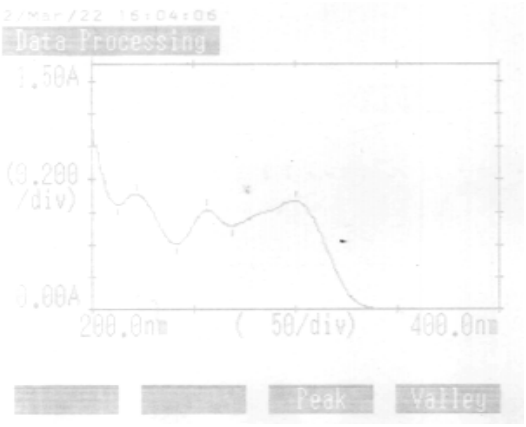




**Fig. 1: Standard calibration curve for ticagrelor, data were shown as mean (n = 3) and SD, where n is the number of observations**

### Screening of polymers for ticagrelor solid dispersion

Copovidone VA 64 is an excellent carrier for solid dispersion and exhibits a low crystallization driving force (CDF). Though amorphization helps enhance solubility and bioavailability to a great extent, its crystallization driving force (CDF) leads to a polymorphic transformation, thereby favouring one thermodynamically stable form during *in vitro* and *in vivo* dissolution. Also, the increased molecular mobility leads to kinetic instability. Low-molecular-weight excipients are known to lower the crystallization driving force (CDF) and elevate the glass transition temperature (Tg). Copovidone VA 64 is a co-polymer composed of a chain structure composed of two monomers, namely N-vinylpyrrolidone (NVP) and vinyl acetate (VAc). It is an excellent crystallization inhibitor and matrix-forming agent with a glass transition temperature (Tg) of 101 °C. Hence, copovidone VA 64 was selected for the solid dispersion formulation of ticagrelor. In a preliminary screening study, different ratios of ticagrelor, vitamin E TPGS, and copovidone VA 64 were taken for amorphization of ticagrelor. A solvent evaporation technique was followed to prepare ticagrelor solid dispersion. The solid dispersions thus obtained were evaluated for powder x-ray diffraction (p-XRD) and differential scanning calorimetry (DSC) to evaluate the extent of amorphization of crystalline ticagrelor. Tablets were prepared with the above solid dispersion (SD), and the dissolution profiles were compared with the tablet formulation prepared by the conventional wet granulation method in line with the reference product BRILINTA® (ticagrelor) tablets, ASTRAZENECA LP [14-17].

### Experimental set-up and process flow for the ticagrelor formulation technologies

### Ticagrelor conventional IR film-coated tablets with wet granulation method

Conventional immediate-release tablet formulations of ticagrelor were prepared by the aqueous wet granulation method. Qualitative (Q1) and quantitative (Q2) composition as well as the manufacturing process flow, was similar to the reference product BRILINTA® ASTRAZENECA LP as given in fig. 2 [3].


**Fig. 2: Process flow for preparation of conventional film-coated ticagrelor tablets**

**Ticagrelor film-coated tablets with solid dispersion (SD) technology**

Ticagrelor solid dispersion (TCG-SD) was prepared by solvent evaporation. Ticagrelor and Vitamin E TPGS were completely dissolved in ethanol under continuous stirring with a magnetic stirrer at about 100–150 rpm for 30 min. In a similar way, copovidone VA 64 was completely dissolved in ethanol and added to the above solution with continuous stirring for about 30 min. The ethanol was evaporated using a rotary evaporator at 50 °C. The dried solid dispersion was then passed through a sieve mesh #30 to get a uniform powder. Blank-SD was prepared by the same method as TCG-SD without ticagrelor [18].

Solid dispersion prepared by the kneading method has an advantage over the solvent evaporation method with respect to the lower risk of residual solvent contamination. However, because ticagrelor is a metastable, polymorphic drug substance, the solvent evaporation method was selected [19].

Solid dispersions (SDs) were prepared with different ratios of ticagrelor, vitamin E TPGS and copovidone VA 64 (CP VA 64), i.e., TCG: TPGS: CP VA 64-1:0.4:1, TCG: TPGS: CP VA 64-1:0.4:2, and TCG: TPGS: CP VA 64-1:0.4:4, as per the process flow described in fig. 3.

**Design of experiments and optimization of ticagrelor amorphous solid dispersion (ASD)**

The experimental design and statistical analysis were performed using Design-Expert 13 (Stat-Ease, Inc.). Based on the screening study, the quantities of copovidone VA 64, Vitamin E TPGS, and Crospovidone XL were set as per the range given in tables 1–3. The data from all 11 DoE runs was fitted to a linear model. The Fit statistics for the linear model were evaluated with parameters such as model p-values, lack of fit, coefficient of determination ($R^2$), adjusted $R^2$, and adequate precision provided by ANOVA. The desirability function was analyzed for the optimization of factors associated with desirable responses after the fitting of the statistical model.


**Fig. 3: Process flow for preparation of film-coated ticagrelor tablets with solid dispersion technology**

**Table 1: Build information for factorial design**

| **File version** | **22.0.6.0** | | |
|---|---|---|---|
| Study Type | Factorial | Subtype | Randomized |
| Design Type | 2 Level Factorial | Runs | 11 |
| Design Model | 3FI | Blocks | No Blocks |

**Table 2: Factors in the factorial design**

| FaFactor | Name | Unit | Type | Subtype | Min | Max | Coded low | Coded high | Mean | STD. Dev. |
|---|---|---|---|---|---|---|---|---|---|---|
| A | Copovidone VA 64 | mg | Numeric | Continuous | 180.0 | 540.0 | -1 ↔ 180.0 | +1 ↔ 540.0 | 360.0 | 161.0 |
| B | Vitamin E TPGS | mg | Numeric | Continuous | 18.0 | 54.0 | -1 ↔ 18.0 | +1 ↔ 54.00 | 36.0 | 16.10 |
| C | Crospovidone XL 10 | mg | Numeric | Continuous | 78.0 | 130.0 | -1 ↔ 78.0 | +1 ↔ 130.0 | 104.0 | 23.26 |

**Table 3: Responses**

| Responses | Units | Observations | Minimum | Maximum | Mean | STD. Dev. | Ratio |
|---|---|---|---|---|---|---|---|
| R1:Dissolution 10 min | % | 11.00 | 80 | 103 | 93.36 | 6.68 | 1.29 |
| R2:Disintegration time | Minutes | 11.00 | 6 | 9 | 7.09 | 1.22 | 1.50 |

**Characterization of ticagrelor and its formulations**

**Powder X-ray diffraction (p-XRD)**

The samples of solid dispersions and their tablet formulations were evaluated for p-XRD. The p-XRD patterns of the samples were recorded using the Rigaku Miniflex 600 XRD System (Tokyo, Japan) with Ni-filtered Cu-K radiation at 1.54 Å powered at 40 kV and 15 MA. The samples were scanned in steps of 0.02 °/s from 5° to 120° (diffraction angle 2) with an increment of 5°/min.

**Differential scanning calorimetry (DSC)**

Thermograms of samples were obtained by a DSC N-60 thermal analyzer (Scinco, Seoul, Korea). Samples (equivalent to 5 mg of TCG and TC SD) were placed into aluminum pans, sealed, and subjected to DSC under nitrogen flow. A DSC thermogram was recorded under heating conditions ranging from 0 to 350 °C at a heating rate of 10 °C/min.

**Assay by HPLC**

Preparation of Standard Stock Solution: A 10 mg Ticagrelor reference standard was transferred to a 10 ml volumetric flask, dissolved with 5 ml methanol, sonicated, and made up to 10 ml methanol and mixed well.

Preparation of Standard Solution: 2 ml of standard stock solution were diluted to 20 ml with mobile phase (Acetonitrile: Ammonium Acetate, 50 mmol (57:43, v/v) with pH adjusted to 8.2).

Test solution Preparation for solid dispersion: Solid dispersion (SD), equivalent to 90 mg of ticagrelor, was dissolved in 25 ml of methanol with sonication. Filter the solution through a 0.45-micron syringe filter. The first few ml of filtered solution were discarded, and the amount of ticagrelor was quantified with an HPLC system at 270 nm.

***In vitro* dissolution study**

An *in vitro* dissolution study was conducted in the USP II paddle apparatus with the aid of a dissolution tester (Electrolab TDT-08 L instrument). The dissolution method includes Phosphate buffer pH 6.8, 900 ml, a paddle, and 75 RPM with sampling times of 10, 20, 30, 45, and 75 min. Comparative dissolution profiles of the formulations prepared with conventional wet granulation and solid dispersion technology Dissolution samples were withdrawn at 5, 15, 30, 60, and 75 min and filtered through a 0.45-m syringe filter. The filtrate was diluted with the mixture of methanol-phosphate buffer 6.8 (70:30), and the absorbance was taken in a UV-visible spectrophotometer (Shimadzu 1900 series with Lab Solution software) at 222 nm to calculate the dissolution of ticagrelor.

**RESULTS**

**Characterization of ticagrelor solid dispersion (SD) formulations**

The solid dispersion of ticagrelor, prepared with a solvent evaporation technique, was evaluated for powder X-Ray diffraction (p-XRD) and differential scanning calorimetry (DSC) to understand the extent of amorphization. Further, the dissolution profile of tablet formulations prepared with solid dispersion was evaluated using a discriminatory dissolution method. The dissolution profiles for all the formulations prepared with solid dispersion technology were compared with the dissolution profiles of conventional immediate-release tablet formulations prepared with similar qualitative (Q1) and quantitative (Q2) compositions and process technologies as those of the reference product BRILINTA® (ticagrelor) tablets, ASTRAZENECA LP.

**Powder X-ray diffraction (p-XRD)**

The p-XRD pattern of crystalline ticagrelor and its solid dispersion prepared with copovidone VA 64 are shown in fig. 9. Sharp crystalline peaks of 2θ at 10.50°, 13.36°, 14.74°, 18.18°, 19.06°, 21.12°, 22.52° and 24.12° were observed for the API ticagrelor, whereas all the crystalline peaks disappeared in the solid dispersion samples, indicating complete conversion of the crystalline ticagrelor into an amorphous form.

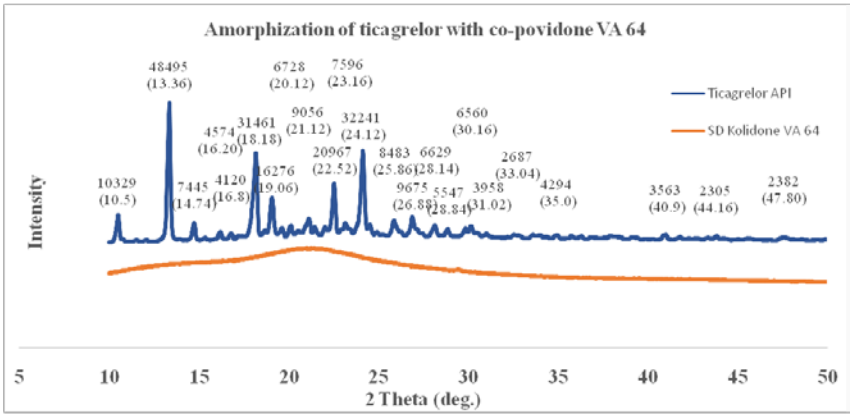
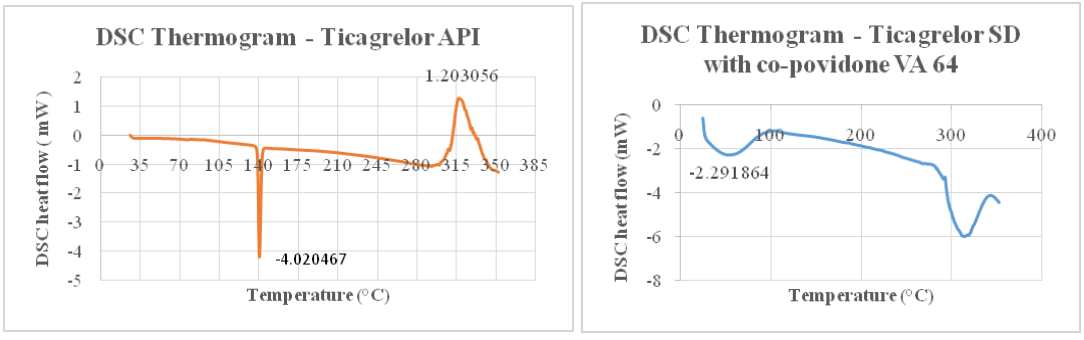



**Fig. 4: Amorphization of ticagrelor with copovidone VA 64 through solid dispersion technology**




**(a)** **(b)**

**Fig. 5: DSC thermogram of pure ticagrelor API (a) and ticagrelor solid dispersion (b)**

**Differential scanning calorimetry (DSC)**

The DSC thermograms of pure ticagrelor and ticagrelor solid dispersion are presented in fig. 10. For ticagrelor API, the endothermic peak at 140 °C and the exothermic peak at 315 °C indicate the melting and crystallization temperatures, respectively, while the endothermic peak of ticagrelor solid dispersion at 52.72 °C indicates the glass transition temperature (Tg) of the solid dispersion.

**Assay by HPLC**

The assay of ticagrelor in the solid dispersion, tablet formulation with solid dispersion and tablet formulation with the conventional wet granulation technique were estimated to be 99.186 %, 99.60 % and 99.876%, respectively. Further, the assay of all the experimental batches taken for optimization of amorphous solid dispersion was in a range of 98 to 102%.

***In vitro* dissolution**

**Discriminatory dissolution**

For evaluation of the dissolution performance of the ticagrelor dosage form, a dissolution study was performed with the USP II paddle apparatus at 75 RPM in 900 ml of phosphate buffer pH 6.8 with and without surfactant. Considering the better discriminatory power, phosphate buffer pH 6.8 without surfactant was considered for the formulation trials designed for dissolution improvement. The sample analysis was carried out with a UV-visible spectrophotometer (Shimadzu 1900 series with Lab Solution software) at 222 nm.

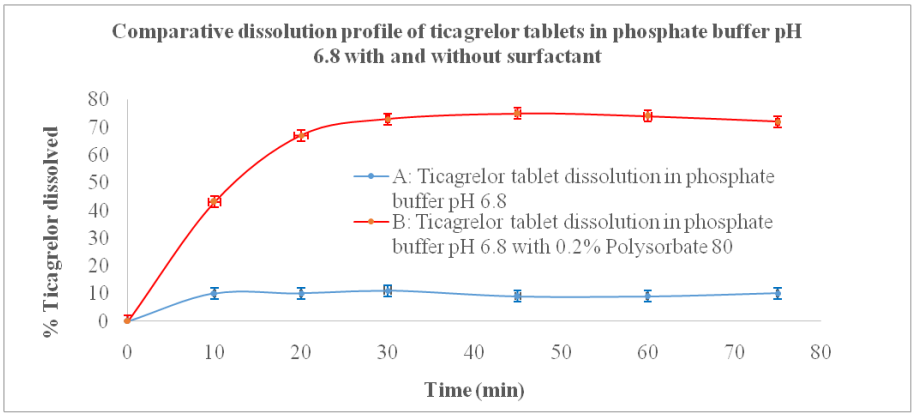



Fig. 6: Comparative dissolution profile of ticagrelor in dissolution medium (Phosphate buffer pH 6.8) with and without surfactant (polysorbate 80) for evaluation of discriminatory power, dissolution data, shown as mean (n = 6)±SD, where n is the number of observations

**Comparative dissolution profiles of ticagrelor formulations**

The dissolution profiles for the formulations of ticagrelor prepared with conventional wet granulation techniques and solid dispersion techniques were generated in phosphate buffer pH 6.8 as presented in fig. 7. As per the result, the dissolution rates at 75 min were found to be 10% (RSD-1.1), 24% (RSD-0.5) and 107% (RSD-0.6) for conventional IR tablets, TCG-SD tablets with copovidone (1:0.4:1), and TCG-SD tablets with copovidone (1:0.4:4), respectively.

Formulation of amorphous solid dispersion (ASD) with polymer copovidone VA 64 [i.e., TCG-SD tablet with copovidone (1:0.4:4)] showed a significantly faster dissolution rate (increased by 10.7 fold) compared to ticagrelor tablet prepared with conventional wet granulation technology in line with the similar Q1/Q2 of reference product BRILINTA® (ticagrelor) tablets, ASTRAZENECA LP. However, TCG-SD tablet with copovidone (1:0.4:1) could only increase the dissolution rate by 2.4 fold.

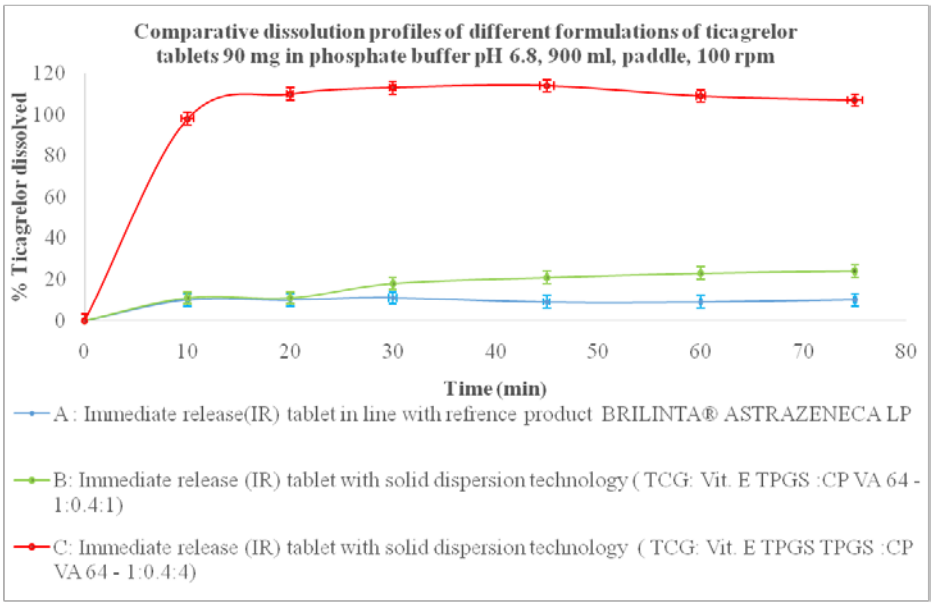



**Fig. 7: Comparative dissolution profile of ticagrelor formulations (A) Immediate release tablet (B) TCG-SD tablet with copovidone (1:0.4:1) (C) TCG-SD tablet with copovidone (1:0.4:4), dissolution data, shown as mean (n = 6)±SD, where n is the number of observations**

**Design of experiment (DoE) and optimization**

The experimental design and statistical analysis were performed using Design-Expert 13 (Stat-Ease, Inc.). Based on the screening study, the quantities of copovidone VA 64, vitamin E TPGS, and crospovidone XL were set as per the range given in table 2. The 8 DoE runs with 3 center points were evaluated for dissolution at 10 min and disintegration time (DT). The results are presented in table 4. The summary of model fitting and statistical analysis is presented in table 5.

**Evaluation of responses**

The responses that are considered to have a very high impact on the clinical performance of the formulations were statistically evaluated.

The following responses were evaluated for optimization of Ticagrelor solid dispersion.

**Dissolution at 10 min (R1)**

The ANOVA for the selected factorial model and the fit statistics for the response R1 Dissolution at 10 min are presented in tables 6 and 7, respectively.

**Disintegration time (R2)**

The ANOVA for the selected factorial model and the fit statistics for the response R1 Dissolution at 10 min are presented in tables 8 and 9, respectively.

**Table 4: Experimental results of the DoE study, levels of factors, and responses**

| DoE runs | Factor A crospovidone VA64 (mg) | Factor B vitamin E TPGS (mg) | Factor C crospovidone XL (mg) | Response 1 dissolution 10 min (%) | | Response 2 disintegration time (DT) (min) |
|---|---|---|---|---|---|---|
| | | | | % Dissolution | % RSD | |
| 1 | 180 | 18 | 78 | 80 | 2.0 | 8 |
| 2 | 540 | 54 | 130 | 102 | 1.4 | 6 |
| 3 | 360 | 36 | 104 | 99 | 1.5 | 7 |
| 4 | 180 | 18 | 130 | 90 | 1.7 | 6 |
| 5 | 360 | 36 | 104 | 98 | 1.8 | 7 |
| 6 | 180 | 54 | 130 | 90 | 0.9 | 6 |
| 7 | 540 | 54 | 78 | 93 | 1.1 | 9 |
| 8 | 180 | 54 | 78 | 90 | 1.0 | 8 |
| 9 | 360 | 36 | 104 | 103 | 0.6 | 6 |
| 10 | 540 | 18 | 78 | 90 | 1.5 | 9 |
| 11 | 540 | 18 | 130 | 92 | 1.1 | 6 |

Dissolution data, were shown as mean (n = 6) and RSD, where n is the number of observations.

**Table 5: Summary of model fitting and statistical analysis**

| Model parameters | R1: Dissolution, 10 min | R2: Disintegration time |
|---|---|---|
| Suggested Model | Linear | Linear |
| Model P-value | 0.0032 | 0.0002 |
| Lack of fit P-value | 0.9448 | 1.0000 |
| $R^2$ | 0.9382 | 0.9529 |
| Adjusted $R^2$ | 0.8887 | 0.9294 |
| Adequate precision | 16.4603 | 13.3492 |
| Standard deviation | 1.81 | 0.3333 |

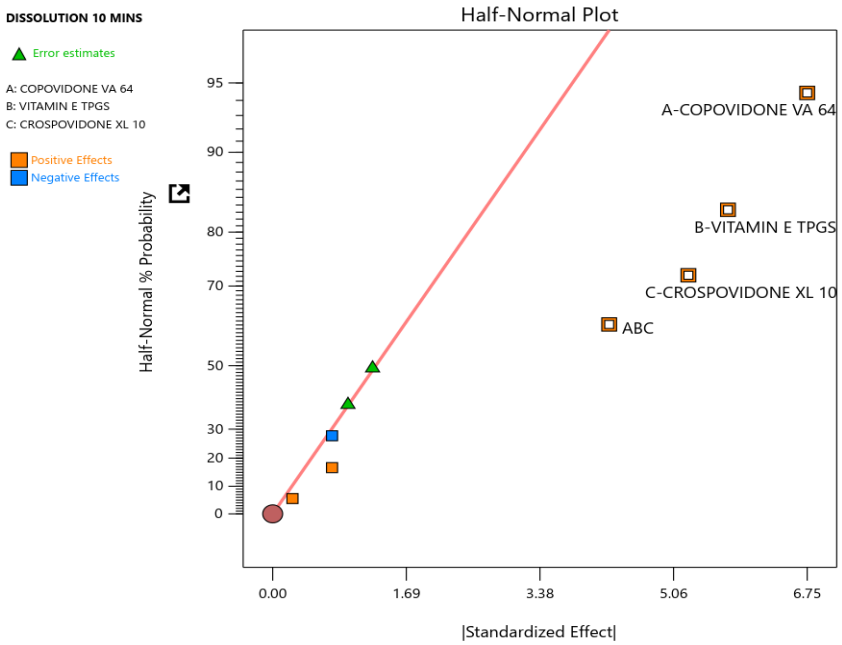



**Fig. 8: Half-normal plot for the formulation variable effects of dissolution in 10 min**

**Table 6: ANOVA for a selected factorial model**

| Source | Sum of squares | DF | Mean square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 248.50 | 4 | 62.12 | 18.97 | 0.0032 | Significant |
| A-COPOVIDONE VA 64 | 91.13 | 1 | 91.13 | 27.82 | 0.0033 | Significant |
| B-VITAMIN E TPGS | 66.13 | 1 | 66.13 | 20.19 | 0.0064 | |
| C-CROSPOVIDONE XL 10 | 55.13 | 1 | 55.13 | 16.83 | 0.0093 | |
| ABC | 36.13 | 1 | 36.13 | 11.03 | 0.0210 | |
| Curvature | 181.67 | 1 | 181.67 | 55.47 | 0.0007 | |
| Residual | 16.37 | 5 | 3.27 | | | |
| Lack of Fit | 2.37 | 3 | 0.7917 | 0.1131 | 0.9448 | Not significant |
| Pure Error | 14.00 | 2 | 7.00 | | | |
| Cor Total | 446.55 | 10 | | | | |

**Table 7: Fit statistics of the factorial model for the response, dissolution at 10 min (R1)**

| STD. Dev. | 1.81 | $R^2$ | 0.9382 |
|---|---|---|---|
| Mean | 93.36 | Adjusted $R^2$ | 0.8887 |
| C. V. % | 1.94 | Predicted $R^2$ | 0.8173 |
| | | Adeq Precision | 16.4603 |

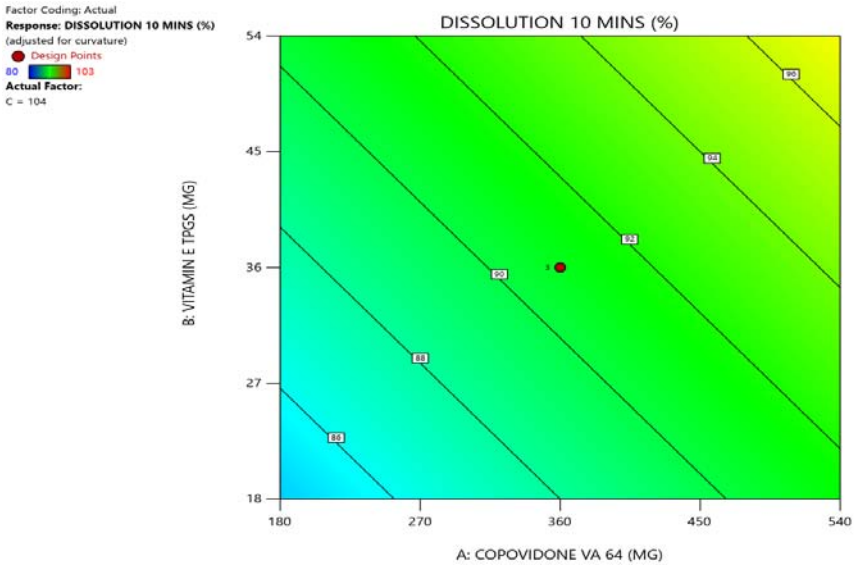



**Fig. 9: Contour plot showing the interaction of copovidone VA 64 and vitamin E TPGS and their impact on dissolution at 10 min**

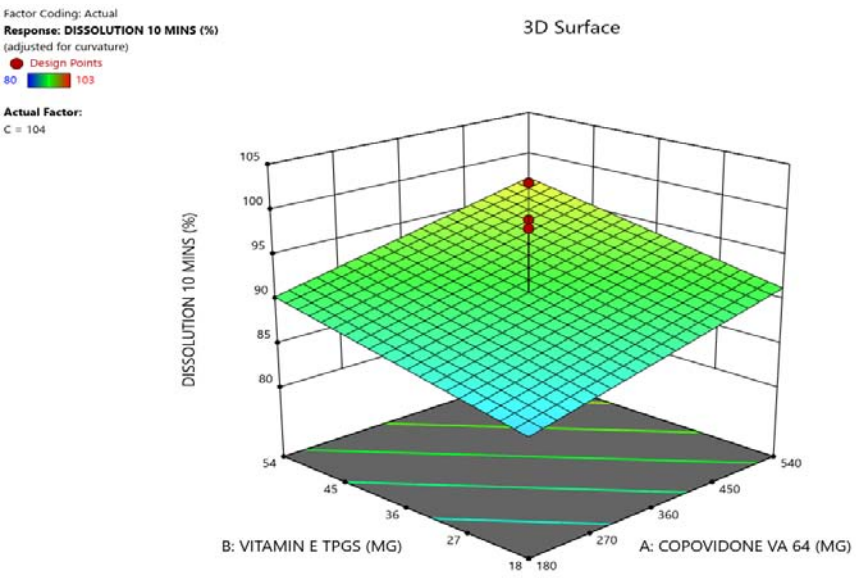



**Fig. 10: 3D plot showing the interaction of copovidone VA 64 and vitamin E TPGS and their impact on dissolution at 10 min**

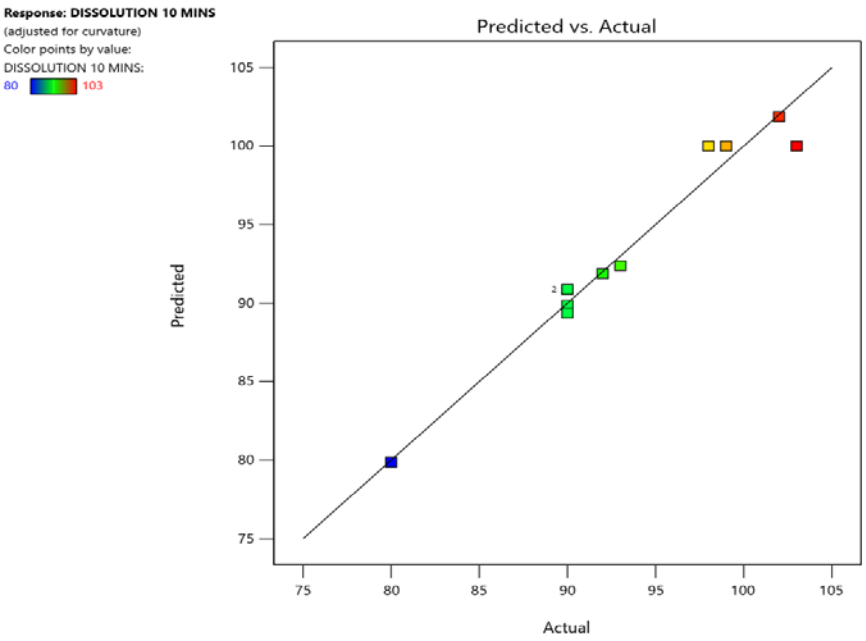



**Fig. 11: Predicted vs. actual response for dissolution at 10 min (R1)**

**Table 8: ANOVA for a selected factorial model**

| Source | Sum of squares | DF | Mean square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 13.50 | 3 | 4.50 | 40.50 | 0.0002 | Significant |
| A-COPOVIDONE VA 64 | 0.5000 | 1 | 0.5000 | 4.50 | 0.0781 | Significant |
| C-CROSPOVIDONE XL 10 | 12.50 | 1 | 12.50 | 112.50 | <0.0001 | |
| AC | 0.5000 | 1 | 0.5000 | 4.50 | 0.0781 | |
| Curvature | 0.7424 | 1 | 0.7424 | 6.68 | 0.0415 | |
| Residual | 0.6667 | 6 | 0.1111 | | | |
| Lack of Fit | 0.0000 | 4 | 0.0000 | 0.0000 | 1.0000 | Not significant |
| Pure Error | 0.6667 | 2 | 0.3333 | | | |
| Cor total | 14.91 | 10 | | | | |

**Table 9: Fit statistics for the factorial model for the response, disintegration time (R2)**

| Std. Dev. | 0.3333 | $R^2$ | 0.9529 |
|---|---|---|---|
| Mean | 7.09 | Adjusted $R^2$ | 0.9294 |
| C. V. % | 4.70 | Predicted $R^2$ | 0.8941 |
| | | Adeq Precision | 13.3492 |

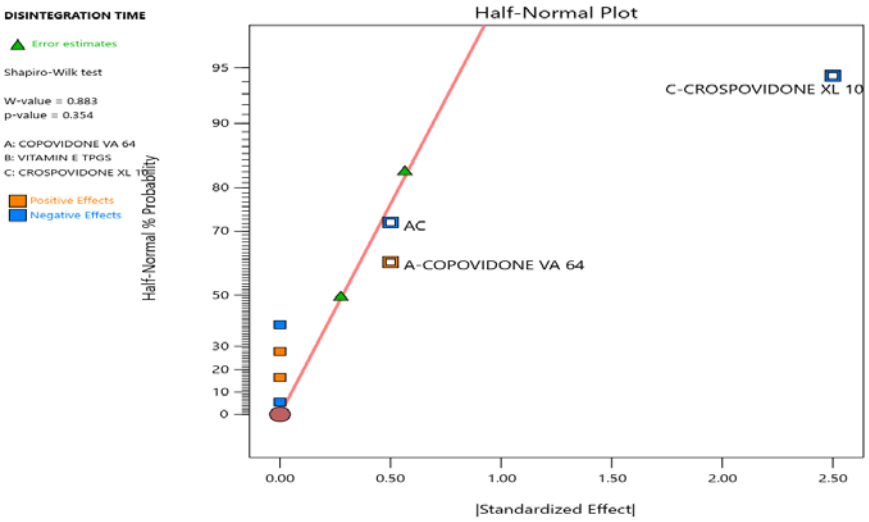



**Fig. 12: Half-normal plot for the formulation variable effects of disintegration time**

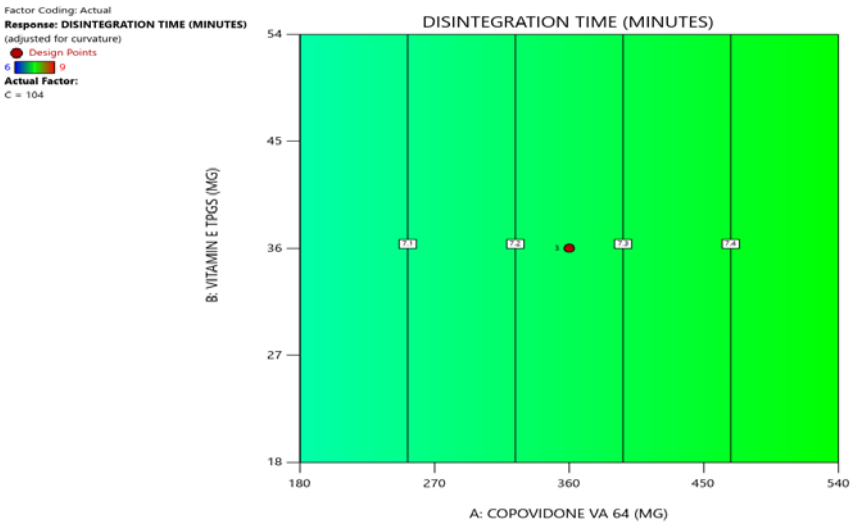



**Fig. 13: Contour plot showing the interaction of copovidone VA 64 and vitamin E TPGS and their impact on disintegration time**

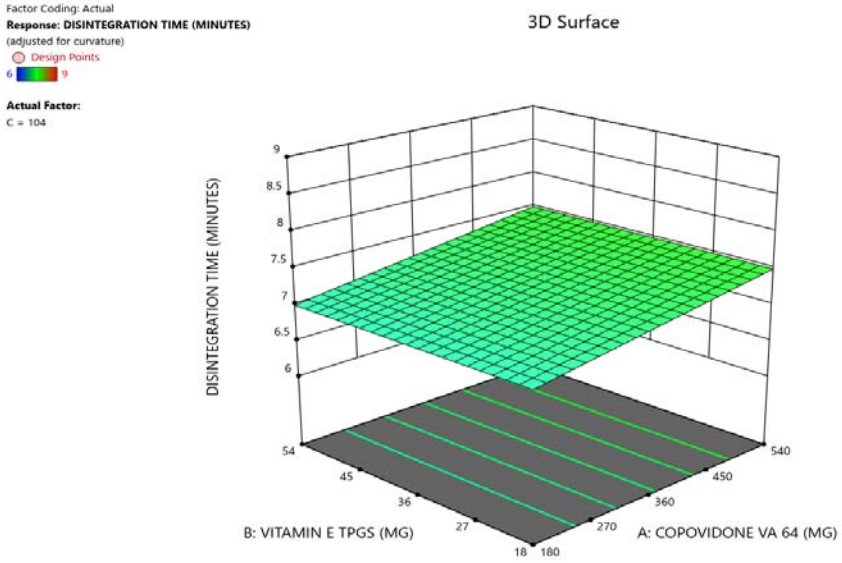



**Fig. 14: 3D surface plot showing the interaction of copovidone VA 64 and vitamin E TPGS and their impact on disintegration time, the predicted vs. actual for disintegration time is presented in fig. 15**

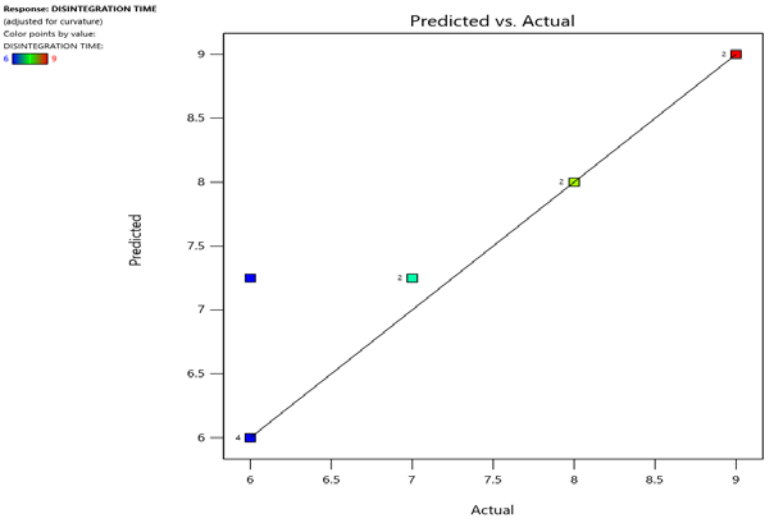



**Fig. 15: Predicted vs. actual response dissolution at 10 min (R1)**

**Optimization**

The graphical and numerical optimization of the critical factors and design space evaluation of the responses R1: dissolution at 10 min and R2: disintegration time are as per the given fig. 16 and 17, respectively. The coded factor level and the responses with the goals are as per table 10.

**Table 10: Coded factor level, the responses, and the goals**

| Factors | Levels | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| X1: Copovidone VA 64 (mg) | 180 | 360 | 540 |
| X2: Vitamin E TPGS (mg) | 18 | 36 | 54 |
| X3:Crospovidone XL (mg) | 78 | 104 | 130 |
| Responses | Goal | | |
| Dissolution at 10 min (%) | Maximize | | |
| Disintegration time (min) | Minimize | | |

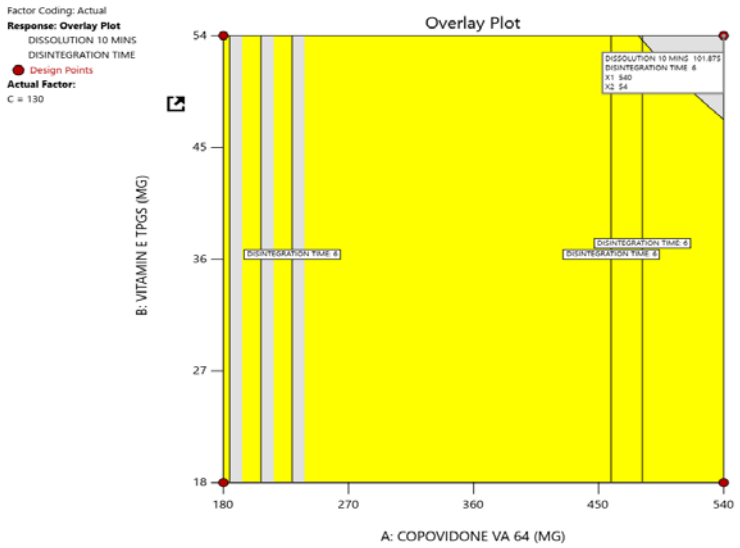



**Fig. 16: Graphical optimization for dissolution at 10 min and disintegration time**

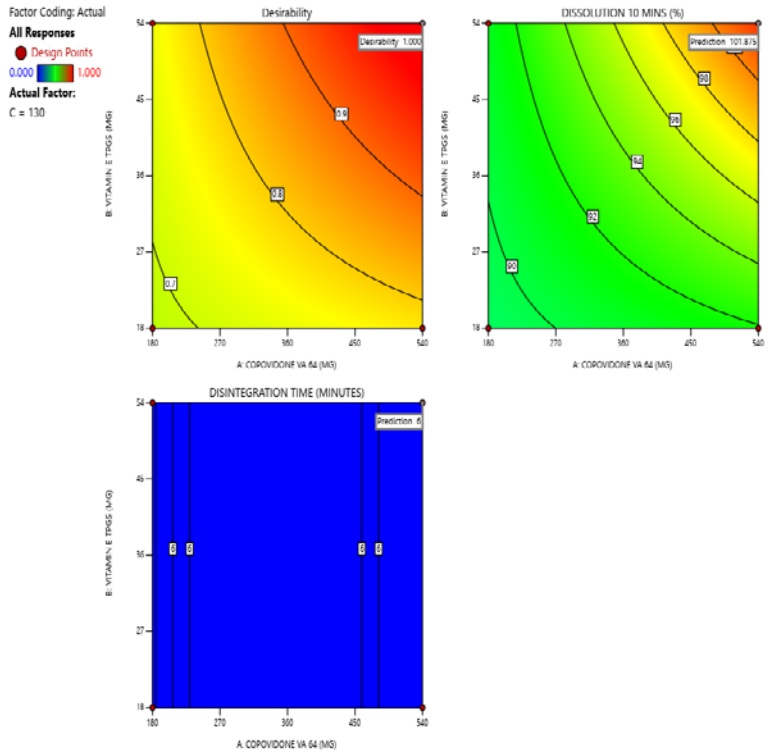



**Fig. 17: Numerical optimization for dissolution at 10 min and disintegration time with an overall desirability function**

## DISCUSSION

From the p-XRD and DSC results, it is evident that complete conversion of ticagrelor from crystalline to amorphous form could be possible through solid dispersion technology with various carriers, namely copovidone VA 64, soluplus, and HPMC acetate succinate. The dissolution rate for the amorphous solid dispersion prepared with copovidone VA 64 was found to be significantly higher compared to formulations prepared with conventional wet granulation techniques. The faster dissolution rate with carrier copovidone VA 64 could be attributed to the low crystallization driving force (CDF). Also, copovidone VA 64 being an excellent crystallization inhibitor and a matrix-forming agent with a glass transition temperature (Tg) of 101 °C, favouring one thermodynamically stable form during *in vitro* and *in vivo* dissolution. The absence of any exothermic peak in the DSC thermogram of the solid dispersion is indicative of a low crystallization driving force (CDF) [17].

Phosphate buffer pH 6.8 without surfactant yielded good discrimination in the dissolution profiles of different formulations and could be explored to differentiate formulations prepared with conventional wet granulation from formulations prepared with solid dispersion technology. Dissolution improved significantly by 10.7 fold, with a dissolution of 107%±0.7% at 75 min for the amorphous solid dispersion formulation (TCG-SD tablet-1:0.4:4) compared to a dissolution of 10.0%±0.7% for tablets prepared with conventional wet granulation technology in line with reference product BRILINTA® (ticagrelor) tablets, ASTRAZENECA LP.

In a study (Cho *et al.*, 2018), the dissolution rate of ticagrelor solid dispersion prepared with vitamin E TPGS and Neusilin® US2 by solvent evaporation technique was reported to be 44.7-fold higher than Brilinta® at 90 min in phosphate buffer pH 6.8. However, the manufacturing process was adopted with difficulty due to the stickiness of vitamin E TPGS [18].

In another study (Srivastava *et al.*, 2023), the dissolution rate of ticagrelor solid dispersion prepared by the hot melt extrusion process with plasdone S 630 as a carrier was reported to be 7.52 fold higher than BRILINTA® (ticagrelor) tablets, ASTRAZENECA LP at 90 min in water [4].

An increase in the dissolution of ticagrelor by 4.18 fold was reported in a study (Bayoumi, A. A., 2018) with a co-grinding approach using PVP K 25 [20].

In the current study, the proposed formulation of ticagrelor solid dispersion with copovidone VA 64 and vitamin E TPGS could yield more than 90% dissolution in 10 min and an overall increase in the dissolution of 10.7 fold compared to the formulation in line with the reference product BRILINTA® (ticagrelor) tablets, ASTRAZENECA LP. Additionally, the formulation and process used in the laboratory provide a suitable and viable method for transferring technology to the manufacturing stage.

The ANOVA and the fit statistics for the selected factorial model were found to be significant for the responses R1: dissolution at 10 min and R2: disintegration time (DT). For dissolution at 10 min (tables 6 and 7), the model F-value is 18.97, indicating the model is significant. P-values less than 0.0500 indicate that model terms are significant. In this case, A, B, C, and ABC are significant model terms. The Lack of Fit F-value of 0.11 implies the Lack of Fit is not significant relative to the pure error. The curvature appears significant and may be due to the center of the design space being poorly modelled. However, considering the fit statistics, the curvature is in a desirable direction and is not recommended to be removed in this situation. To understand the cause of curvature, it is further augmented by a response surface. A non-significant lack of fit is good; hence, the model is good to fit. The predicted R2 of 0.8173 is in reasonable agreement with the Adjusted R2 of 0.8887, as the difference is less than 0.2.

As per the ANOVA results (table 6) and half-normal plots (fig. 8), the factors A (copovidone VA 64), B (vitamin E TPGS), C (crospovidone XL 10), and ABC are affecting dissolution and hence are considered significant.

An interaction is represented by a non-linear response, so contour and 3D views of the interaction are helpful to get a feel for the non-linearity. Fig. 9 and 10 show the interaction of factors copovidone VA 64 and vitamin E TPGS and their impact on dissolution at 10 min. The impact of the level of copovidone VA 64 on dissolution was significantly higher compared to the impact of the vitamin E TPGS Level. The comparison of the predicted response versus the actual response is given in fig. 11.

Similarly, for a disintegration time (tables 8 and 9), the model F-value of 40.50 implies the model is significant. P-values less than 0.0500 indicate that model terms are significant. In this case, C is a significant model term. The lack of fit F-value of 0.00 implies the lack of fit is not significant relative to the pure error. The curvature appears significant and may be due to the fact that the centre of the design space is poorly modelled. However, considering the fit statistics, the curvature is in a desirable direction and is not recommended to be removed in this situation. To understand the cause of curvature, it is further augmented by a response surface. A non-significant lack of fit is good; hence, the model is good to fit. The predicted $R^2$ of 0.8941 is in reasonable agreement with the adjusted $R^2$ of 0.9294, i.e., the difference is less than 0.2.

As shown in table 8, the model and model terms C are significant. Hence, as per the above ANOVA results and half-normal plots (fig. 12), the significant factor affecting the disintegration time was C (crospovidone XL 10).

Fig. 12 and 13 show the interaction of copovidone VA 64 and vitamin E TPGS and their impact on disintegration time. The level of vitamin E TPGS had no significant impact on disintegration time.

The numerical and graphical optimization of the critical factors was quite helpful in establishing the design space. The goals are combined into an overall desirability function. There is more than one maximum because of the curvature of the response surfaces and their combination with the desirability function. The contour and 3D surface of the desirability function at each optimum are used to explore the function in factor space.

In the overlay plot (fig. 16), the edge of failure of the critical quality attributes (CQAs), i.e., dissolution at 10 min and disintegration, are represented by a grey zone. Considering a target dissolution of more than 90% in 10 min and a target disintegration time of less than 7 min. Hence, failures are not considered essential parts of establishing a design space. Within the design space, normal operating range (NOR) and control space (CS) can be further established based on further requirements with respect to clinical outcomes.

## CONCLUSION

Ticagrelor solid dispersion (TCG-SD) with copovidone VA 64 and vitamin E TPGS prepared by solvent evaporation technique could enhance the dissolution of ticagrelor significantly by 10.7 fold as compared to formulation prepared by a conventional wet granulation technique. Factorial design could be suitably adapted to optimize critical formulation factors with adequate $R^2$and be useful in establishing design space for critical formulation parameters. Understanding factor interactions and their impact on drug product CQAs can be achieved by using the multivariate factorial design.

Factorial design and DoE play an important role in QbD-based development and are essential for ensuring clinical safety and efficacy of life-saving drug formulations.

## LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance, API: Active pharmaceutical ingredients, ASD: Amorphous solid dispersion, BCS: Biopharmaceutical classification system, CDF: crystallization driving force, CS: Control space, CQAs: Critical quality attributes, CV: Coefficient of variation, df: degree of freedom, DoE: Design of experiment, DSC: Differential scanning calorimetry, DT: Disintegration time, EMA: European Medicines Agency, FD: Factorial design, HPLC: high-performance liquid chromatography, HPMCAS: Hydroxypropyl methylcellulose acetate succinate, NOR: Normal operating range, OGD: Office of Generic Drugs, PDA: Photodiode array, PMDA: Pharmaceutical and Medical Devices Agency, p-XRD: powder X-ray diffraction, QbD: quality by design, RSD: relative standard deviation, SD: solid

dispersion, TCG: Ticagrelor, Tg: Glass transition temperature, TPGS: Tocopheryl polyethylene glycol succinate, USFDA: United States Food and Drug Administration

**FUNDING**

This research work has not received any grants from any funding agencies.

**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

**CONFLICT OF INTERESTS**

The authors declare no conflict of interest

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