RASĀYAN *J. Chem.*
Vol. 17 | No. 3 |1371-1376| July - September | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

INVESTIGATION OF CRYSTAL FORMS OF METHYL(+)-(S)-O-(2-CHLOROPHENYL)-4,5,6,7-TETRAHYDROTHIENO [3.2-C] PYRIDINE-5-ACETATE

Roselin Pavithra A. and S.I. Davis Presley✉
Department of Chemistry, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam-603110, (Tamil Nadu) India
✉Corresponding Author: davispresleysi@ssn.edu.in

ABSTRACT

Polymorphism refers to a substance's ability to exist in multiple crystalline phases with distinct molecular arrangements within the crystal lattice. This phenomenon plays a significant role in influencing product performance across various industries such as pharmaceuticals, chemicals, and food. However, it poses challenges to ensuring consistent product quality, particularly in pharmaceutical manufacturing. Our study focused on the conversion of from-II to from-I of clopidogrel bisulfate (Methyl (+) -(S)-O-(2-chlorophenyl)-4,5,6,7-tetrahydrothieno [3.2-c] pyridine-5-acetate) which is used as an anti-thinning drug. This conversion is crucial as form-I typically demonstrates superior bioavailability compared to form-II. Different solvents, including acetone, ethyl acetate, and isopropyl alcohol, are utilized to induce this conversion, each resulting in distinct crystal arrangements. To confirm the crystallographic outcomes, we employed techniques such as XRD, FT-IR, and DSC analyses. Our investigation primarily revolves around varying solvent-modifying conditions during experiments, such as temperature and stirring periods. The transition from crystals of form II to those of form I is governed by various thermodynamic parameters, necessitating precise regulation of the reaction conditions.

Keywords: Polymorphs, Crystallization, Clopidogrel Bisulfate, Bioavailability

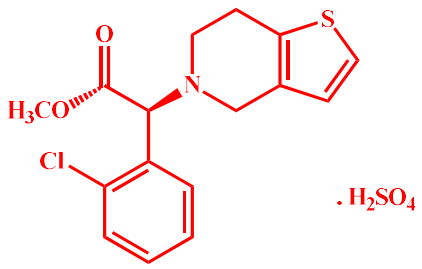
RASĀYAN *J. Chem.*, Vol. 17, No.3, 2024

INTRODUCTION

The term "polymorphism" refers to the ability of a compound to exist in multiple crystalline forms, where the molecules display unique arrangements either in packing or conformation within the crystal lattice at different times.[1] Put different, crystalline structures exhibit identical molecular conformations, alternative lattice structures, and/or diverse chemical compositions.[2] More than half of all active pharmacological compounds exhibit polymorphism, a common phenomenon.[3] Thus far, only a limited number of physiologically active substances—such as aspirin—can be regarded as practically non-polymorphic.[4] Non-polymorphic organic compounds could still have polymorphs, according to theory. Drug stability and bioavailability vary depending on the physical and chemical characteristics of polymorphs.[5] By varying the temperature range, cooling rate, or overall supersaturation level, as well as the concentrations and seed crystals utilized, the crystallization process may be used to influence the creation of polymorphs.[6] We concentrate on converting form-II to form-I of clopidogrel hydrogen sulphate, or its hydrogen sulphate counterpart of (methyl (+) -(S)-O-(2-chlorophenyl)-4,5,6,7-tetrahydrothieno [3.2-c] pyridine-5-acetate). Clopidogrel hydrogen sulphate is a platelet aggregation inhibitor. It has recently been shown that clopidogrel hydrogen sulfate may exist in a variety of polymorphic crystalline forms that vary from one another in terms of stability, physical features, and spectral characteristics.[7] Patients frequently receive active pharmaceutical ingredients (APIs) in solid form within dosage forms like tablets, capsules, granules, powders, etc. These APIs can exist in various solid states, encompassing polymorphs, pseudo-polymorphs (solvates and hydrates), salts, co-crystals, and amorphous solids, whether utilized as pure medicinal ingredients or within manufactured formulations. Each form could possess distinct mechanical, thermal, physical, and chemical characteristics, which greatly influence the drug's solubility, bioavailability, hygroscopicity, melting point, stability, compressibility, and other performance aspects.[9] Hence, selecting the optimal form of an API for development into a medicinal product necessitates a comprehensive understanding of the correlation between the functional properties of the API and its particular solid state.[10] As a medication having antiplatelet properties, clopidogrel bisulfate (CPG) is used to treat several platelet-related blood vessel disorders, including myocardial infarction, stroke, angina, arrhythmia, and peripheral artery occlusive disease.[11] As seen in Fig.-1. Six different forms of CPG polymorphs have been identified so

http://doi.org/10.31788/RJC.2024.1738915

far; form-I and form-II crystals are specifically employed in the production of CPG medications.[12-13] Enantiotropic polymorphs of form-I and form-II crystals exist. According to the statement, form-II crystals are thermodynamically more stable than form-I crystals at room temperature. This is due to form-II crystals remaining stable at temperatures below their transition temperature, while form-I crystals remain stable at temperatures above it. Additionally, it has been noted that monoclinic form-I crystals exhibit greater density and solubility compared to orthorhombic form-II crystals.[15] For this reason, form-II crystals are less preferred by the pharmaceutical industry than form-I [16]. Form-I crystals have been obtained through a highly intricate synthetic process; however, owing to the rapid polymorphic transition from form-I to form-II crystals, this transformation happens in less than 10 minutes. In addition, microcrystalline powder is formed by irregular, rectangular-shaped form-I crystals.[17]



Clopidogrel bisulphate

Fig.-1: Molecular Structure of Clopidogrel Bisulphate (CPG)

EXPERIMENTAL

Materials

Clopidogrel bisulfate (purity greater than 99.9%; Form-II) was supplied by A.R. Life Sciences Pvt Ltd., Hyderabad. Acetone, ethyl acetate, and isopropyl alcohol were supplied by Rankem, and sodium hydrogen carbonate, activated charcoal, and concentrated sulphuric acid were supplied by Thermo Fisher Scientific India Pvt Ltd.

Free Base Method Using Acetone, Ethyl Acetate, and Isopropyl Alcohol as Solvent

Place 5 grams of CPG form-II into a round bottom flask and add 5 milliliters of acetone. Stir the mixture at 50 degrees Celsius for 5 minutes. Slowly add sodium hydrogen carbonate until reaching a neutral pH. Stir the resulting mixture and transfer it into a separating funnel. Allow the layers to separate, then collect the organic layer. Dry the organic layer with sodium sulfate, filter it, and distill the organic layer using a rotary evaporator to obtain a viscous solid base. Treat the base with acetone and a small amount of charcoal, then stir the mixture for 5 minutes. Filter the resulting CPG base solution using a celite bed. To the obtained solution, add a mixture of sulfuric acid and water while maintaining a temperature below -5 degrees Celsius for 2 hours. Stir the solution at room temperature for 12 hours. Filter the precipitate formed and wash it with acetone. Afterwards, instead of using acetone, we experimented with ethyl acetate and isopropyl alcohol in the free base method, while ensuring all physiochemical parameters remained constant. The only notable difference observed was that when using ethyl acetate, a precipitate formed, whereas, with isopropyl alcohol, no precipitate was observed. The experimental setup employed in this investigation is illustrated in Fig.-2.

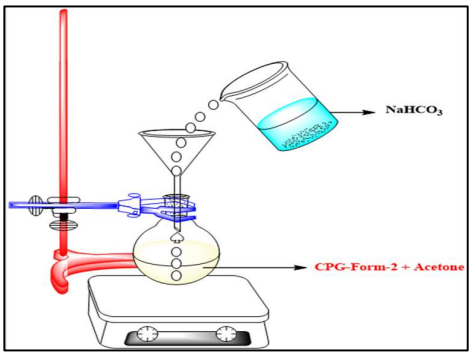


Fig.-2: Schematic Representation of the Experimental Setup

Detection Method

XRPD (X-ray Powder Diffraction)

By comparing, the computed diffract grams of Form I and II with the gathered X-ray powder diffraction (XRPD) patterns of the samples, the repeatability of the two preparation techniques was optimized. Measurements of X-ray powder diffraction was performed with an empyrean diffract meter that was supplied with Cu Kα radiation (λ = 1.54178 Å) at 45 kV and 40 mA of current. The Kβ filter was utilized together with the following optics: a 0.2 mm receiving slit, a 1 mm divergence, and a scattering slit. Diffraction data were obtained using a slow-scan rate at a constant rate of 3^0/min over a 2θ range from 5 to 50 degrees.

Thermal Analysis

In order to verify unique sensitivity in aluminium standard 40ul with an effective temperature range of 25.0 to 500.0 °C, differential scanning calorimetry (DSC) measurements have been performed on a (METTLER TOLEDO, Switzerland) sensor with 56/120 thermocouples. This method was accomplished at a heating rate of 30.00 K/min under an active atmosphere of nitrogen (60 mL/min).

Vibrational Spectroscopic Analysis

The Fourier transform infrared (FT-IR) spectra were obtained using the (Alpha T, Brucker, Germany) spectrometer. Using a range of 4000–400 cm^{-1}. The KBr pellet (resolution 4 cm^{-1}, 16 scans per spectrum) was made with a target polymorphic powder content of about 2%. Each sample's spectrum was captured at a resolution of 4 cm^{-1}1720X across the 500–4000 cm^{-1} spectrum area.

RESULTS AND DISCUSSION

The procedure outlined in materials and methods describes the conversion of Form-II to Form-I. However, when ethyl acetate is used as the solvent, only Form-II precipitate is obtained. Similarly, when isopropyl alcohol is used, no precipitate is observed even after 24 hours of stirring. To address this, the conditions were optimized using acetone as the solvent. It was found that maintaining the temperature below 5 degrees Celsius when using acetone is crucial. Furthermore, it was observed that the addition temperature of sulfuric acid is critical for the conversion of Form-II to Form-I. When added at room temperature, no precipitate formation is observed. This emphasizes the importance of controlling the additional temperature. Additionally, the duration for the solid to precipitate plays a critical role. When the precipitate is filtered within 6 hours, a mixture of Form-I and Form-II is obtained. However, with longer stirring hours, a complete conversion of Form-II to Form-I is observed. Form-I was characterized using various experimental techniques to confirm its identity.

Differential Scanning Colorimetry (DSC)

Colorimetric analysis was utilized to examine the temperature-sensitive parameters and any physicochemical reactions associated with the drug substances, Figure 3 displays the DSC thermogram of clopidogrel form I and II bisulfate. The peak corresponding to form-I of clopidogrel hydrogen sulphate appeared as a clear, sharp endothermic peak at 184°C, exhibiting an enthalpy range of 32± 2 kJ mol-1, indicating its melting point. The use of pure crystalline clopidogrel bisulfate was demonstrated by a pronounced endothermic peak. The DSC thermogram of the sample compact indicated the characteristic peak of clopidogrel's melting point, strongly suggesting that the drug had undergone a transition from its crystalline state to an amorphous form. Form II of clopidogrel hydrogen sulfate displayed an endothermic peak at 178°C, with an enthalpy range of 35± 1 kJ mol-1, as indicated by the drug's peak. A crucial aspect of a practical insert for a polymorphic substance is the comparative thermodynamic stability of its forms. The melting point of form I suggests that higher melting points entail lower heats of fusion, while lower melting points correspond to higher heats of fusion.[19]

Fourier Transforms Infrared Spectroscopy (FTIR)

One of the spectroscopic methods for characterizing solid states that have been published the most is FT-IR, and it is useful. Using this approach, several medicinal substances have been investigated.[20] The FTIR spectral analysis of clopidogrel bisulfate forms I and II are displayed in Fig.-4. The strong peak intensity is evident in Forms I and II, where the frequency range originating from the C=O carbonyl group's band exhibits a stretching vibration at 1755 cm^{-1}. The stretching vibration of the amine C-N group at 1222 cm^{-1} exhibits medium and weak intensity. The thiophene five-membered heterocyclic system has a peak range of 709 cm^{-1}. The above-mentioned bands were observed in both the Forms I and II. Aromatic C-H vibrations of stretching was found at 3121 cm^{-1}, in form II but in form I, it was

displaced to 3103 cm^{-1}. It implies that form II of the chlorophenyl ring has a stronger C-H bond than form I. A large absorbance band was observed in both forms at approximately 2509–2549 cm^{-1} that was related to bound N–H vibrations of stretching caused by the formation of salts across clopidogrel's quaternary nitrogen. In form I with a small peak, the band connected to C-O stretching occurred at 1064 cm^{-1}, and in form II, it displayed at 1165 cm^{-1}. The structure of form II revealed that Cl and O (of the -OCH3 group) are close to each other, leading to an electron-repellent effect across their lone pair of electrons and strengthening the C-O bond in form II. Moreover, forms I and II showed distinct absorption bands at 841 and 2989 cm^{-1}, in addition to 1031 and 1165 cm^{-1}.[21]

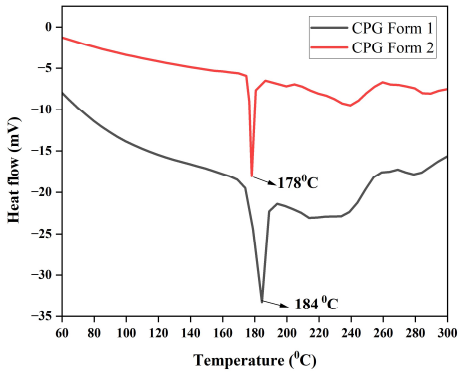


Fig.-3: DSC Thermogram of Clopidogrel bisulfate form-1 and form-II

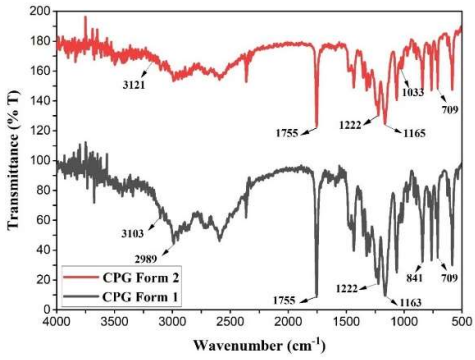


Fig.-4: FT-IR Spectrum of Clopidogrel bisulphate form-1 and form-II

X-ray Powder Diffraction (XRPD)

The XRPD patterns for clopidogrel forms I and II are displayed in Fig.-5. Form II displayed three peaks in the 12.33°–13.67° range that were less intense. Two high peaks of intensity are observed at 17.74° and 18.53°. A single peak of maximum intensity at 21.65°. Form II displayed three smaller intensity peaks at 22.99° to 24.72°, while Form I displayed the maximum intensity peak at 23.600°. At 25.96°, one less intense peak was noted. This implies that when compared to Form II, form I is more tightly packed. This resulted in a strong correlation with published data demonstrating that form II's crystalline structure is form II is less dense with a density of 1.462 g/cm^3 compared to form I, which has a density of 1.505 g/cm^3.[22]

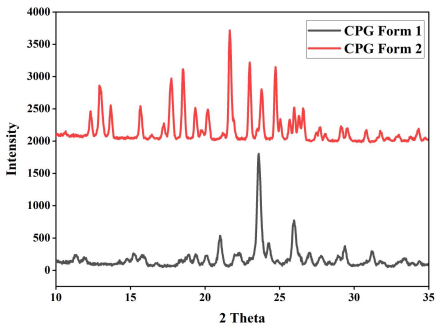


Fig.-5: Powder x-ray Diffraction Pattern of Clopidogrel Bisulphate form-1 and form-II

CONCLUSION

As a result, Forms I and II of CPG have different melting points as well as distinct vibrational peaks and peak intensities (i.e. 2 theta values), respectively. It is feasible to differentiate between these using thermal, phase identification, and spectroscopic techniques. During the DSC examination, there is no loss, and both forms are pure. Forms I and II in powder mixtures have been successfully quantified using differences in their spectral signatures. Form II changes into Form I, displaying the crystal nature (i.e., Form II is crystalline whereas Form I is amorphous in nature). As a result, FTIR is a secure method for detecting CPG. The most effective technique for determining the phase is XRD. The most accurate technique confirms that clopidogrel's melting point is displayed through DSC.

ACKNOWLEDGEMENTS

This work was financially supported and received from the Junior Research Fellowship (JRF) From SSN College of Engineering, Chengalpattu- 603110, Tamil Nadu, India (Licence no. SSN CE /2023).

CONFLICT OF INTERESTS

The author declares that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the author can be verified from their ORCHID ID given below:

Roselin Pavithra A. https://orcid.org/0009-0008-5621-0222
S.I. Davis Presley https://orcid.org/0000-0001-6101-6004

REFERENCES

1. M. Saifee, N. Inamda, D. Dhamecha, and A. Rathi, *International Journal of Health Research*, **2(4)**, 291(2009), https://doi.org/10.4314/ijhr.v2i4.55423
2. J. Bernstein, *Polymorphism in Molecular Crystals 2e*, International Union of Crystallography, 30(2020), https://doi.org/10.1093/oso/9780199655441.003.0002
3. P.J. Larkin, M. Dabros, B. Sarsfield, E. Chan, J.T. Carriere, and B.C. Smith, *Applied Spectroscopy*, **68(7)**, 758(2014), https://doi.org/10.1366/15-07926
4. J. Lu, *African Journal of Pharmacy Pharmacology*, **6(9)**, 581(2012), https://doi.org/10.5897/AJPP12.044
5. J. Lu, and S. Rohani, *Current Medicinal Chemistry*, **16(7)**, 884(2009), https://doi.org/10.2174/092986709787549299
6. H.G. Brittain, *Drugs and the pharmaceutical sciences 2e*, Polymorphism in Pharmaceutical Solids, New York, **192(95),** 183(1999), https://doi.org/10.3109/9781420073225
7. S.H. Wang, X. Li, F.L. Hou, Y.J. Tian, Y.H. Liu, and S.L. Zheng, *Genetics and Molecular Research*, **15(2)**, 15(2016), https://doi.org/10.4238/gmr.15027136
8. X. Zhang, H. Xing, Y. Zhao, and Z. MaZ, *Pharmaceutics*, **10(3)**, 74(2018), https://doi.org/10.3390/pharmaceutics10030074
9. E. Jurczak, A.H. Mazurek, L. Szeleszczuk, D.M. Pisklak, and M. Zielińska-Pisklak, *Pharmaceutics*, **12(10)**, 959(2020), https://doi.org/10.3390/pharmaceutics12100959
10. P. Narayan, *Current Protocols in Pharmacology*, **55(1)**, 7(2011), https://doi.org/10.1002/0471141755.ph0703s55
11. B. Jarvis, and K. Simpson, *Drugs*, **60(2)**, 347(2000), https://doi.org/10.2165/00003495-200060020-00012
12. L. Song, Li Minxu, and J. Gong, *Journal of Chemical Engineering Data,* **55(9)**, 4016(2010), https://doi.org/10.1021/je100022w
13. M.D. Ward, *Israel Journal of Chemistry*, **57(1-2)**, 82(2017), https://doi.org/10.1002/ijch.201600071

14. M.B. Alsirawan, and A. Paradkar, *Innovative Dosage Forms: Design and Development at Early Stage*,1(2019), https://doi.org/10.1002/9783527812172.ch1
15. O.M.M. Santos, J.T.J. Freitas, E.C.L. Cazedey, M.B.D. Araújo, and A.C. Doriguetto, *Molecules*, **21(3)**, 328(2016), https://doi.org/10.3390/molecules21030328
16. H.J. Kim, and K.J. Kim, *Industrial & Engineering Chemistry Research*, **48(24)**, 11133(2009), https://doi.org/10.1021/ie900715w
17. An, Ji-Hun, F. Jin, H.S. Kim, H.C. Ryu, J.S. Kim, H.M. Kim, K.H. Kim, A.N. Kiyonga, and K. Jung, *Crystal Growth Design,* **16(4)**, 1829(2016), https://doi.org/10.1021/acs.cgd.5b01079
18. H.H. Tong, B.Y. Shekunov, J.P. Chan, C.K. Mok, H.C. Hung, and A.H. Chow, *International Journal of Pharmaceutics*, **295(1-2)**, 191(2005), https://doi.org/10.1016/j.ijpharm.2005.02.024
19. Y. Zhou, J. Wang, Y. Xiao, T. Wang, and X. Huang, *Current Pharmaceutical Design*, **24(21)**, 2375(2018), https://doi.org/10.2174/1381612824666180515155425
20. S. Yamamura, and Y. Momose, *International Journal of Pharmaceutics*, **212(2)**, 203(2001), https://doi.org/10.1016/S0378-5173(00)00605-0
21. D.A. Roston, M.C. Walters, R.R. Rhinebarger, and L.J. Ferro, Journal *of Pharmaceutical and Biomedical Analysis,* **11(4-5),** 293(1993), https://doi.org/10.1016/0731-7085(93)80020-2
22. Ji-Hun An, A.N. Kiyonga, E. H. Lee, and K. Jung, *Crystals*, **9(1)**, 53(2019), https://doi.org/10.3390/cryst9010053

[RJC-8915/2024]