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INSILICO SCREENING OF COFORMERS, DEVOLPOMENT OF COCRYSTAL TO ENHANCE DISSOLUTION AND SOLUBILITY OF FAMOTIDINE

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Abstract:
The solubility and dissolution profile of the drug directly affects the therapeutic efficacy of the drug. Cocrystallization offers a promising method to improve the dissolution and solubility of the drug. Pharmaceutical cocrystals are multicomponent systems made of API and acceptable cocrystal formers in the same lattice. Cocrystals are increasingly popular in the pharmaceutical industry since extending the opportunity to improve the physicochemical property of drugs without affecting the internal activity of the compound. In this study famotidine, an antiulcer agent taken as a model drug. It is very slightly soluble in water, due to its low solubility categorized in BCS class 2. Here a very effective method of Insilico screening applied to the screening of coformers and cocrystal formation. The virtual screening technique, a computational tool developed the efficiency of screening the coformers to limited the coformer for experimentally study. Seven coformers from the GRAS list were screened with API. Binding affinity and the possibility of formation of H- bond interaction were computationally determined. Autodock was used for docking. Based on molecular docking citric acid (Ei -2.6kcal/mol0 was selected as the best coformer. The work was continued by the cocrystallization process using the solvent evaporation method. Assay of solubility in water and dissolution at different pH like 2.5,3.5,6.4 was performed using UV spectrophotometric. Characterization of cocrystal was done by FTIR, DSC, Powder XRD. A morphological study was done by trinocular microscopy and SEM. The prepared cocrystal of famotidine- citric acid showed remarkable improvement in dissolution and solubility.

Keywords: Molecular docking, Coformers, Famotidine,Cocrystal , Dissolution

抽象的：
药物的溶解度和溶出度直接影响药物的疗效。共结晶提供了一种有前途的方法来改善药物的溶解度和溶解度。药物共晶是由相同晶格中的 API 和可接受的共晶形成剂制成的多组分系统。共晶在制药行业中越来越受欢迎，因为它可以在不影响化合物内部活性的情况下扩大改善

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药物理化性质的机会。在这项研究中，法莫替丁是一种抗溃疡药，作为模型药物。由于其属于 BCS 类别 2 的低溶解度，它极微溶于水。这是一种非常有效的 Insilico 筛选方法，用于筛选共形成物和共晶形成。虚拟筛选技术，一种计算工具，提高了筛选共形成物的效率，以限制共形成物进行实验研究。使用 API 筛选了 GRAS 列表中的 7 名共形成者。结合亲和力和形成 H-键相互作用的可能性通过计算确定。 Autodock 用于对接。基于分子对接柠檬酸（Ei -2.6kcal/mol0 被选为最佳共形成剂。通过使用溶剂蒸发法的共结晶过程继续工作。在水中的溶解度和在不同pH值下的溶解度分析，如2.5、3.5、6.4采用紫外分光光度法进行共晶表征，通过FTIR、DSC、粉末XRD进行表征，通过三目显微镜和SEM进行形态学研究，制备的法莫替丁-柠檬酸共晶在溶解度和溶解度方面均有显着改善。

关键词：分子对接，共形成物，法莫替丁，共晶，溶解

Introduction:

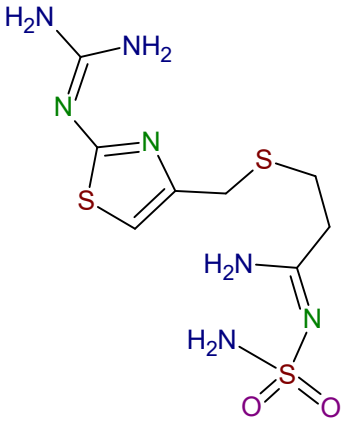
The poor aqueous solubility of the drug is becoming more prevalent in the R&D of most of the company. Currently, the major issue of the orally administered drug is their limited solubility in water. Approx. 40% of the new compound to reach the market have poor solubility. The solubility and dissolution of a drug are directly affecting the oral bioavailability profile of the drug. Numerous modifications have been developed to overcome the solubility and dissolution of the drug in an aqueous medium [1].

Pharmaceutical co-crystallization is an advanced method to improve the physicochemical properties of the drugs like solubility, dissolution, stability. The term co-crystallization refers to a multi-component system, consisting of two or more components in a single specific crystallization without interfering with their internal chemical structure [2]. In a cocrystallization system, the heterogeneous components interact via non-covalent interactions such as H-bonding, wander wall interaction, ionic interaction, and pi-interaction [3]. The resulting assembled structure has a new physical and chemical property from the parent molecule.

The co-crystal former plays a major role in the co-crystallization process. The selection of a co-former is a key challenge in cocrystallization. Various methods such as Hansen's solubility parameter, Cambridge structure database (CSD), hydrogen bond propensity, and Supramolecular synthon have been developed for the selection of co-former [4]. In recent years, molecular docking pay more attention to the screening of co-former with a compatible particular drug. It is a computational method that can easily screen the numerous co-formers at the same time [5].

Famotidine is widely used as an H2 receptor antagonist, commonly marketed under the Pepcid trade name. Molecular formula C8H15N7O2S3 and IUPAC name is (3-[({2-[Hydrazonomethyl) amino] thiazol-4yl} methyl) thio]-N-sulfamoyl-propionamide). It is pale yellow crystalline powder in nature. It is commonly used in the treatment of various kinds of peptic ulcer-like gastric and duodenal ulcers, Zollinger Allison syndrome, prophylaxis, gastritis, and gastrointestinal hemorrhage, and GERD [6]. Famotidine is given orally 20-40mg to decrease stomach acid production. It is also given for production against the pulmonary target acid during anesthesia. Due to extremely low solubility in water, it has unfavorable pharmacokinetic properties like low

bioavailability (40-45%) and short plasma of half-life (2.5-3.5hrs) It is more potent than ranitidine cimetidine in this class of drug [7]. A recent study has been reported that famotidine is given to the Covid-19 bed patient to improve their symptoms of Covid-19 SARs-COV2 [8].



Famotidine

Fig. 1. Molecular structure of Famotidine

1. Material and method:

1.1. Molecular Docking:

1.1.1. *Personal Computer*:

Dell laptop, Intel Core i3, window 10 operating system with DDR4 8 GB RAM

1.1.2. *Programmes*:

PyRx (Vina), Open babel 2.3.2/GUI, and Biovia discovery studio 2021 client version tool to analyzing the result.

Famotidine is provided by pharmacy institute, NIET, Greater Noida, U.P., and Citric acid (co-former) was supplied by CDH chemicals, New Delhi.

1.1.3. *Drug and coformers preparation*:

The 3d structure of famotidine and numerous co-formers were obtained from Pub Chem (http://pubchem.ncbi.nih.gov./) in the comfortable SDF format. These all SDF file formats are converted into PDB file format using the Babel tool (Open Babel 2.3.2/GUI). PDB files of drug and co-formers were opened in the virtual screening tool PyRx (Vina) and converted into a pdbqt format file. For docking studies minimized the energy of all structures they were used.

1.1.4. *Molecular docking study*:

Autodock Vina docking algorithm was used to analyze the binding confirmation between API and co-formers molecules. Lamarckian genetic algorithm method is applying to perform the calculation in docking [9]. Docking was repeated 5 times best confirmation pose was selected on the behalf of least binding energy. Types of interactions such as H-bond, π-bond interaction parameters, and binding affinity, Ei Kcal/mol, were observed. The interaction between drug and co-formers were analyzed by using the Biovia discovery studio 2021 client version.

1.2. *Preparation of Famotidine co-crystal*:

The famotidine co-crystal was prepared by a solvent evaporation method using methanol as solvent. Drug and co-former took in 1:1 molar ratio. Famotidine (338mg, 1 mol) dissolved in 30 ml methanol and continuous stirring at 40º C till a clear solution is obtained. The citric acid (193mg, 1 mol) was added and stirred for 25-30 min. The resulting solution was filtered with Whatman filter paper. The solution is covered with an aluminum foil by piercing 4-5 pinholes and allowed to slowly evaporate at room temperature for 2-3 days. The obtained co-crystals were stored in a desiccator for characterization by various analytical parameters [10].

2. Characterization of co-crystal:

2.1. FTIR:

FTIR spectra were obtained for the drug and prepared co-crystal. Spectra were recorded in the

range of 4000-400 cm^{-1} by perking Elmer diamond ATR FTIR spectrophotometer. All the spectra were analyzed by the KBr pellet method with $4cm^{-1}$ spectral resolution.

2.2. Microscopy morphology:

The morphology of the cocrystal was examined by trinocular microscopy (RXLr–3T) adding a camera to a computer monitor. Samples were sprinkled on the glass slide and placed under the microscope, examined at 60X resolution.

2.3. DSC:

The thermal behavior of pure famotidine, citric acid, and co-crystal were analyzed by Setaram labysis DSC 1700. 8-10 mg samples were placed in an alumina crucible and heated at room temperature at 200℃ at the rate of 10℃/min under nitrogen purge.

2.4. Powder XRD:

PXRD of famotidine citric acid and co-crystal were performed by a D8 Bruker X-ray powder diffractometer (Bruker, Madison, U.S.A). The samples were placed in an aluminum sample holder. Data were recorded at room temperature from 10-80º, 2θ range at a 10º/min. scanning rate.

2.5. SEM:

The surface morphological analysis of the pure drug and co-crystal were examined under the scanning electron microscope. (JEOL, JSMN.40A, TOKYO, JAPAN) at 1000x and 1400x resolution. The samples were sprinkled on copper stubs secured double side adhesive tape. The samples were analyzed using an SE detector at 10kV.

2.6. Solubility studies:

20 mg of pure famotidine and its equivalent weight of co-crystal were placed in Erlenmeyer containing aquadest. These were agitated in BOD shaker at room temp. for next 24 hrs. The solutions were filtered through a 0.45µm membrane filter [11]. The solubility of drug co-crystal was determined by UV spectrophotometrically at 288 nm.

2.7. In-vitro dissolution study:

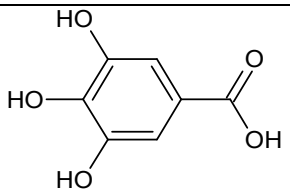
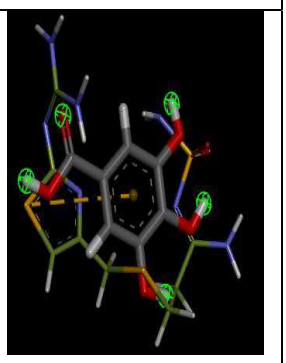
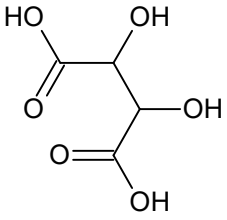
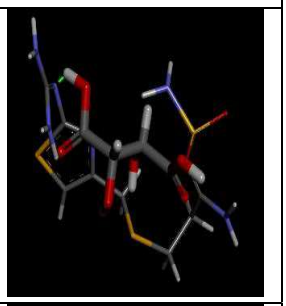
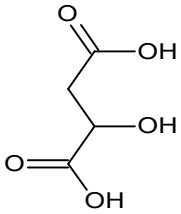
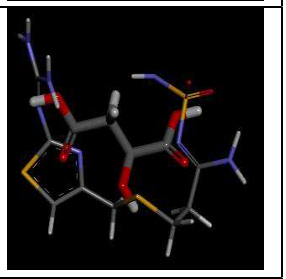
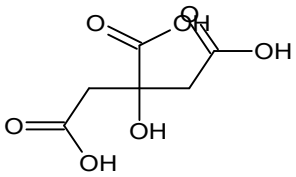
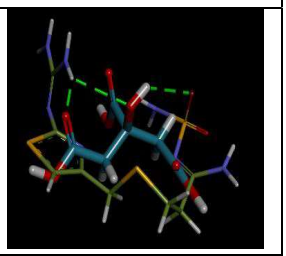
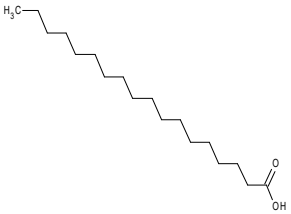
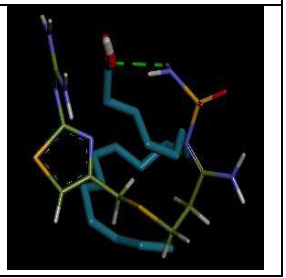
The dissolution test of pure famotidine and FMT-PABA cocrystal were performed using USP type II apparatus (paddle type) [12]. 900 ml of 0.1 N HCL solution with pH 2.5, 3.5, and 6.4 were used as dissolution medium. The temperature of the dissolution medium was set at $37\pm$ 0.5 ºC with 75 rpm paddle rotation speed. 5 ml samples were withdrawn at a predetermined time interval, immediately filter through a 0.45 µm membrane filter. 5 ml blank solution was added to the dissolution medium of replenishing. The samples were analyzed by UV spectrophotometric at 288 nm.

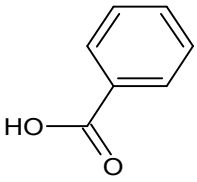
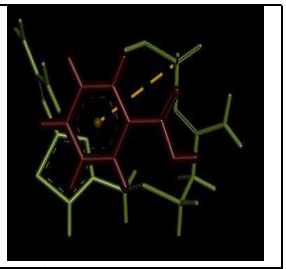
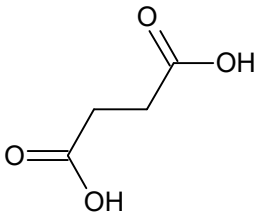
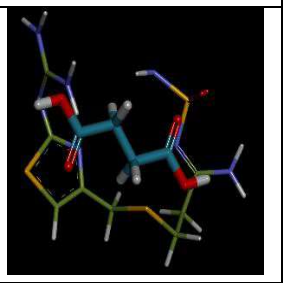
3. Result and Discussion:

3.1. Molecular Docking:

Famotidine is a member of 1, 3-thiazole, sulphonamide, and guanidine. The Chemical Structure of famotidine is shown in figure 1. The molecules of famotidine have 8 H- bond acceptor, 4 h- bond donor, and 7 rotational bond count, so it can easily interact with H-bond from cocrystal formers. Gallic acid, Tartaric acid, Malic acid, Citric acid, Stearic acid, Benzoic acid, and Succinic acid were selected for molecular docking with Famotidine. Binding affinity parameters and the number of H-bond were used for the selection of the best coformer. In molecular docking out of 7 coformers were screened with the drug (shown in table 1). Citric acid was proved to be the best coformers with the least binding energy -2.6 Ei (Kcal/mol). Citric acid was selected for the preparation of cocrystal and various analytical characterization such as FTIR, Powder XRD, DSC, and SEM.

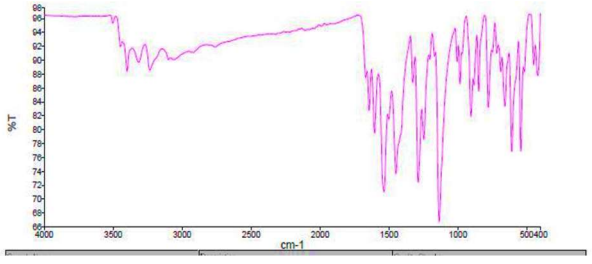
Table 1. Insilico screening of coformers with famotidine.

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	

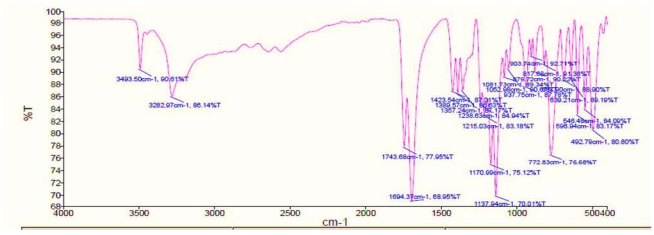
Benzoic acid		-2.0 π - bond	
Succinic acid		-1.9 no,any interaction	

3.2. FTIR:

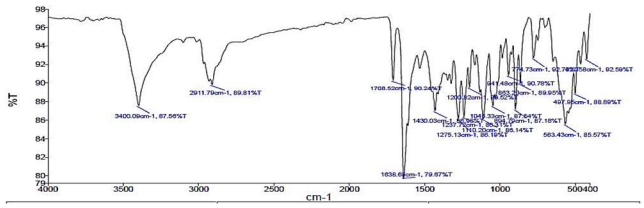
The vibrational change in FTIR is an important tool for confirmation of a cocrystal formation. The pure famotidine showed (–NH) stretching peaks at 3504, 3400, and 3236 cm^{-1}. Citric acid showed (– OH) stretching at 3493cm^{-1}, 3282cm^{-1} and (−COOH) stretching at 1743cm^{-1}, and 1694cm^{-1}. In the cocrystal (–OH) stretching shifted at 3400cm^{-1}, and (−COOH) stretching shifted at 1708 and 1638cm^{-1}. A new peak (C−H stretching) was detected at 2911.79cm^{-1}. (Spectra are shown in fig.2) The shifting of hydroxyl and carboxyl groups and detection of the new peak indicate the formation of a cocrystal.



(a)



(b)



(c)

Fig. 2. FTIR spectrum of (a) pure famotidine (b) citric acid (c) famotidine citric acid cocrystal

3.3. DSC:

The DSC thermogram of pure drug, co-crystal former, and prepared co-crystal is shown in fig. (3). The thermogram of pure famotidine showed a well-defined endothermic peak at 164℃. The Thermogram of citric acid showed an endothermic peak at 158℃, whereas the thermogram of FMT-citric acid prepared co-crystal is showed an endothermic peak at 165.6℃, which shows different endothermic peak from the drug as well as co-former that indicates the formation of a new co-crystal.

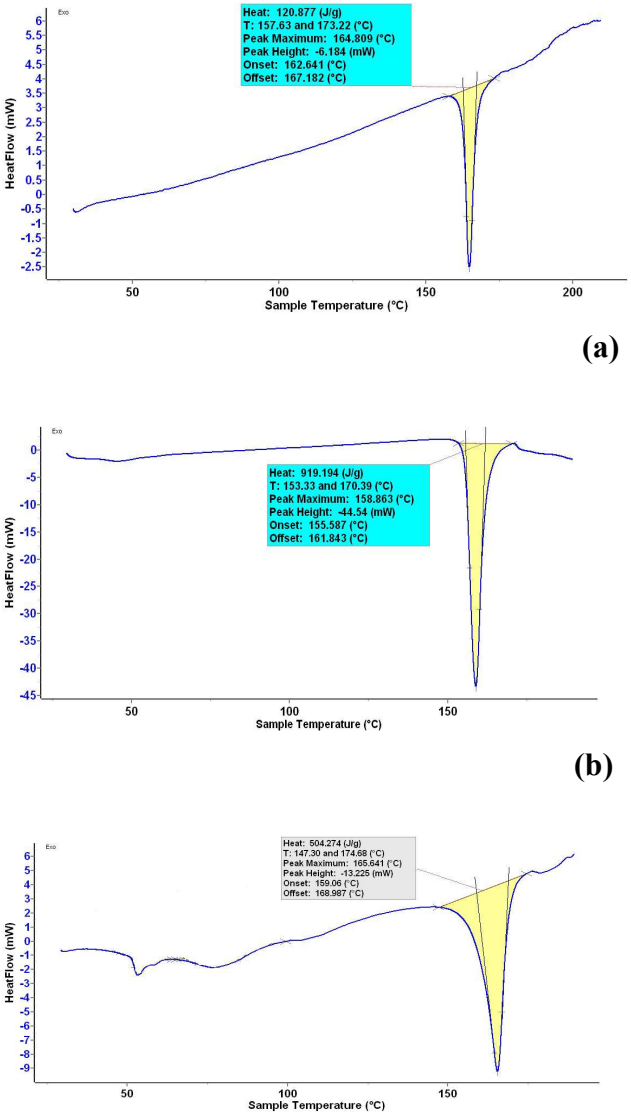


Fig.3. DSC thermogram of (a) famotidine (b) citric acid (c) famotidine -citric acid cocrystal

3.4. Powder XRD:

PXRD diffractogram is usually for justification of a new solid phase like co-crystal or amorphous. If the resultant diffractogram pattern is different from starting material such as drug and coformer, it can be concluded that a new solid phase or co-crystal formed. PXRD results showed different diffractograms of drug, co-former, and prepare co-crystals (shown in fig. 4). The characteristics diffractogram of famotidine shows at 2θ values 11.6, 15.1, 18.0, 19.2, 20.1, 10.9, 22.3, 23.2, and 24.1.The diffractogram of citric acid shows at 2θ values 18.5, 19.5, 37, 24, 33.4. But the diffractogram of co-crystal shows a new peak at 2θ values 14.9, 15.8, 21. The change in relative intensity of PXRD peaks of cocrystal as well as the appearance of new peaks indicates the formation of a new co-crystal.

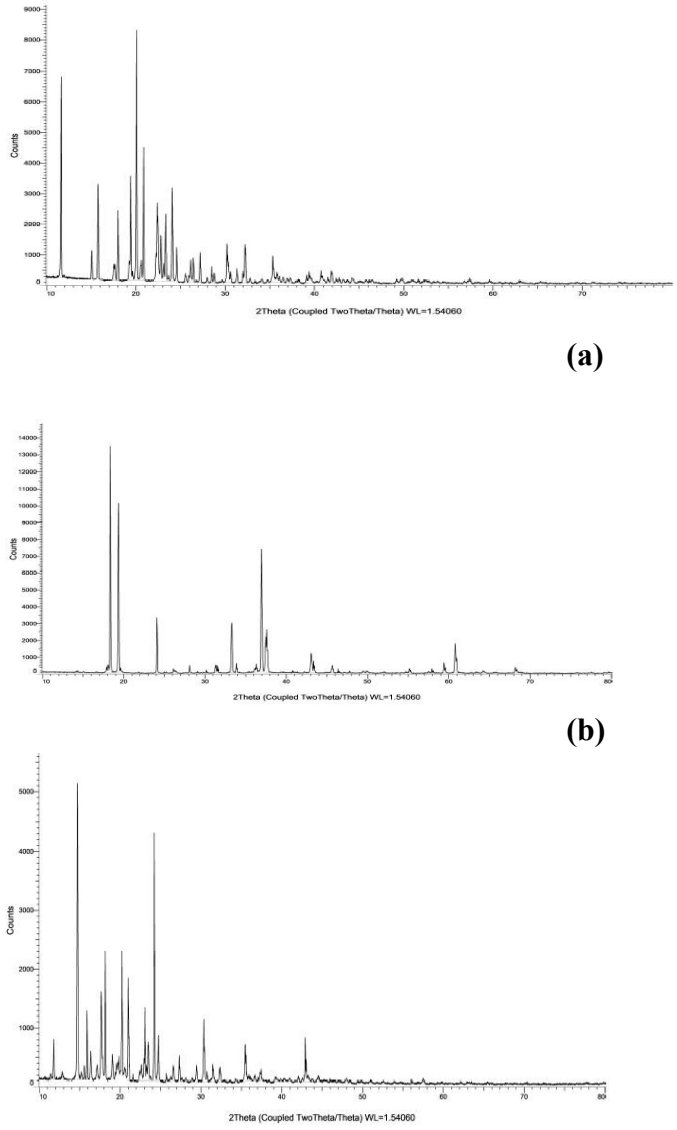
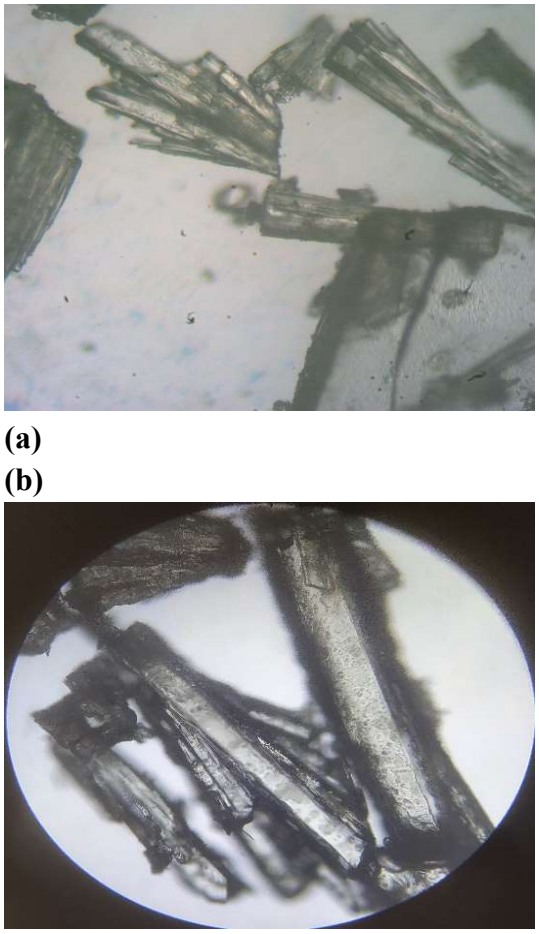


Fig.4. Powder XRD Diffractogram (a) famotidine (b) citric acid (c) famotidine - citric acid cocrystal

3.5. Trinocular Microscopy:

In microscopy morphology of cocrystal was found to be irregular and flattened in structure with well-defined morphology (shown in fig.5).



(a)

(b)

Fig. 5. Microscopic morphology of prepared cocrystal (a) and (b)

3.6. Solubility:

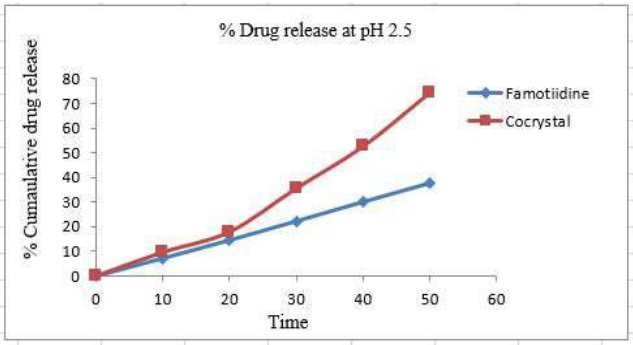
The solubility of pure famotidine was found to b 0.080±0.13mg/ml (80.1±0.13 μg/ml) an famotidine-citric acid prepared cocrystal wa 0.306±0.19mg/ml (306±0.19 μg/ml) in CO_2 fre distilled water (represent in table 2). All th calculations were performed as n=3(mean±SD). Th solubility of cocrystal was 3.8 times increased in a aqueous medium as compare to pure famotidine.

3.7. In-vitro dissolution:

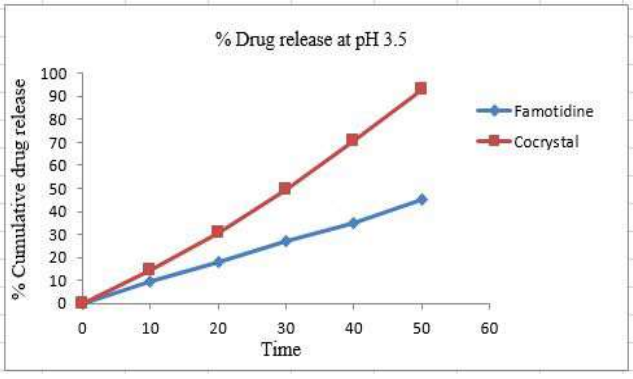
The Invitro dissolution test of pure famotidine and cocrystal were performed at different pH. The result of the dissolution test is shown in table 3 and the curve of cumulative % release and time profile represent in fig. (6). The pure famotidine showed drug release 37.5% ±0.08 at pH 2.5, 44.8% ±0.57 at pH 3.5, and 26.08% ±0.72, at pH 6.4, whereas the cocrystal showed 74.5% ±0.01 at pH 2.5, 92.75% ±0.01 at pH 3.5, and 61.7% 0.71 at pH 6.4. All the calculations were performed as n=3(mean±SD). The above results illustrate that the drug release increased 2 times at pH 2.5, 2.07 times at pH 3.5, and 2.3 times at pH 6.4 in cocrystal as compare to pure drug.

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

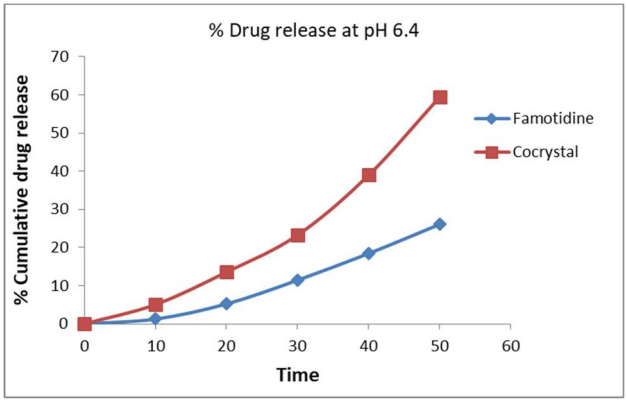
S.N.	pH	Pure famotidi	Cocrystal
1.	2.5	37.5% ±0.08	74.5% ±0.0
2.	3.5	44.8% ±0.57	92.75% ±0.
3.	6.4	26.08% ±0.72	61.7% ±0.7



n=3(mean±SD) **(a)**



n=3(mean±SD) **(b)**

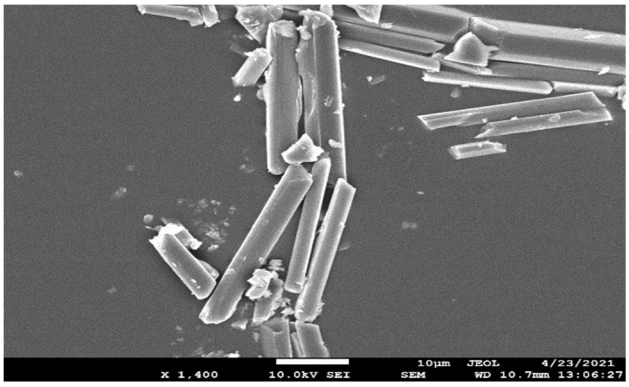


n=3(mean±SD) **(c)**

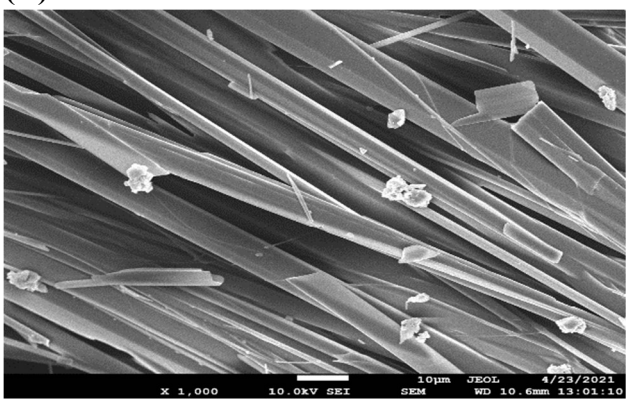
Fig. 6. Invitro dissolution test n=3(mean±SD) (a) at pH 2.5 (b) at pH 3.5 (c) at 6.4 pH

3.8. Scanning electron microscopy:

The surface morphology of the cocrystal examined smooth surface, linear structure with 10 µm in size (shown in fig. 7).



(A)



(B)

Fig.7. SEM image of Cocrystal (a) at 1400x and (b) at 1000x

Conclusion:

Insilico screening of coformers by Autodock using PyRx (Vina) is a very effective tool for screening numerous compounds at the same time. Citric acid was selected as a coformer for the preparation of cocrystal with the least binding energy Ei= -2.6 Kcal/mol. The cocrystal was prepared by a solvent evaporation method using methanol as solvent. The cocrystal was characterized by various analytical techniques such as FTIR, Powder XRD, DSC, and the morphological study was done by optical microscopy and SEM. The solubility results showed that the solubility of cocrystal 3.8 folds increased from pure famotidine in an aqueous medium at 24 hrs. The dissolution (% drug release) result of cocrystal also increased at different pH as compare to pure famotidine. These results confirm that the physicochemical properties such as solubility, dissolution, and bioavailability of famotidine can increase by cocrystallization technique.

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