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COFORMER DEVELOPMENT AND IN SILICO SCREENING TO IMPROVE THE SOLUBILITY AND DISSOLUTION OF FAMOTIDINE.

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Abstract

The solubility of a medicine is influenced by the excipients employed in its preparation. The incorporation of binders in tablet formulation plays a pivotal role in enhancing the solubility of both the dose form and the active pharmaceutical ingredient. This study aimed to explore the utilisation of natural excipients in order to enhance the solubility and dissolving rate of a pharmaceutical compound. A novel and stable cocrystal of famotidine (FMT) was successfully synthesised using crystal engineering techniques. The coformer (CCF) selected for this cocrystallization process was m-nitrobenzoic acid (MNBA), which is a pharmaceutical intermediate containing a carboxylic acid group. The salt cocrystals were characterised using various analytical techniques, including scanning electron microscopy, differential scanning calorimetry, thermogravimetric analysis, infrared spectroscopy, powder X-ray diffraction, and

X-ray single crystal diffraction. The utilisation of drug-drug cocrystals is increasingly gaining popularity in contemporary times.

Keywords: Co crystal, Famotidine, Molecules, Drug, famotidine

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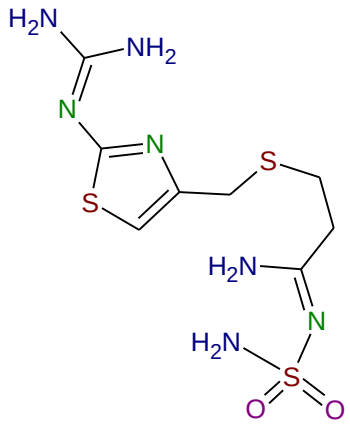
1. Introduction:

The utilisation of multicomponent crystals [1] is regarded as a highly versatile and potent approach for altering the functional characteristics of solid-state materials. This approach entails the intentional manipulation of intermolecular interactions, together with the modification of the crystalline environment, achieved by carefully selecting appropriate coformers. In the realm of pharmaceutics, the process of cocrystallization has been seen to yield notable improvements in many physicochemical characteristics of an active pharmaceutical ingredient (API), leading to the development of a novel formulation with heightened therapeutic effectiveness [2]. In addition to the inherent complexity, the pharmaceutical industry often has the additional difficulty of having a multitude of viable coformers at its disposal for the purpose of cocrystallizing a certain active pharmaceutical ingredient (API). The aforementioned aspect significantly increases the complexity of the rational design of pharmaceutical cocrystals. The method of experimental screening for potential coformers is frequently characterised by a time-intensive trial and error approach, which entails numerous experimental procedures and a restricted selection of coformers [3]. Medications with low water solubility often exhibit common occurrences of reduced bioavailability and limited dissolution absorption. According to recent estimations, it has been suggested that a significant proportion, maybe up to 75%, of chemicals now undergoing development exhibit limited solubility in aqueous solutions. Consequently, enhancing the solubility of these active pharmaceutical ingredients (APIs) in aqueous solutions is a significant challenge for researchers in the field of pharmaceutical science [4]. In the realm of API development, salts have conventionally functioned as the primary means of mitigating issues related to low solubility and sluggish dissolving rates. In recent years, there has been an increased scholarly focus on the examination of cocrystals [5].

Over the last decade, the utilisation of cocrystal technology has gained prominence as an innovative approach to enhance the solubility of pharmaceuticals with limited water solubility, through the application of crystal engineering principles [6]. The preservation of the medications' inherent chemical composition has been successfully achieved. The combination of an active pharmaceutical ingredient (API) with one or more coformers in a pharmaceutical

cocrystal is achieved by carefully selecting a specific stoichiometric ratio, primarily driven by non-covalent interactions [7]. The aforementioned interactions encompass hydrogen bonding, van der Waals forces, stacking, and electrostatic interactions. The distinguishing feature that sets salts apart from cocrystals is the occurrence or absence of proton transfer between an acid and a base [8]. However, the identification of whether a salt or a cocrystal has been formed is often a complex task that lacks simplicity. Although the determination of crystal structure can sometimes provide exact proton placements, other methods are often necessary to achieve precise localization of protons [9]. Researchers have dedicated a significant portion of the past two decades to developing and studying various qualitative and quantitative theoretical methods [10] in order to rationally pre-select the most promising coformers before doing experimental screening. The supramolecular synthon approach is frequently employed in the selection of coformers for cocrystallization. Due to its predominantly descriptive nature and absence of quantitative criteria, the anticipation of novel multicomponent systems devoid of significant particular interactions or competition can pose challenges. A recent study conducted by Cappuccino et al. has shown that utilising known supramolecular synthons and relative frequencies obtained from the Cambridge Structural Database (CSD) does not yield more accurate predictions compared to a random screening search. The user did not provide any text to rewrite. The aforementioned discovery was disseminated in the scholarly publication known as Chemical Science. Consequently, the coformer selection process has witnessed the development of several novel quantitative computational approaches that exhibit enhanced reliability. The methodologies employed in this study can be broadly categorised into three main approaches: knowledge-based, physicsbased, and chemically-specific [11]. The knowledge-based approach encompasses structural informatics and thermodynamic approaches. The physics-based approach incorporates various techniques such as Hansen solubility parameters, molecular electrostatic potential map, COSMO-RS, crystal structure prediction, and machine learning approaches. Lastly, the chemically-specific approach focuses on the unique characteristics of the chemical compounds under investigation. Each of the aforementioned methodologies possesses both merits and constraints, and the efficacy of predictive outcomes might significantly fluctuate based on the application programming interface (API) employed and the magnitude of the test dataset. Famotidine, an H2 blocker and antagonist targeting the histamine type 2 receptor, is employed for the treatment of many medical disorders. The use of this substance has been found to provide relief for heartburn and other acid-peptic diseases. Although the incidence of this consequence is exceedingly low, a correlation exists between the administration of famotidine and the occurrence of acute liver

injury with discernible clinical manifestations. Famotidine is employed in the management of acid-related gastrointestinal disorders due to its pharmacological property of reducing gastric acid production. Famotidine is available in both prescribed and over-the-counter formulations [12]. The FDA has granted a licence for the treatment of pathological hypersecretory illnesses in adults, as well as for the treatment of gastroesophageal reflux disease (GERD) in both paediatric and adult populations. This encompasses the management of duodenal ulcers, gastric ulcers, and gastroesophageal reflux disease (GERD). Famotidine, an over-the-counter medication, is indicated for individuals across all age groups as a therapeutic option for alleviating the discomfort associated with heartburn resulting from gastroesophageal reflux disease (GERD) [13]. Additionally, there exist applications that have not been sanctioned by the maker. This exercise elucidates the indications for the utilisation of famotidine, delineates its mechanism of action, outlines the various routes of administration, highlights the principal bad effects, identifies the contraindications, discusses the recommended monitoring practises, and examines the potential toxicity associated with the drug. The purpose of this comprehensive analysis is to ensure the delivery of appropriate patient care and facilitate favourable treatment outcomes [14].



Famotidine

Fig. 1. Molecular structure of Famotidine

2. Materials and method:

2.1. Molecular Docking:

2.1.1. Personal Computer:

A 4GB RAM, Windows 7–running HP laptop with an Intel Core i5 processor.

2.1.2. Programmes:

For this analysis, we used PyRx (Vina), Open babel 2.3.2/GUI, and the client version of Biovia discovery studio (2023). Cipla pvt. ltd. supplies famotidine, and CDH chemicals, New Delhi provided citric acid (co-former).

2.1.3. Drug and coformers preparation:

Research needs to be done to determine whether or not the coformer and the drug are compatible with one another before any attempt can be made to construct a pharmacological co-crystal. This is a prerequisite for the process. A "coformer screen" is performed so that it may be identified which coformer will work correctly in a co-crystal formulation with the medicine. This helps ensure that the drug receives the best possible treatment. After a suitable candidate has been located, its physicochemical and pharmacological properties are studied in order to design an effective dosage form for the medicine. This process takes place after a suitable candidate has been located. The US Food and Drug Administration (FDA) maintains a list of chemicals that are "generally recognised as safe" (GRAS), and the addition of these coformers does not change the pharmacological properties of an active pharmaceutical ingredient (API). Coformers are typically chosen from this list.

2.1.4. Molecular docking study:

The basic objective of the molecular docking methodology is to computationally anticipate the structure of the complex formed between a ligand and its receptor. The achievement of this objective can be facilitated by using technological advancements. The docking procedure comprises two fundamental components: the exploration of diverse ligand conformations within the active site of the protein, and the subsequent evaluation and prioritisation of these conformations through the utilisation of a scoring function. Collectively, these characteristics constitute the docking procedure. The optimal result is achieved when the scoring system assigns higher importance to the generated conformation that exhibits the highest level of accuracy in approximating the experimental binding mode. This is the situation in the optimal scenario. In the subsequent paragraphs, a comprehensive description of the docking theory will be provided, encompassing multiple perspectives.

2.2. Preparation of Famotidine co-crystal:

The FMT compound (340 mg, 1 mmol) was subjected to heating and agitation at a temperature of 70 °C for a duration of one hour. This process was carried out while the FMT compound was dissolved in a mixture consisting of 7 mL of methanol and 3 mL of dimethylformamide.

Following the addition of MAL (104 mg, 1 mmol), the mixture was subjected to heating at a temperature of 70 degrees Celsius and subsequently agitated for a duration of two hours. The resulting solution underwent filtration and was thereafter allowed to undergo gradual evaporation at room temperature for a duration of two days. A meticulous assortment of individual crystals was conducted under microscopic examination, followed by their subsequent preservation in mineral oil.

3. Characterization of co-crystal:

3.1. FTIR:

The Fourier Transform Infrared (FTIR) analyses of the examined co-crystals were conducted using a Thermo Fischer Scientific spectrometer, namely the Nicolet 380 model, situated in Madison, Wisconsin, United States. This spectrometer was equipped with a DTGS KBr detector and OMNIC software for data acquisition and analysis. The experimental procedure involved the combination of 1 mg of the sample with 100 mg of KBr (Merck, Darmstadt, Germany). This process necessitated the utilisation of a hydraulic press produced by Specac, located in Orpington, United Kingdom. FTIR spectra were acquired at ambient temperature, with a spectral range spanning from 4000 to 400 cm^-1, with a spectral resolution of 4 cm^-1. Prior to commencing each measurement, the spectral characteristics of the background were evaluated to ensure precision and reliability.

3.2. Microscopy morphology:

The majority of the EVs that are present in biofluids are too small to be detected by even the most cutting-edge optical microscopes. This is due to the fact that EVs are found in biological fluids. Consequently, EM is applied in order to evaluate their structure as a consequence of this fact. The two most commonly utilised types of electron microscopes are the transmission electron microscope (TEM) and the scanning electron microscope (SEM). The scanning electron microscope (SEM) and the transmission electron microscope (TEM) are both classified as electron microscopes. Both methodologies produce a picture by putting the specimen to significantly reduced pressures, typically ranging from 104 to 105 pascals, and depending on the interactions between the electrons in the beam and the atoms in the sample. Hence, both the electron microscope and the transmission electron microscope share three fundamental elements: (i) an electron gun responsible for generating an electron beam, (ii) magnetic lenses (ML) and apertures (A) contained within metal diaphragms, which serve to manipulate and direct the electron beam towards either the sample (S) or a detector, and (iii)

detectors that capture signals arising from the interactions between the electron beam and the sample, subsequently converting them int

3.3. DSC:

Nevertheless, in most instances, the chemical of interest is examined in buffered aqueous solutions. The methodology for sample preparation is contingent upon the specific nature of the material under analysis. Nevertheless, it remains consistent that aqueous buffers are employed in this process. The observation of distinct peaks, such as the gel-to-liquid crystalline phase transition (L) in high purity phosphatidylcholines (PC) [4], necessitates the use of extremely slow scan speeds, often about 0.1 K min-1 or lower. The implementation of this measure is necessary to mitigate the phenomenon of increased scope commonly associated with higher scan rates. The resolution can be enhanced by reducing the scan rates, so enabling the differentiation of closely spaced DSC peaks that may arise from distinct phospholipid phase transitions. Another benefit of utilising slower scan speeds is observed. Nevertheless, this phenomenon results in a reduction of the signals, hence requiring the utilisation of calorimeters with a heightened level of sensitivity. The user's text is already academic and does not need to be rewritten.

3.4. Powder XRD:

Characterization of nanoscale materials frequently makes use of powder X-ray diffraction, abbreviated as XRD. Powder XRD analysis is a technique that complements other microscopic and spectroscopic methods by providing information on phase identification, sample purity, crystallite size, and in some cases shape. These are only some of the insights received from this technique. The information that is obtained through the use of this bulk technique can be coupled with the data that is obtained through the use of microscopy in order to assess whether or not the microscopic observations made on a limited number of particles are typical of the majority of the sample. The availability of powder X-ray diffraction (XRD) data for nanoscale materials is commonly seen, while its utilisation is often accompanied by misconceptions or neglect. The objective of this Editorial is to offer a comprehensive introduction to key elements of powder X-ray diffraction (XRD) data that are commonly encountered during the analysis of nanoscale materials. The emphasis is placed on inorganic nanoparticles with diverse sizes, shapes, and dimensionalities, catering to the wider communities of nanoscience and nanotechnology. This Editorial will specifically focus on the characterization and properties of inorganic nanoparticles with diverse sizes, shapes, and dimensionalities.

3.5. SEM:

In order to conduct a microscopic analysis of the pure drug as well as the created co-crystals, a scanning electron microscope with the model number EVO® LS 10 and manufactured by Carl Zeiss in Jena, Germany was utilised. Sputtered atoms of gold were used to create the coating.

3.6. Solubility studies:

The primary aim of this study was to assess the solubility of famotidine in various physical combinations (PM1, PM2) and solid dispersions (XS1, XS2, XS3, HS4, HS5, HS6). Conical flasks containing an excessive amount of formulations, along with 10 ml of distilled water, were subjected to incubation and agitation in an incubator shaker at a temperature of 25 degrees Celsius for a duration of 48 hours. After reaching equilibrium, aliquots of 5 millilitres were removed and passed through filter paper with a pore size of 0.45 millimetres made by Millipore. At a maximum wavelength of 280 nm, spectrophotometric analysis [16] was carried out on the filtrate. We each did three separate readings, and then we meticulously took notes on what we discovered.

3.7. In-vitro dissolution study:

A study was conducted to investigate the dissolution of famotidine tablets in an in vitro setting. The experiment was conducted at a temperature of 37 ± 0.5 degrees Celsius using simulated gastric fluid (SGF) with a pH of 1.2. The dissolution was measured over a volume of 900 millilitres. The experimental setup employed in this study consisted of a USP apparatus (model TDT-08T, Electrolab, Mumbai, India), equipped with a paddle that was set to rotate at a constant speed of fifty revolutions per minute. Samples of 10 millilitres were collected at regular intervals and subsequently filtered via a membrane filter with a pore size of 0.45 microns. The filtered samples were then diluted and their absorbance at 265 nanometers was measured using a Shimadzu UV 1800 double-beam spectrophotometer, manufactured by Shimadzu in Kyoto, Japan. The determination of the cumulative percentage of drug release involved the utilisation of an equation derived from the calibration curve, which was afterwards employed in the calculating process.

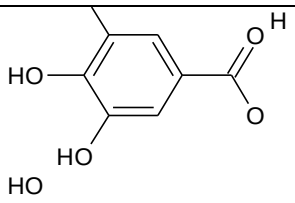
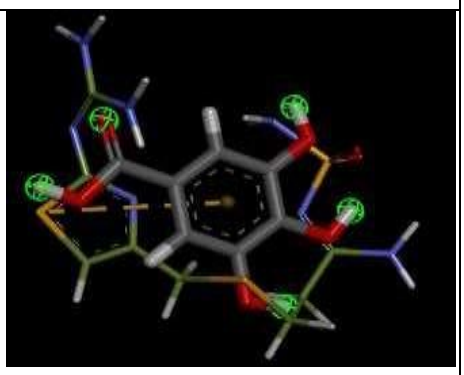
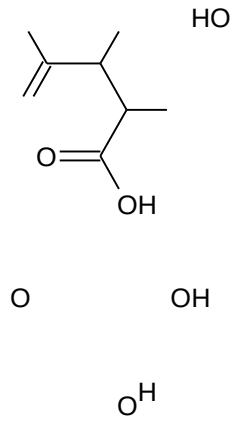
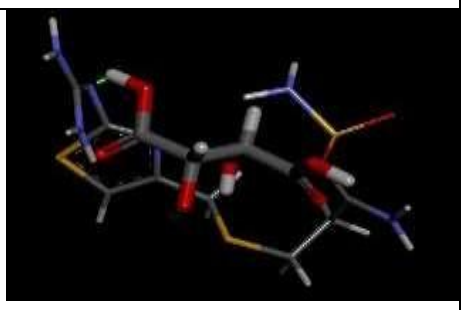
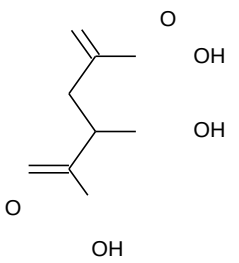
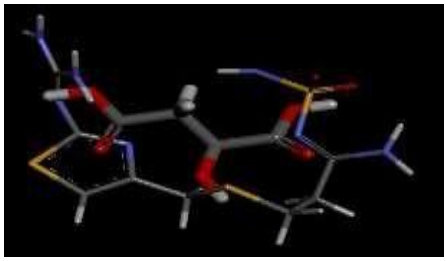
4. Result & Discussion

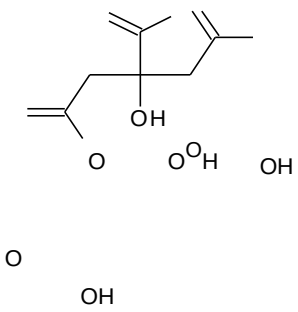
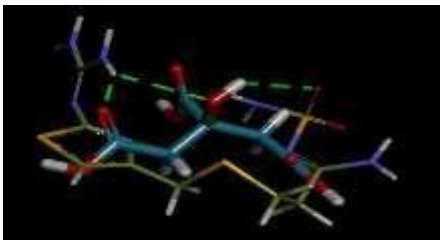
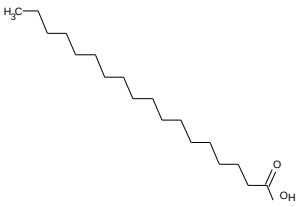
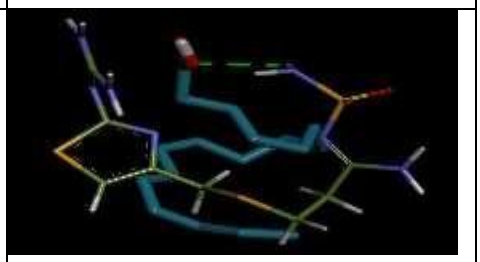
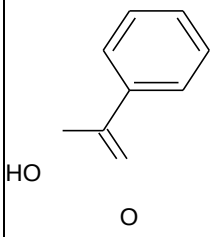
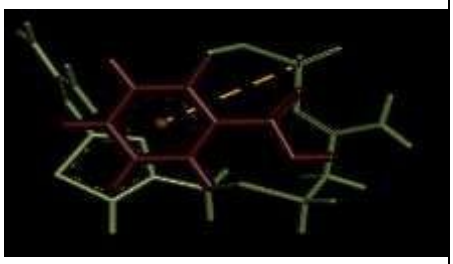
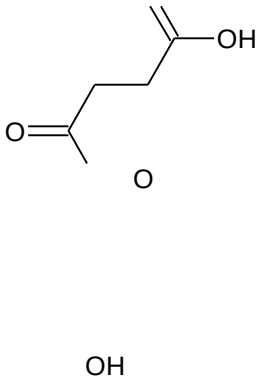
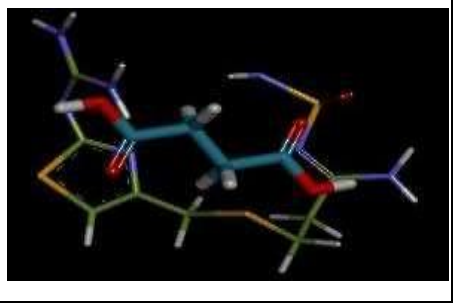
4.1. Molecular docking

Out of all of the H2RAs that were examined for their capacity to bind to coronavirus proteins, famotidine was found to have the highest binding affinity. This finding was made possible by the fact that famotidine was found to be the most potent. The docking technique was repeated

three times with the same coordinates for each ligand, and each iteration gave results that were extremely similar to the results produced by the preceding iterations. The results of docking replications demonstrated that the binding energy of famotidine to NSP3 was 6.4, 6.7, and 6.4 kcal/mol; the binding energy of cimetidine to NSP3 was 6.3, 6.1, and 6.2 kcal/mol.

Table 1. Insilico screening of coformers with famotidine.

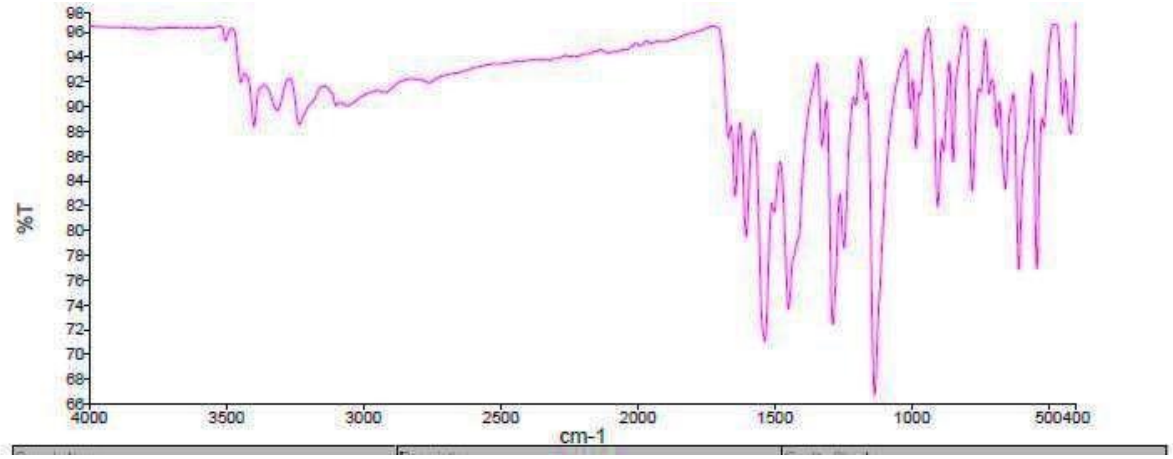
Coformers	**2D structure**	**Binding affinity(Ei kcal/mol)**	**Results**
Gallic acid		-2.2 $\pi - bond$	
Tartaric acid		-2.4 H- bond	
Malic acid		-2.4 no, any interaction	

Citric acid		-2.6 H- bond	
Stearic acid		-1.3 H-bond	
Benzoic acid		-2.0 π - bond	
Succinic acid		-1.9 no,any interaction	

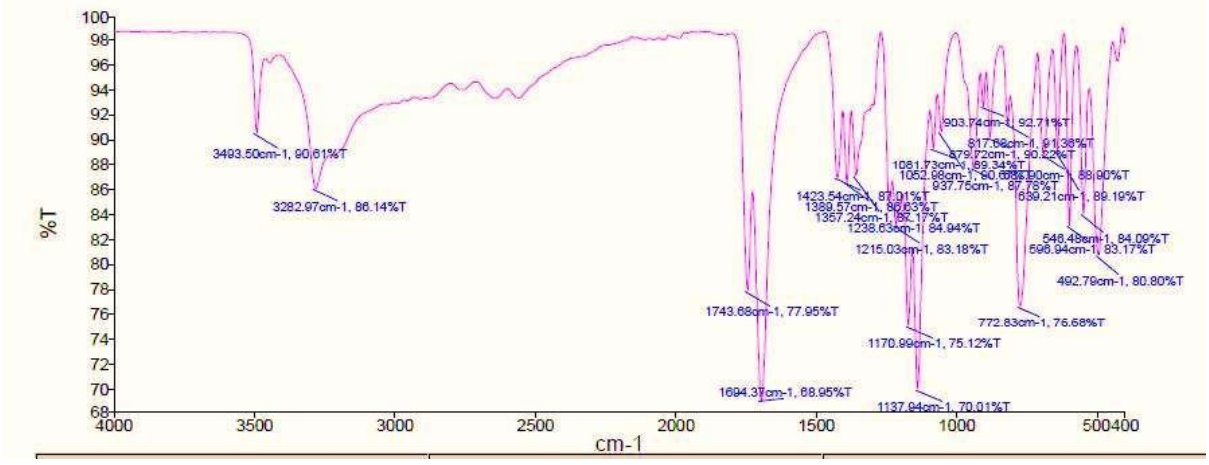
4.2. FTIR

In the cocrystals that were generated, the results of the FT-IR spectroscopy and the determination of the crystal structure show that the hydrogen-bonded network is formed in an unexpected manner. Crystal systems with the names orthorhombic PNCB and triclinic P 1 are both capable of cocrystallizing FSOR, however FSY can only do so with FSOR. FSOR is the

only crystal system that can cocrystallize FSY. In addition to this, the cocrystalline form of FMT demonstrated significantly enhanced solubility (9–5-fold), dissolution (8–5-fold), peak plasma concentration (2–1.5-fold), and relative bioavailability (about 200%–135%). As a direct result of this element, their ability to defend themselves against ulcers and free radicals has significantly been boosted. The results of this investigation provide confidence to the possibility of cocrystallization as a strategy for the efficient delivery of neutral chemicals under settings that present considerable solubility problems. The outcomes of this study can be found here.



(a)



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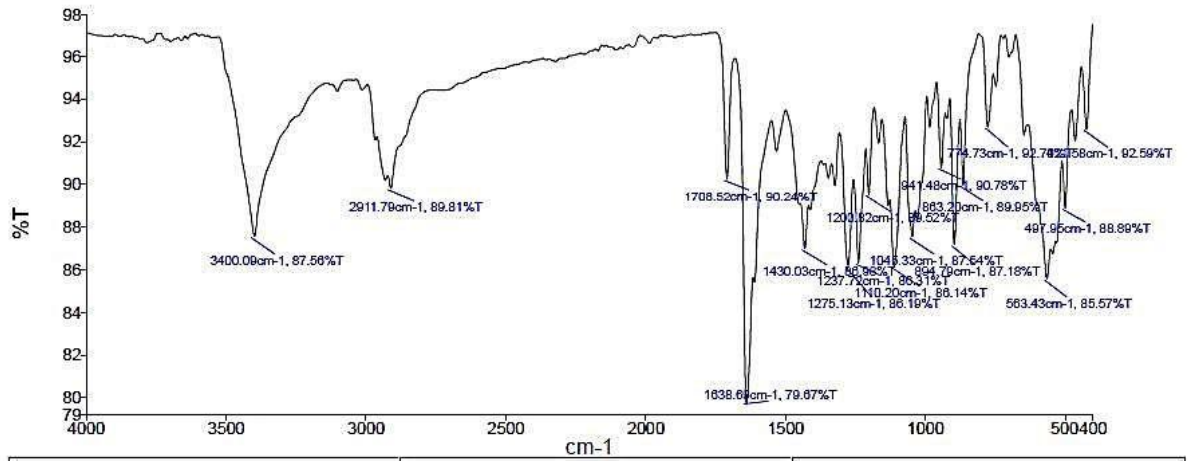
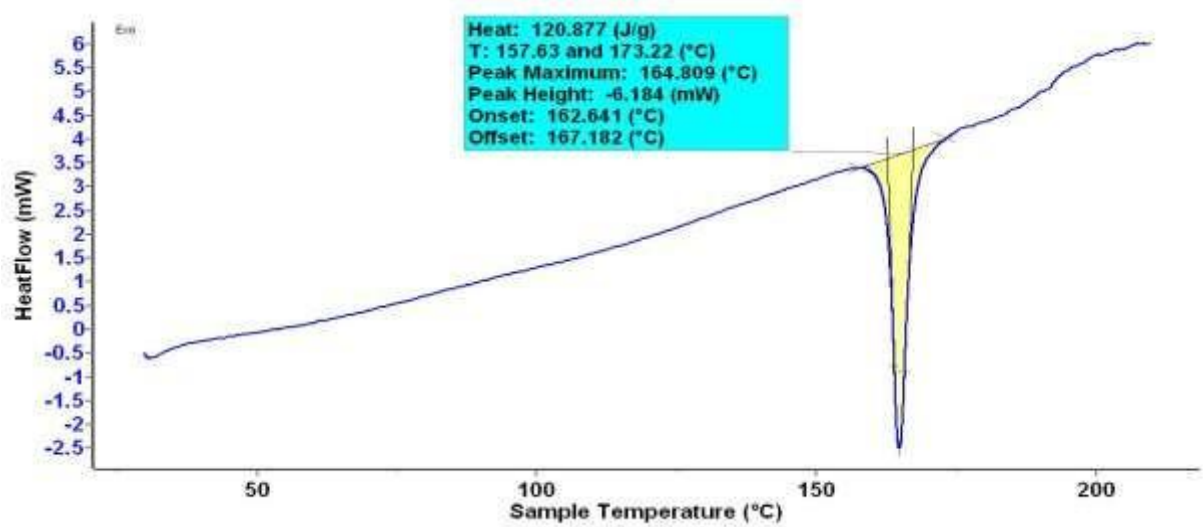


(c)

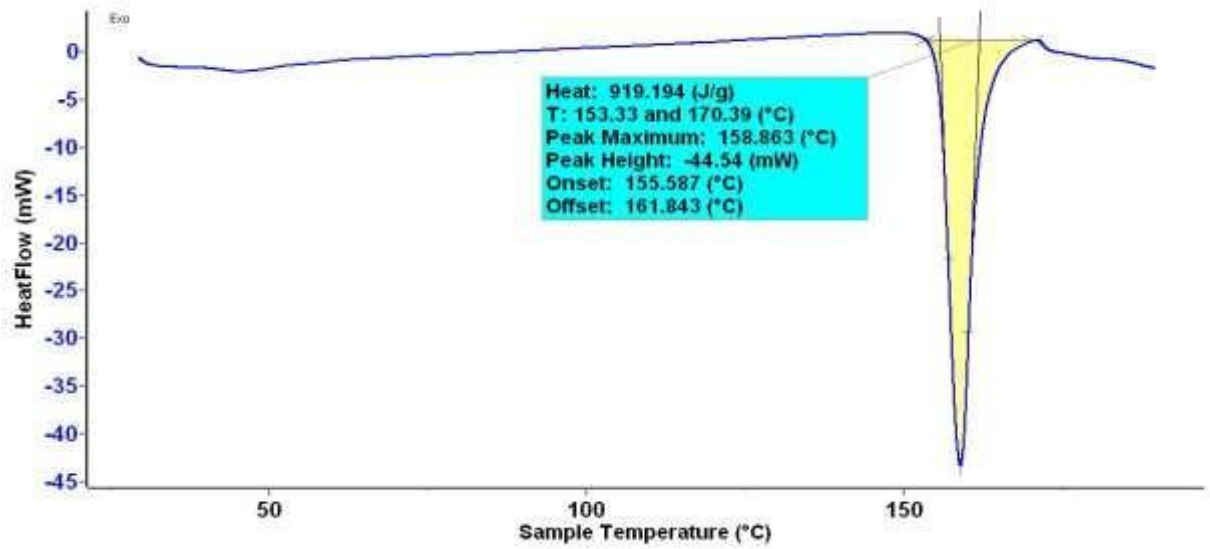
Figure 2: The Fourier Transform Infrared (FTIR) spectra of (a) famotidine in its pure form, (b) citric acid, and (c) the cocrystal formed by famotidine and citric acid were analysed in this study.

4.3. DSC:

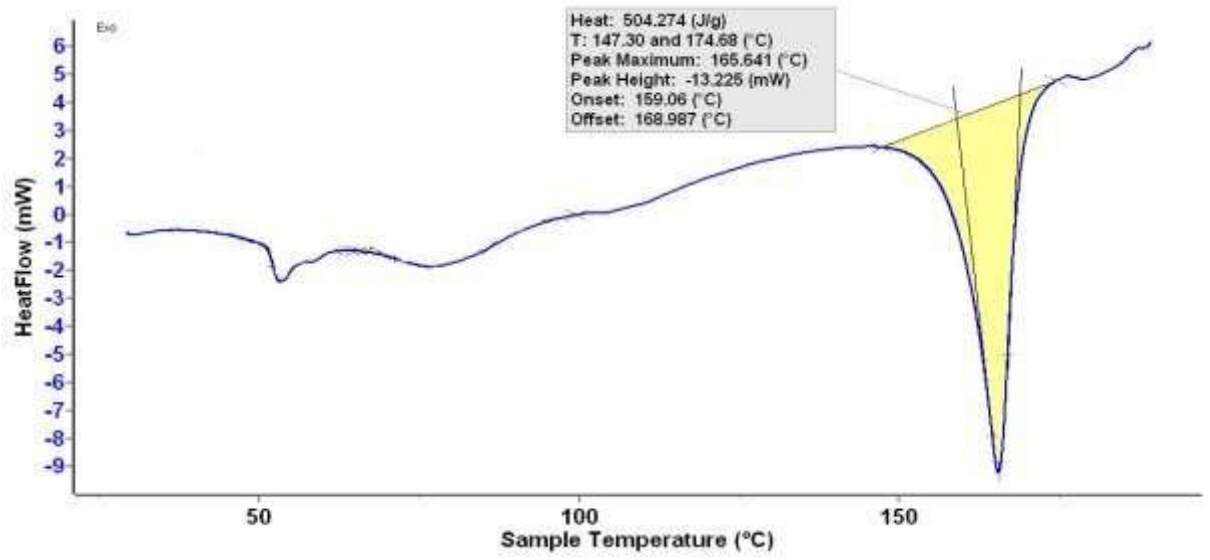
Figure 3 presents the DSC thermograms of the pristine drug, the in-progress co-crystal, and the fully produced co-crystal. A distinct endothermic peak was readily identifiable at a temperature of 164 degrees Celsius on the thermogram obtained from the pure famotidine sample. The thermogram of citric acid exhibited an endothermic peak at a temperature of 158 degrees. In contrast, the thermogram of the FMT-citric acid co-crystal had an endothermic peak at a slightly higher temperature of 165.6 degrees. This observation indicates that the drug and the co-former exhibit distinct endothermic peaks, supporting the hypothesis that a novel co-crystal has been formed.



(a)



(b)



(c)

Figure 3. The differential scanning calorimetry (DSC) thermogram showcases the thermal behaviour of three substances: (a) famotidine, (b) citric acid, and (c) the cocrystal formed by famotidine and citric acid.

4.4. Trinocular Microscopy:

The analysis of figure 4 reveals that the cocrystal had an uneven and flattened shape, while still maintaining a distinct morphology.

(a) **(b)**

Figure 4. (a) and (b) show images of the microscopic morphology of a prepared cocrystal.

4.5. Solubility:

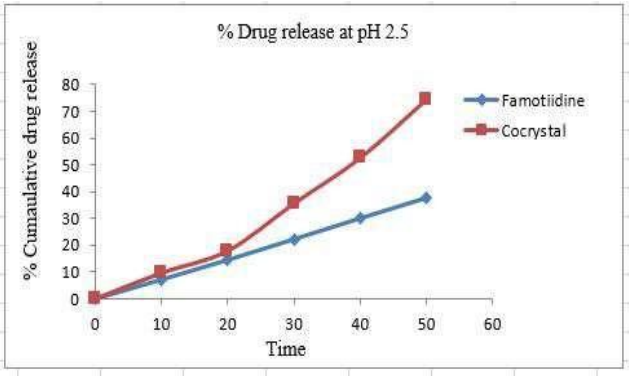
The solubility of pure famotidine in CO2-free distilled water was found to be 0.080-0.13 mg/ml (80.1 g/ml), but the solubility of the famotidine-citric acid cocrystal was determined to be 0.3060.19 mg/ml (306 g/ml). The computations (mean and standard deviation) were carried out with a sample size of three. The cocrystal exhibited a water solubility that was approximately 3.8 times greater than that of the pure famotidine.

4.6. In-vitro dissolution:

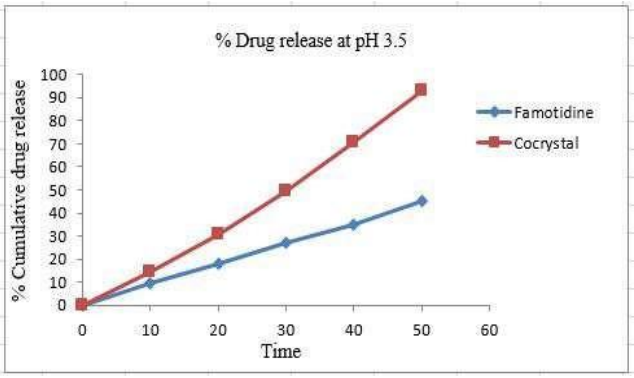
Various pH values were employed during the in vitro dissolution test for both the pure famotidine and cocrystal samples. Table 2 presents the outcomes of the dissolution test, whereas figures 5 and 6 depict the cumulative percent release curve and time profile, respectively. The drug release of pure famotidine was shown to be 37.5% at pH 2.5, 44.8% at pH 3.5, and 26.08% at pH 6.4. In contrast to the cocrystal, the drug release percentages observed were 74.5% at pH 2.5, 92.75% at pH 3.5, and 61.7% at pH 6.4. In order to conduct the computations, it was expected that a sample size of three would be utilised. Based on the aforementioned data, it can be observed that the cocrystal exhibits a release of drug at pH levels of 2.5, 2.07, and 2.3 times greater than that of the pure drug.

Table 2. Invitro % release of pure famotidine and FMT: PABA cocrystal.

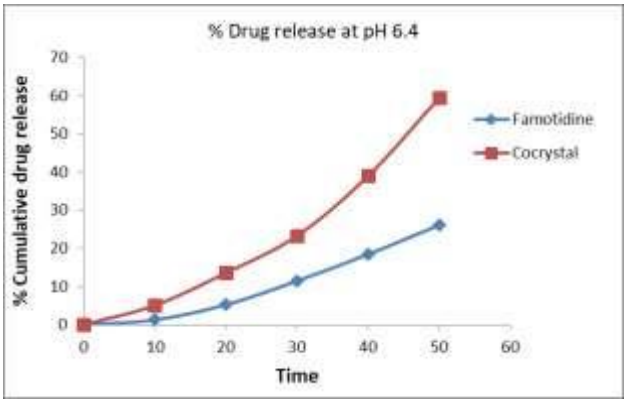
S.N.	pH	Pure famotidine	Cocrystal
1.	2.5	37.5% ±0.08	74.5% ±0.01
2.	3.5	44.8% ±0.57	92.75% ±0.01
3.	6.4	26.08% ±0.72	61.7% ±0.71



(a) n=3(mean±SD)



(b) n=3(mean±SD)

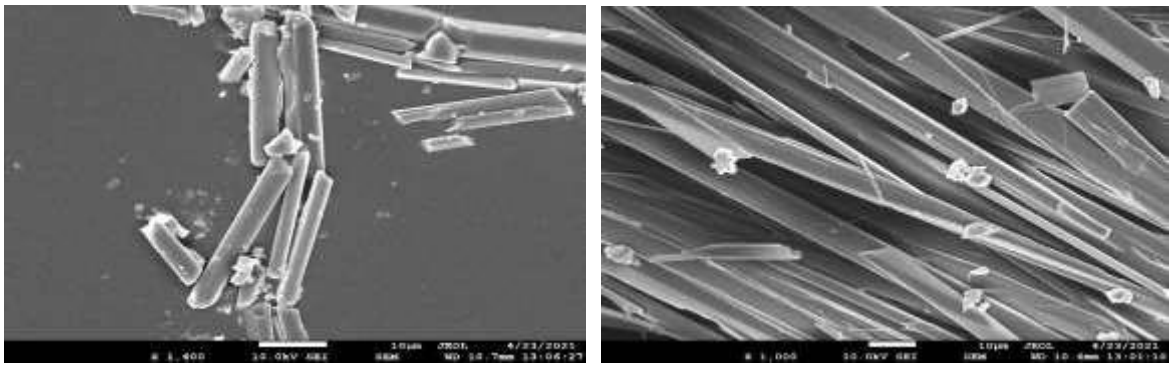


(c) n=3(mean±SD)

Figure 5: Invitro dissolution test n=3(mean±SD) (a) at pH 2.5 (b) at pH 3.5 (c) at 6.4 pH

4.7. Scanning electron microscopy:

A look at the surface morphology of the cocrystal revealed a linear and planar structure that was ten metres long (refer to Figure 6).



(a) **(b)**

Figure 6: This study presents scanning electron microscopy (SEM) images of a Cocrystal specimen, captured at two different magnifications: (a) 1400x and (b) 1000x.

5. Conclusion:

In the context of in silico screening of coformers, the utilisation of PyRx (Vina) coupled with the advanced technique of autodock enables the efficient screening of a considerable number of compounds within a very little timeframe. This enables the identification and exclusion of potential coformers. The choice of citric acid as the coformer for the synthesis of cocrystals was based on its favourable binding energy of -2.6 Kcal/mol, as determined through calculations. This led to citric acid being the optimal choice. It was able to generate cocrystals, which was the reason for this. An evaporation method that included the use of methanol was utilised at some point over the course of the synthesis procedure. The fabrication of the cocrystal was the ultimate result of utilising this technology. In order to carry out the morphological analysis, both optical and scanning electron microscopy were utilised as analytical tools. On the other hand, FTIR, Powder XRD, and DSC were applied in order to characterise the cocrystal. Following a testing period of twenty-four hours, the cocrystal demonstrated a solubility in aqueous solutions that was three and a half times higher than that of famotidine in its purest form. The discovery of this conclusion was brought about by doing a comparison between the two distinct types of substance. while compared to the percentage of drug that was released while the famotidine was in its pure form, the proportion of the drug that was released during the process of dissolving the cocrystal was greater at all pH levels. This was the case regardless of the level of pH. The results of this study offer empirical support for the notion that the utilisation of cocrystallization technology holds promise in enhancing the physicochemical characteristics of famotidine.

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