Improving Mesalazine Solubility Via, Cocrystallization: Formulation and Characterization

Dr. Bhavya Ekambaram, Ph.D[1], P. Inigo[2,3], Mr. Arun Kumar MS[4], Dr. A. Rajalakshmi[5], Kannan S[6], Jothimani R[7], Yashwanth M[8], Tamilselvi M[9]

[1]Professor and Head, Department of Pharmacy Practice, Saveetha College of Pharmacy.
Saveetha Institute of Medical and Technical Sciences, Saveetha Nagar, Chennai 602125, Tamil Nadu, India. Email: bhavyavivek24@gmail.com; bhavyae.scop@saveetha.com. ORCID ID: 0000-0002-5360-4110
[2]Research scholar, Saveetha college of pharmacy, Saveetha Institute of Medical and Technical Science (SIMATS) Saveetha Nagar, Chennai 602125, Tamilnadu, India.
[3]Associate professor, Department of Pharmaceutics, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi 626 130, Tamilnadu, India. Email: inigoaslin@gmail.com ORCID ID: 0009-0000-4627-9287
[4]Assistant Professor, Faculty of Pharmacy, Karpagam Academy of Higher Education, Pollachi Main Road, Eachanari Post, Coimbatore, Tamil Nadu -641021, India. Email: arunmaruthai907@gmail.com, ORCID ID: 0009-0003-6920-9160
[5]Assistant Professor, Sri Kaliswari College (Autonomous), Sivakasi, Tamil Nadu -641021, India. Email: rajimullai291289@gmail.com
[6]Pharmacist, Apollo Pharmacy, Rameswaram, Tamil Nadu, India. Email: sethukannan178@gmail.com
[7]Pharmacist, Apollo Pharmacy, Rameswaram, Tamil Nadu, India. Email: rjothimani111@gmail.com
[8]Student, M.Pharm (Pharmaceutics), Karpagam College of Pharmacy, Coimbatore-641032. Tamil Nadu, India. Email: yashwanth7598@gmail.com
[9]Lecture, Dept of Pharmacognosy, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi, Tamil Nadu, India. Email: mtamilselvi432@gmail.com
Corresponding author
Dr. Bhavya Ekambaram, Ph.D
[1]Professor and Head, Department of Pharmacy Practice, Saveetha College of Pharmacy Saveetha Institute of Medical and Technical Sciences, Saveetha Nagar, Chennai 602125, Tamil Nadu, India. Email: bhavyavivek24@gmail.com; bhavyae.scop@saveetha.com
ORCID ID: 0000-0002-5360-4110

Abstract

This research project addresses the challenge of improving the solubility of the BCS class IV drug, mesalazine, through innovative co-crystallization techniques. By focusing on the formulation of cocrystals, the study aims to enhance the drug's solubility, bioavailability and therapeutic efficacy. The research involves the design and characterization of mesalazine cocrystals using selected conformers. The Solvent Evaporation technique is employed to successfully formulate Mesalazine nano cocrystals with conformers such as Mannitol, Benzoic acid, Urea and Ascorbic acid at a 1:1 molar ratio. Comprehensive evaluations, including physico-chemical characterization, saturated solubility studies, X-ray diffraction, melting point analysis, SEM and in vitro dissolution testing are conducted. Through systematic formulation and thorough characterization, this research aims to provide valuable insights into the development of enhanced Mesalazine formulations.

Keywords: Absorption, Bioavailability, Co-crystals, Co-former, Mesalazine.

Introduction

The Bio-pharmaceutical Classification System (BCS) designates drugs with solubility problems into Class II and Class IV[1]. The low aqueous solubility observed in 40% of drugs underscores the ongoing necessity for innovative formulations aimed at enhancing bioavailability and therapeutic efficacy[2]. Cocrystallization, a notable focus in pharmaceutical and materials science, has attracted attention for its ability to enhance the physicochemical properties of various molecules[3].

Mesalazine

Mesalazine, identified as a BCS Class IV drug, demonstrates low solubility and permeability. It is used to treat ulcerative colitis and inflammatory bowel disease. Its chemical name is 5-amino salicylic acid, with a molecular weight of 153.135 g/mol and a molecular formula of $C_7H_7NO_3$. The melting point range for Mesalazine is 275 to 280º C. It is very slightly soluble in water but dissolves in dilute solutions of alkali hydroxides and in dilute hydrochloric acid. The mechanism of mesalazine involves the reduction of prostaglandin and leukotriene synthesis, thereby modulating the inflammatory response through the inhibition of the cyclooxygenase and lipoxygenase pathways[4].

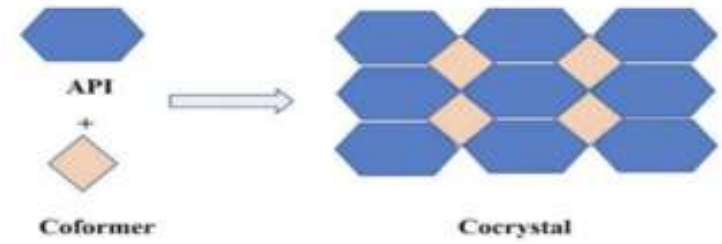
Cocrystalization:

Cocrystals, also called multicomponent crystals or coordination compounds are solid-state structures formed by non-covalent interactions between two or more distinct molecules[5].

Co-crystal is composed of

- Active Pharmaceutical Ingredient
- Co-former
- Solvent

Figure:1. Schematic Representation of Co-crystal Formation



Materials And Methods

Mesalazine was procured from BEC Chemicals, Chennai. Ethanol was procured from Rankem Chemicals Pvt.Ltd., Haryana. Benzoic acid was procured from Loba Chemie PvtLtd, Maharashtra. Urea was procured from Chempure Private Limited, Bengaluru. Ascorbic acid was procured from Rankem Chemicals PvtLtd, Haryana. Mannitol was procured from Lark Chemicals Pvt.Ltd, Mumbai. All other chemicals and reagents used were of analytical grade.

Preformulation Studies

The purpose of preformulation studies is to gather essential information about the drug substance, which helps in the formulation and optimization of the final dosage form. The API was evaluated for its color and odour[6].

Solubility Study: [7]

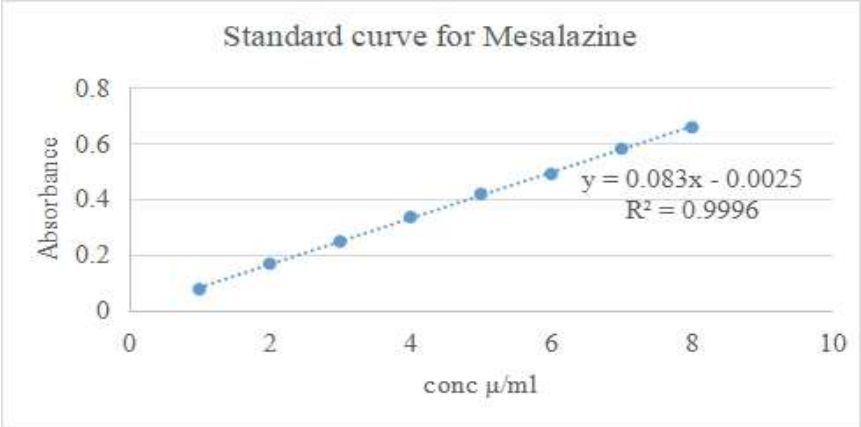
Table:1. Solubility Parameter

Solubility	Approximate volume of solvent in ml per gm of solute
Very soluble	Less than 1
Freely soluble	1 to10
Soluble	10 to 30
Sparingly soluble	30 to100
Slightly soluble	100 to 1000
Very slightly soluble	1000 to 10000
Practically insoluble	Morethan10000

Calibration curve of Mesalazine [8]

Mesalazine samples, ranging from 10 to 400µg (0.4-16 ppm), are prepared in 25ml volumetric flasks by adding 1N HCl, 1% sodium nitrite, 3% sulfuric acid, and 1N sodium hydroxide sequentially. After 10 minutes, absorbance is measured at 471nm against a blank solution using 1cm matched cells. Figure:2. The calibration curve reveals adherence to Beer's law within the concentration range of 10-300 µg/25ml (0.4-12 ppm), with concentrations exceeding 300µg/25ml exhibiting negative deviation. The molar absorptivity is determined to be 2.9480×10^4 $l.mol^{-1}.cm^{-1}$.

Figure :2. Standard Calibration Curve of Mesalazine



The UV absorbance of Mesalazine standard solution in the range of 1-9µg/ ml of drug in 0.1N Hcl buffer pH 1.2 showed linearity at λ max 232nm. The linearity was plotted for absorbance against concentration with R2 value 0.9996 and with the slope.

FT-IR Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was utilized to assess the physicochemical compatibility and interactions between drug and excipients [9].

Methods Of Preparation Of Mesalazine Co-Crystal

Co-formers such as ascorbic acid, mannitol, urea, benzoic acid and tartaric acid were selected for the preparation of Mesalazine co-crystals. These co-formers were chosen from substances that are listed as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (USFDA). This selection ensures that the co-formers used in the co-crystal preparation are considered safe for human consumption. The purpose of using these co-formers is solely to form co-crystals with Mesalazine and improve their physical properties. In Table: 1. The composition of the co-crystal was presented, indicating the respective molar ratio of the drug to the co-former.

Solvent Evaporation Method:

In the solvent evaporation method, Mesalazine and Co-formers such as ascorbic acid, mannitol, urea, and benzoic acid were weighed in a 1:1 molar ratio. The drug Mesalazine and co-formers were dissolved separately in ethanol to form clear solutions, which were then combined and mixed for 5-20 minutes. The solution was allowed to evaporate at 40°C in a hot air oven. The resulting co-crystals were collected and stored at room temperature for further studies[10].

Table 1: The Ratio and Quantity of The Drug and Co-former In The Co-crystal Composition.

S.No.	Co-crystal components (Drug + Co-former)	Molar ratio of Mesalazine: Co-former	Quantity of Mesalazine: Co-former (in mg)
1	Mesalazine + Ascorbic acid	1:1	156:176
2	Mesalazine+ Mannitol	1:1	156:182
3	Mesalazine+ Urea	1:1	156:60
4	Mesalazine+ Benzoic acid	1:1	156:122

Characterization Of Mesalazine Co-Crystals

Saturated Solubility Studies

In the saturated solubility study, the prepared co-crystals were tested in 0.1N hydrochloric acid solution. Excessive amounts of the samples were added to 10 mL of the 0.1N HCL dissolution media and continuously agitated. After 24 hours of equilibration, the solutions were filtered and the filtrates were diluted and analyzed using a UV-visible spectrophotometer at 240 nm. This allowed to determine the solubility of Mesalazine and the co-crystals in the 0.1N HCl solution[11].

X-Ray Diffraction

X-ray diffraction (XRD) is indeed a valuable technique for determining the crystalline nature of substances. It exploits the unique diffractograms exhibited by different compounds. In this study, diffractograms of both the pure drug Mesalazine and the co-crystal were obtained using an X-ray diffractometer. Powder samples of Mesalazine and the co-crystal, weighing between 100-200 mg, were taken. The samples were placed in a suitable sample holder. The XRD instrument was set up with a step time of 7.0000 deg./min with the appropriate measurement parameters, including the from 3-800 range of diffraction angles, 2Θ to be scanned. The resulting data were collected to generate diffractograms, which are graphical representations of the intensity peaks corresponding to different diffraction angles[12].

Melting Point Determination

In the determination of the melting point, a small amount of the sample, such as Mesalazine co-crystals were placed into an open capillary tube or melting point capillary. The capillary is then inserted into the melting point apparatus, which allows for controlled

heating of the sample. As the temperature rises, the solid sample begins to melt, and the transition from solid to liquid phase occurs. The temperature at which this transition occurs is recorded as the melting point[13].

SEM Analysis

It uses a focused beam of high energy electrons to generate a variety of signals at the surface of Mesalazine co-crystals. In most SEM microscopy applications, data is collected over a selected area of the surface of the Mesalazine co-crystal and a two dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the Mesalazine. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations[14].

IN VITRO Dissolution's Study

Preparation of 0.1N HCL: To prepare 0.1 N HCL, dilute 8.5 ml of HCL with distilled water to make up the volume to 1000 ml.

Procedure: The dissolution test was performed using 900 ml of 0.1N HCL (dissolution media) at 37±0.50C at 50 rpm. Samples (10 ml) were collected at predetermined time intervals (5, 10, 15, 20, 25 Upto 60mins) and replaced with an equal volume of 0.1 N HCL fresh medium. The study was conducted for 60 min, the samples were then filtered and analyzed at 232 nm using a UV spectrophotometer[15].

Results And Discussion

Predormulation Study

Organoleptic Properties

Mesalazine pure drug is white to grayish and slightly pink in color. The solid is odorless, or can exhibit a slight characteristics odor.

Solubility study

Mesalazine pure drug is Very slightly soluble in water and it is dissolve in dilute hydrochloric acid.

Drug-excipients interaction studies by using FT-IR.

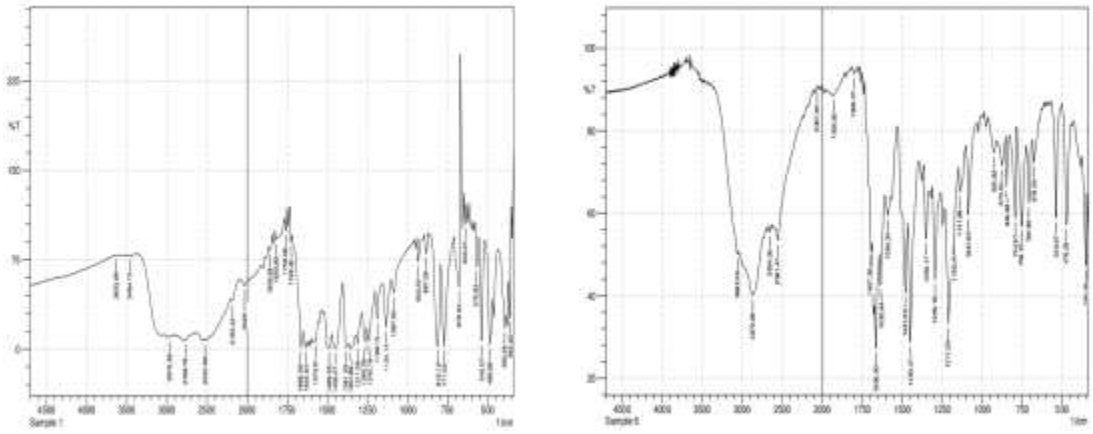


Figure :3. FT-IR Spectra of Mesalazine and FT-IR Spectra of Mesalazine with Benzoic Acid Co-crystal

It is observed here there is no appreciable information exists between API and other ingredients in the formulation therefore the formulation does not have any interaction between other ingredients and API. Hence, FT-IR studies further supported the absence of incompatibility between drug and excipients.

Formulation Of Co-Crystals

Mesalazine Co-crystals prepared by solvent evaporation method in the ratios of (1:1, and 1:1).

Figure :4. Formulation of mesalazine co-crystals was achieved through the solvent evaporation method, employing various coformers.

Characterization

Saturated Solubility Study:

The saturated solubility of Mesalazine pure drug and mesalazine cocrystals was determined and is reported in Table 2.

Table 2: Saturated Solubility of Pure drug Mesalazine and Mesalazine Cocrystals in 0.1 N HCL

S.No	Sample	Solubility (mg/ml)	Increase in Solubility
1.	Mesalazine Pure Drug	2.935	-
2.	Mesalazine Mannitol Cocrystals	0.923	0.3144
3.	Mesalazine Ascorbic acid Cocrystal	1.708	0.5819
4.	Mesalazine Urea Cocrystals	1.462	0.4981
5.	Mesalazine Benzoic acid Cocrystals	1.140	0.3884

Discussion

Cocrystals prepared with various coformers have demonstrated their potential to enhance the solubility of the drug. Co-crystals of Mesalazine with Ascorbic acid, prepared using the solvent evaporation method, exhibited higher solubility compared to other cocrystals. The results of solubility studies revealed that the solubility of the drug increased in cocrystal form.

Melting Point:

The Melting point of Mesalazine pure drug and mesalazine cocrystals was determined and is reported in Table 3.

Table 3: Melting Point of Pure drug Mesalazine and Mesalazine Cocrystals

S.No	Sample	Melting point
1.	Mesalazine Pure Drug	270^0C
2.	Mesalazine Mannitol Cocrystals	140^0C
3.	Mesalazine Ascorbic acid Cocrystal	125^0C
4.	Mesalazine Urea Cocrystals	135^0C
5.	Mesalazine Benzoic acid Cocrystals	120^0C

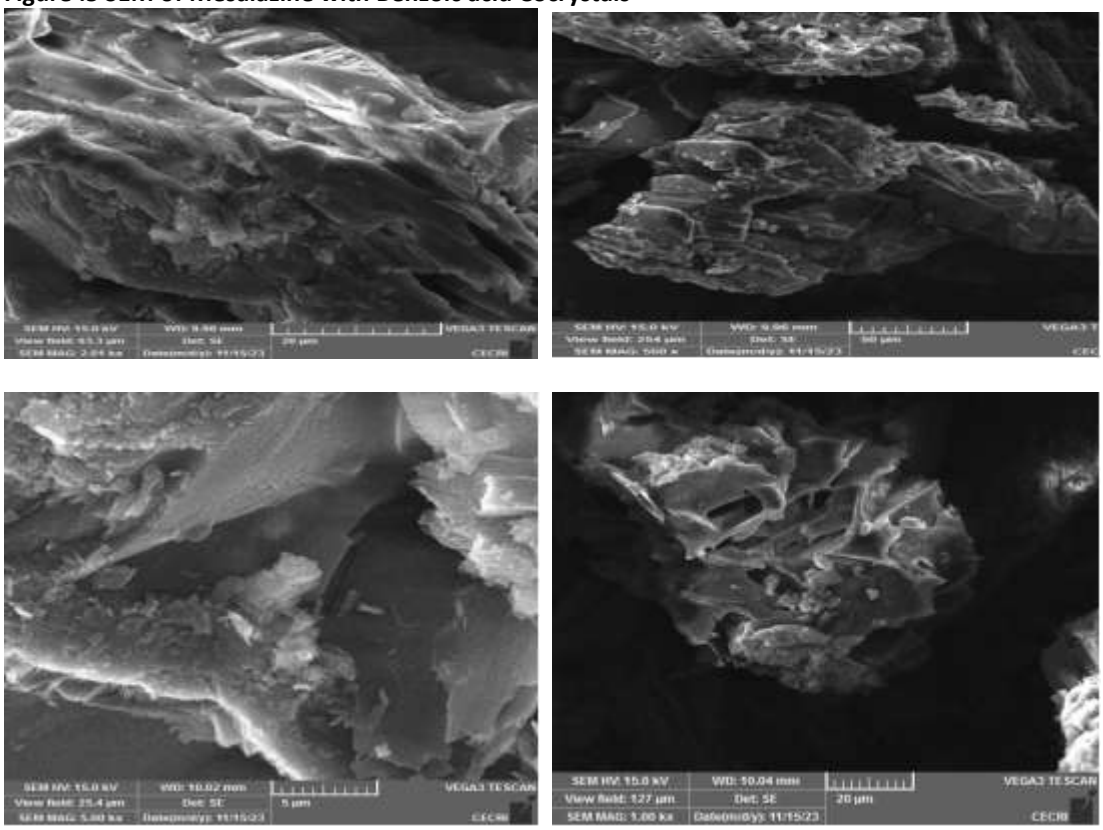
Discussion

The melting point of the drug and cocrystals was determined using the capillary method. This study revealed that all cocrystals shows an increase in melting point compared with the pure drug Mesalazine.

SEM Analysis:

Scanning electron microscopic analysis of Mesalazine with benzoic acid cocrystals was conducted, and the results are presented in Figure :5.

Figure :5 SEM of Mesalazine with Benzoic acid Cocrystals



Discussion

The SEM view of the Mesalazine with Benzoic acid cocrystals indicated the presence of crystal shape.

X-Ray Diffraction:

The XRD patterns of the pure drug Mesalazine and Mesalazine cocrystals obtained from the solvent evaporation method were presented in **Figures :6 - 10.**

Figure :6. XRD of Pure Mesalazine

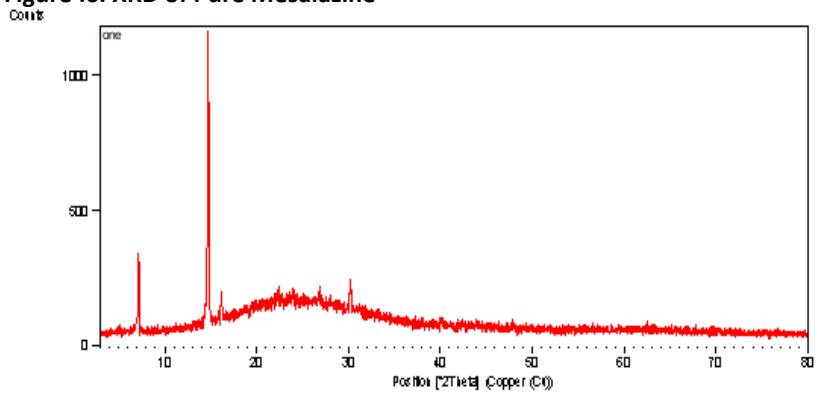


Figure: 7. XRD of Mesalazine with Mannitol Cocrystals

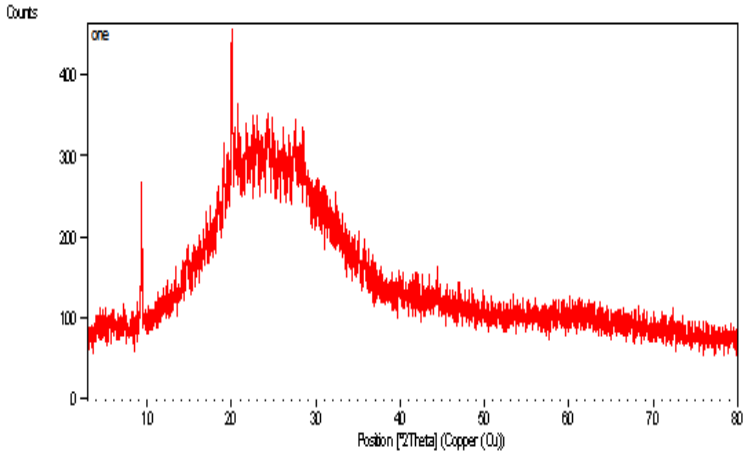


Figure: 8. XRD of Mesalazine with Ascorbic acid Cocrystals

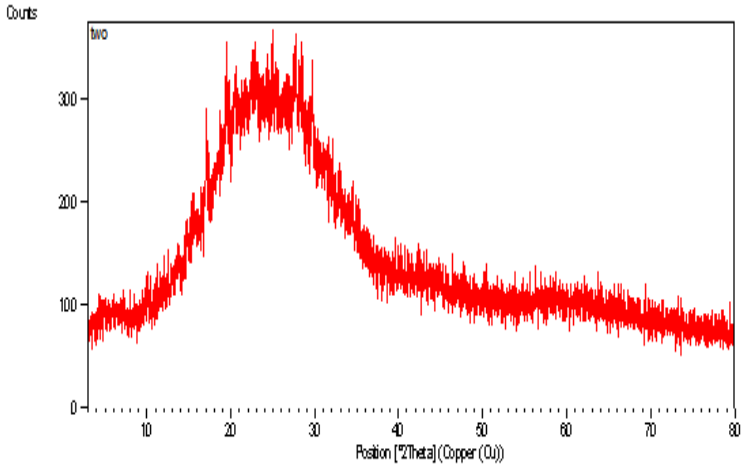


Figure: 9. XRD of Mesalazine with Urea Cocrystals

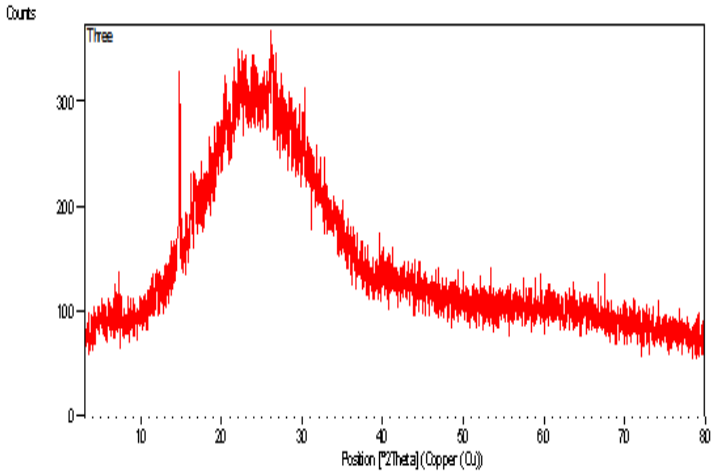
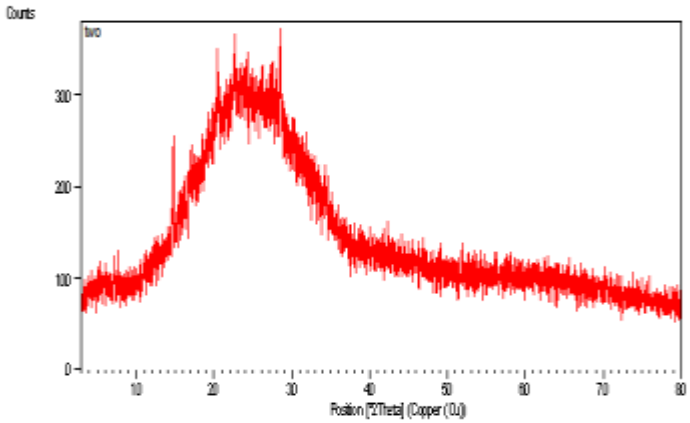


Figure: 10. XRD of Mesalazine with Benzoic acid Cocrystals



Discussion

- The XRD pattern of the pure Mesalazine exhibited characteristic diffraction lines to 2θ values at 4.31,9.22,15.10 and 30 respectively.
- The XRD pattern of Mesalazine and mannitol cocrystal by using the solvent evaporation method exhibited characteristic diffraction lines to 2θ at 7.8, 10.01,15. 12 and 22.87
- The XRD pattern of Mesalazine and ascorbic acid cocrystal by using the solvent evaporation method exhibited characteristic diffraction lines to 2θ at 8.90, 11.02, 15.15, 22.24, 24.20, 29.10 and 30.
- The XRD pattern of Mesalazine and urea cocrystal by using the solvent evaporation method exhibited characteristic diffraction lines to 2θ at 5.24, 16.20, 21.04, 23. 65, 26.81, 30.31 and 32.
- The XRD pattern of Mesalazine and benzoic acid cocrystal by using the solvent evaporation method exhibited characteristic diffraction lines to 2θ at 4.33, 15.25, 19.31 ,22.27, 24.90, 29.00 and 30.1
- The XRD results demonstrated a more crystalline form of Mesalazine cocrystals obtained through a co-crystallization method. The emergence of new peaks positions compared to the pure drug indicates the formation of a new crystalline phase, showing the formation of cocrystals.

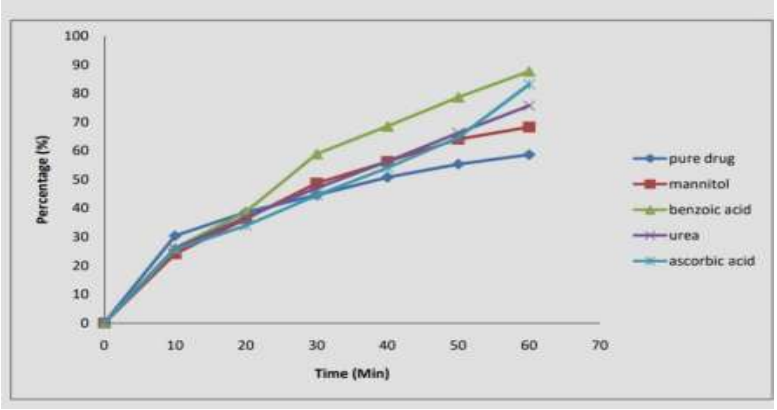
In Vitro Drug Release Study

The in vitro drug release studies of pure mesalazine and mesalazine with various cocrystals are presented in Table 4, accompanied by Figure: 11.

Table: 4. Cumulative percentage drug released of mesalazine pure drug and mesalazine cocrystals

S.NO	TIME (MINS)	PURE DRUG	Mesalazine with Mannitol Cocrystal	Mesalazine with Benzoic acid Cocrystal	Mesalazine with Urea Cocrystal	Mesalazine with Ascorbic acid Cocrystal
	0	0	0	0	0	0
	10	31.01	22.34	23.74	21.54	23.12
	20	36.25	32.63	34.22	32.37	31.56
	30	41.36	47.42	37.56	41.32	40.83
	40	47.57	53.92	66.21	51.46	51.74
	50	49.32	59.43	77.55	59.23	59.86
	60	51.74	60.61	87.56	69.75	79.32

Figure :11. In vitro Drug release of pure Mesalazine and various Mesalazine cocrystals



Discussion

The percentage drug release for all formulation Mesalazine with Mannitol cocrystal, Mesalazine with Benzoic acid cocrystal, Mesalazine with Urea cocrystal and Mesalazine with Ascorbic acid cocrystal were found to be 51.74, 60.61, 87.56, 69.75 and 79.32 respectively in 1 hour. The formulation of Mesalazine with Benzoic acid cocrystal show faster release than other cocrystal formulation.

Conclusion

This study concludes that enhancing the solubility of Mesalazine, belonging to BCS Class IV category, is crucial. Therefore, cocrystallization is pursued to improve its therapeutic effectiveness. Prior to formulation, organoleptic properties, solubility studies, and calibration curves of the pure Mesalazine drug were meticulously examined. FT-IR studies further supported that the absence of incompatibility between drug and coformers. Mesalazine cocrystals were formulated using the Solvent Evaporation technique. We successfully formulated Mesalazine nano cocrystals using various coformers, such as Mannitol, Benzoic acid, Urea, and Ascorbic acid, in a molar ratio of 1:1 concentration.

The comprehensive analysis, including saturated solubility measurements, SEM analysis, X-ray diffraction (XRD) and melting point studies, collectively provided strong evidence confirming the successful formation of co-crystals in the study. The in vitro drug release studies, as detailed in the case of Mesalazine with benzoic acid cocrystals, offer valuable insights into the performance of all other formulations. The findings suggest promising avenues for improving the therapeutic efficacy of Mesalazine, opening new possibilities for enhanced drug delivery and bioavailability.

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