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Research Paper

Enhancing In Vitro Dissolution of Ferulic Acid Through Co-Crystal Formation Using Malonic Acid and Nicotinamide Co-formers

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Abstract
Ferulic acid is categorized as a Biopharmaceutical Classification System (BCS) class II drug, which exhibits low solubility in water (0.91 mg/mL). The formation of a co-crystal using malonic acid and nicotinamide as co-formers by the microwave irradiation method is an approach to enhance its solubility and dissolution. This study aims to evaluate the effect of co-crystal formation using these two co-formers at a 1:1 molar ratio on the solubility and dissolution of ferulic acid. The result emphasizes the formation of new peaks and peak shifting compared to the pure materials characterized from the Fourier Transform Infrared (FT-IR) spectrum. Moreover, the Differential Scanning Calorimetry (DSC) thermogram exhibits the differences in the co-crystal melting point compared to the pure drug and co-former, indicating the alteration of molecular structure on the crystal lattice of ferulic acid caused by the strong interaction between supramolecular homomer and heteromeric synthon. The formation of a new crystalline phase is also observed from the X-ray Diffraction (XRD) diffractogram, suggesting the formation of a different phase from its co-crystal component. The morphology characterization using Scanning Electron Microscope (SEM) revealed that the ferulic acid crystal habit changes into different forms, which is acclaimed as a co-crystal formation. The results of this study also disclosed that the co-crystal formation of ferulic acid significantly enhances ferulic acid solubility and dissolution characteristics compared to the pure drug and physical mixture (p<0.05). The enhancement of solubility is 11.85% and 10.39% for ferulic acid-malonic acid and ferulic acid-nicotinamide co-crystal, respectively. Moreover, the dissolution rate of ferulic acid increases 3.50-fold and 3.61-fold from the formation of those co-crystals. Therefore, the formation of ferulic acid-malonic acid as well as ferulic acid-nicotinamide co-crystals in a 1:1 molar ratio by the microwave irradiation method is effective in improving ferulic acid solubility and dissolution.

Keywords
Ferulic Acid, Co-Crystal, Microwave Irradiation, Solubility, Dissolution Rate

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1. INTRODUCTION

Dissolution is a process that describes the solid solute entering a solution. In the pharmaceutical industry, it is defined as the amount of drug substance that passes into solution per unit of time under standard conditions of liquid or solid interface, temperature, and solvent composition. Drug dissolution test plays an essential role as a routine quality control test, to characterize product quality and drug development (Zhang, 2022). Dissolution is a pivotal aspect related to the bioavailability of solid dosage forms, especially for low-solubility drugs (Bhalani et al., 2022).

The poor bioavailability of drugs is primarily caused by the low solubility and dissolution of drugs, as well as their inability to penetrate the cell membrane. Drugs with low solubility will exhibit a poor absorption profile and bioavailability. Poor bioavailability will result in suboptimal therapeutic efficacy with high intersubject variability in plasma concentrations (Emami et al., 2019; Lennernäs et al., 2024). Drugs must have sufficient bioavailability to be clinically effective. Therefore, some approaches are needed to improve the solubility and dissolution rate of low-solubility drugs such as ferulic acid to increase their

bioavailability.

Ferulic acid is a phenolic compound that exhibits several physiological activities, including anti-inflammation, antimicrobial, antioxidant, antidiabetic, cardioprotective, anticancer, and hepatic cholesterol inhibition (Han et al., 2020; Zhai et al., 2023; Hernández-Jaime et al., 2025; Bekheit et al., 2025). However, ferulic acid possesses low solubility in water (Han et al., 2020). The solubility of ferulic acid in water is 0.91 mg/mL, or soluble in water with a 1:1099 ratio (Contardi et al., 2021). Most researchers classify ferulic acid as a BCS Class II drug, since it has low solubility. However, some report it as a BCS Class III drug, which correlates with its low permeability (Shukla et al., 2022). Generally, ferulic acid has low bioavailability and is difficult to penetrate lipid bilayer biological membranes (Han et al., 2020).

An approach to improve the solubility of ferulic acid is by forming co-crystals. According to the FDA, a co-crystal is a crystalline material consisting of two or more different molecules, usually an active pharmaceutical ingredient (API) and a co-former, in the same crystal lattice. Co-crystals are generally formed by more than two solid molecules that form a single crystal lattice, traditionally connected by noncovalent bonds, such as Van der Waals forces and hydrogen bonds. The crystal components interact with noncovalent bonds such as hydrogen bonds, Van der Waals forces, π-π interactions, and electrostatic interactions. Co-crystals can modify drug properties such as solubility, dissolution rate, stability, mechanical properties, permeability, and bioavailability of a drug without altering the intrinsic pharmacological activity of the molecule (Agostini et al., 2025). In addition, the crystalline form is preferred because it is stable, reproducible, and easy to purify compared to other solid forms, such as amorphous.

The reduction of crystal lattice energy and better hydrophilicity of the co-former are the main mechanism that induces solubility enhancement of the drug through co-crystal formation. The crystal lattice controls the solubility of API within the solvent via solvation resistance. Meanwhile, co-formers also contribute to enhancing co-crystal solubility by reducing the solvation hindrance, which predominantly impacts the dissolution rate of the co-crystal. Several considerations must be made in co-crystal formation, such as determining the co-former that employs a functional group for hydrogen bonding with the API (Lässer and Braun, 2025). The other consideration is designating a suitable and effective method for co-crystal formation (Sakhiya and Borkhataria, 2024). However, there are still limited studies that develop ferulic acid in co-crystal formation. Chaves Junior et al. prepared a co-crystal of ferulic acid-nicotinamide using the solvent evaporation method in a 1:1 ratio. The result revealed that the formation of a co-crystal increased the solubility of ferulic acid by approximately 70% and exhibited a better dissolution profile in dissolution medium pH 6.8 than pure ferulic acid (Chaves Júnior et al., 2020). Pujiono et al. (2025) also successfully developed a ferulic acid-nicotinamide co-crystal prepared by the solvent evaporation method. The intermolecular hydrogen bond was formed between the carbonyl (C=O) group of ferulic acid and the amine (N−H) group of nicotinamide as co-formers. The computational study also predicted that hydrogen bonds enhanced reactivity and interactions with solvents, including the dissolution medium. However, the development of ferulic acid co-crystals using different co-formers and preparation methods is required to emphasize the effectiveness of co-crystal formation for improving the solubility and dissolution profile of ferulic acid. Furthermore, the selection of the optimal co-former and preparation method can be conducted to further develop the ferulic acid co-crystal.

Malonic acid and nicotinamide are selected in this study as co-formers for co-crystal formation. Both co-formers are generally recognized as safe (GRAS) compounds and are safe to use as co-formers. Malonic acid has two carboxylic groups that can form hydrogen bonds with drug molecules, and this functional group can serve as a proton donor or acceptor. Malonic acid has been reported to increase the solubility of several drugs, such as caffeine, carbamazepine, glibenclamide, and ketoprofen (Verma et al., 2021; Wasim et al., 2021; Srivastava et al., 2022; Wicaksono et al., 2020). The preparation of glibenclamide-malonic acid co-crystal by the solution crystallization method was reported to enhance the solubility of glibenclamide by twofold and presented a significant enhancement of glibenclamide dissolved in the dissolution study. The pharmacokinetics parameter of glibenclamide co-crystal showed approximately 1.45-fold enhancement of AUC_{0-24} and 1.36-fold increment of the C-max compared to the pure drug (Srivastava et al., 2022). Meanwhile, nicotinamide is inert and has an amide group and a pyridine ring to form the intermolecular hydrogen bonds necessary for co-crystal formation (Wichianphong and Charoenchaitrakool, 2018). Nicotinamide has been reported to increase the solubility of simvastatin, 5-fluorouracil, and carbamazepine (Khan et al., 2020; Zhang et al., 2020; Ying et al., 2021). Nicotinamide was also employed in the previous study to prepare azilsartan co-crystal using a 1:2 molar ratio. The solubility of azilsartan increased 3.39 times compared to the pure drug, and the cumulative dissolution increased from 10% for the pure drug to 90% for the co-crystal in the pH 6.8 phosphate buffer medium. The formation of a co-crystal also successfully improved the bioavailability of azilsartan, resulting in approximately 3.5 times higher bioavailability than pure azilsartan (Xiao et al., 2022). Moreover, the results of the co-formers virtual screening using the Gaussian program revealed that malonic acid and nicotinamide were two co-formers that presented the lowest Gibbs free energy with ferulic acid. The more negative value of the Gibbs free energy indicated a higher substance polarity and electronegativity, then this substance was easier to bind with water molecules. Thus, the consideration to select these two co-formers was established due to the virtual screening of Gibbs free energy and its superior performance in the previous study to improve drug solubility and dissolution. These two co-formers also showed a difference of pKa value not more than 2, hence it can be predicted that co-crystal formation will occur, not the salt formation (Xie et al., 2022). The

formation of ferulic acid co-crystal using acidic (malonic acid) and basic (nicotinamide) as co-formers also required evaluating the effect of different co-former characteristics on the physicochemical properties of the co-crystal.

The microwave irradiation method was employed in this study to prepare a ferulic acid co-crystal with malonic acid and nicotinamide, using a 1:1 molar ratio. Microwaves are currently widely used in chemical and industrial research. Generally, microwaves work by attracting rotational energy using wavelengths on the micrometer scale (Sulistyowaty et al., 2024). Microwave energy increases molecular mobility by exciting molecular rotation through the interaction between microwave radiation and molecular dipole rotation. This method accelerates co-crystal formation due to the molecular rearrangement caused by microwave radiation, resulting in a greater number of co-crystals. Microwave irradiation is considered a green chemistry method, which emphasizes minimizing the use of environmentally hazardous materials. Therefore, this method is more environmentally friendly and sustainable due to the minimal use of solvents (Solares-Briones et al., 2021). Other advantages of this method include higher yields due to the minimization of by-products, high synthesis rates, a narrow particle size distribution, high purity, and improved physicochemical properties (Kumar et al., 2020).

There have been limited studies to develop ferulic acid co-crystals, especially those that employ the microwave irradiation method. The novelty of this work is the application of an environmentally friendly method, microwave irradiation, for preparing a ferulic acid co-crystal. Moreover, this study also revealed the comparison of malonic acid and nicotinamide as co-formers on the physical and chemical properties of ferulic acid co-crystal that formed with a similar molar ratio (1:1). The prepared co-crystals will also be compared for their solubility and dissolution rates with pure ferulic acid and their physical mixture. From these results, the effect of each co-former on increasing the solubility and the dissolution rate will be emphasized.

2. EXPERIMENTAL SECTION

2.1 Materials

Materials used in this study consist of ferulic acid (trans-ferulic acid) (Sigma-Aldrich®, USA, Lot number: BCCF2460), malonic acid (Merck Schuchardt® OHG, Germany, Lot number: S7006387 529), and nicotinamide (Sigma-Aldrich®, USA, Lot number: SLCF7687). Ethanol 96% (pro analysis), sodium hydroxide (NaOH), hydrochloric acid (HCl) 37%, $NaH_2PO_4.H_2O$, and $Na_2HPO_4.2H_2O$ were obtained from Merck®, Germany, and distilled water.

2.2 Instruments

The instruments utilized in this study include the analytical balance (Mettler Toledo AL 204), microwave equipped with output power micro 900 watt and 25 liter capacity (Sharp R-728(W)-IN), FT-IR ATR spectrophotometer (Perkin Elmer®), Differential Scanning Calorimeter (Mettler Toledo® DSC 3), Powder X-ray Diffractometer (Rigaku® type Miniflex 600C), Scanning Electron Microscope (Thermoscientific Phenom Pro X) , and dissolution tester (Erweka® DT 700).

2.3 Methods

2.3.1 Preparation of Ferulic Acid-Malonic Acid and Ferulic Acid-Nicotinamide Physical Mixture

A physical mixture was prepared by sieving ferulic acid, malonic acid, and nicotinamide using a sieve (Retsch Type ASTM, USA) with an 80-mesh screen. Physical mixture of ferulic acid and each co-former (malonic acid and nicotinamide) was designed at a 1:1 ratio. The amount of each physical mixture prepared in this study was 1 gram. Hence, for the ferulic acid-malonic acid physical mixture, the amounts of ferulic acid and malonic acid were 651.08 mg and 348.92 mg, respectively. Whereas, for preparing the physical mixture of ferulic acid and nicotinamide, ferulic acid and nicotinamide were weighed at 613.92 mg and 386.08 mg, respectively. Each component was weighed using a Mettler Toledo AL 204 analytical balance (Ervasti et al., 2015).

2.3.2 Preparation of Ferulic Acid-Malonic Acid and Ferulic Acid-Nicotinamide Co-Crystal

The preparation of a ferulic acid co-crystal was employed using two different co-formers, namely malonic acid and nicotinamide, both in a 1:1 ratio. To prepare 1 gram of ferulic acid-malonic acid co-crystal, ferulic acid and malonic acid were weighed at 651.09 mg and 348.91 mg, respectively. Meanwhile, ferulic acid and nicotinamide were weighed at 613.91 mg and 386.09 mg, respectively, for preparing the ferulic acid-nicotinamide co-crystal. The preparation of ferulic acid co-crystal using malonic acid and nicotinamide as co-formers was conducted by slight modification from the method established by Sulistyowati et al. The modification was performed for the power and time exposure to microwave radiation. Initially, the physical mixture of ferulic acid with each co-former was prepared by mixing those components in an evaporating dish using a stirring rod, by the addition of distilled water (10% w/w). This mixture was then transferred into a microwave instrument (Sharp R-728(W)-IN) under the influence of 450 W microwave energy for 20 minutes (Sulistyowaty et al., 2024). The power and time exposure of microwave radiation were set from the results of a preliminary study, since these power and time exposures did not influence the alteration of powder color and the melting process.

2.3.3 Ferulic Acid Co-Crystal Characterization

Characterization of ferulic acid-malonic acid and ferulic acid-nicotinamide co-crystal was employed using Attenuated Total Reflectance-Fourier Transform Infrared (ATR FT-IR) (Perkin Elmer®), thermal characterization using Differential Scanning Calorimeter (DSC) (Mettler Toledo® DSC 3), morphology characterization using Scanning Electron Microscope (Phenom ProX, Thermo Fischer Scientific®), and X-ray diffractometer (XRD) (Rigaku® type Miniflex 600C).

2.3.3.1 Fourier-Transform Infrared (FT-IR) Spectroscopy Characterization

FT-IR characterization of the ferulic acid, co-former, physical mixture, and co-crystal was conducted by applying the powder sample on the Attenuated Total Reflectance (ATR) crystal until the entire surface was covered by the sample. Afterward, the sample was closed by applying pressure to the sample compartment. The infrared (IR) spectrum of the sample was then recorded at 400 to 4000 cm^{-1} wavenumber (Setyawan et al., 2018a).

2.3.3.2 Differential Scanning Calorimetry Characterization

Differential scanning calorimetry (DSC) analysis was performed to determine the melting point and thermal characteristics of ferulic acid, malonic acid, nicotinamide, physical mixture, and co-crystal. The analysis was carried out using a DSC (Mettler Toledo®) instrument, which has been calibrated for temperature and heat flow performance by indium. The sample was weighed at 2 mg and placed onto an air-tight aluminum pan. The measurement then took place at a 50°-300°C temperature range, and the heating rate was 10°C/minute (Setyawan et al., 2018b; Abdullah et al., 2022).

2.3.3.3 X-ray Diffractometry (XRD) Characterization

XRD analysis was carried out using an X-ray diffractometer (Rigaku® type Miniflex 600C) by setting the voltage and electric current at 30 kV and 30 mA. The powder sample of ferulic acid, malonic acid, nicotinamide, physical mixture, and co-crystal was placed in the sample container, then simultaneously scanned with an angle from 2θ position 5°-50° and scanning rate 10°/minute (Ikeda et al., 2020).

2.3.3.4 Scanning Electron Microscopy (SEM) Characterization

SEM characterization was performed to examine the morphology and particle size of ferulic acid, co-formers, and the prepared co-crystal of ferulic acid (Han et al., 2020). The powder samples were placed on the carbon tube adhesive, and then the gold powder was coated on the surface of the powder. The prepared samples were analyzed using the Thermo Scientific® Phenom ProX Scanning Electron Microscope at 15 kV accelerating voltage and 5000× magnification (Xiao et al., 2022).

2.3.4 Determination of Ferulic Acid Content in Physical Mixtures and Co-Crystals of Ferulic Acid-Malonic Acid and Ferulic Acid-Nicotinamide

The determination of ferulic acid content in the physical mixtures and co-crystals was performed using a spectroscopic method for flavonoid content according to Sulastri et al, with slight modification (Sulastri et al., 2018). The physical mixture and co-crystal of ferulic acid-malonic acid and ferulic acid-nicotinamide were weighed with an equivalent amount of 25.0 mg. The powder was then dissolved with 10.0 mL of ethanol 96% (pro analysis) in a 25.0 mL volumetric flask, and afterward, ultrasonicated until the powder completely dissolved. Ethanol 96% was then added until the mark, and the solution with a 1000 mg/l concentration was obtained. The absorbance measurement of this solution was carried out using a UV-Vis spectrophotometer (HP 8452 A) at the maximum wavelength of ferulic acid and replicated three times. The ferulic acid content in the physical mixture and co-crystal preparation was determined by extrapolating the absorbance into the standard regression equation (Tian et al., 2021).

2.3.5 Solubility Study

The solubility study was conducted by weighing a sample consisting of 25.0 mg of ferulic acid, a physical mixture, and a co-crystal, which is equivalent to 25.0 mg of ferulic acid, using an analytical balance. The phosphate buffer pH 6.8 was used as a solubility medium, with a 25.0 mL volume for each sample. The sample was placed in a solubility vessel, then phosphate buffer medium was added and stirred using a magnetic stirrer at 600 rpm speed, and the temperature was maintained constant at 25 ± 0.5°C. Afterwards, 2.0 mL of the sample solution was taken after 6 hours. The sample solution was then filtered using a 0.45 μm filter membrane embedded in a filter holder. The absorbance of the solution was analysed by a UV-Vis spectrophotometer (HP 8452 A) at the maximum wavelength. The solubility determination for each sample was performed in triplicate (Setyawan et al., 2018a).

2.3.6 In Vitro Dissolution Study

Approximately 50 mg of ferulic acid, then the physical mixture and co-crystal of ferulic acid equivalent to 50 mg of ferulic acid were weighed for the in vitro dissolution sample test. The amount of physical mixture and co-crystal of ferulic acid-malonic acid was 76.80 mg, whereas for the physical mixture and co-crystal of ferulic acid-nicotinamide was 81.44 mg. All the samples were weighed using an analytical balance. The dissolution medium was 900 mL of phosphate buffer (pH 6.8) in each vessel. Type II apparatus (paddle) was utilized in the dissolution instrument (Erweka DT 700, Germany) and configured at a 75 rpm stirring rate. The temperature of the medium was maintained constant at 37 ± 0.5°C. The 5.0 mL of samples were taken at 0, 5, 10, 15, 30, 45, and 60-minute intervals. The dissolution medium was subsequently added to the dissolution medium with the same volume to maintain the medium volume at 900 mL. Then, the sample was filtered using a 0.45 μm filter paper equipped in a filter holder. 500 μL of the filtered sample was then pipetted and diluted with phosphate buffer pH 6.8 in a 5.0 mL volumetric flask. For the next step, the absorbance of the sample was analyzed using a UV-Vis spectrophotometer (HP 8452 A) at the maximum wavelength to quantify the amount of ferulic acid dissolved for each time interval (Setyawan et al., 2018b).

3. RESULTS AND DISCUSSION

3.1 Preparation of Ferulic Acid Physical Mixture and Co-Crystal Using Nicotinamide and Malonic Acid as Co-Former

The qualitative analysis of all the materials was conducted through organoleptic, melting point determination, and FT-IR spectrum observations. The results indicated that ferulic acid, malonic acid, and nicotinamide exhibited organoleptic properties, melting point, and FT-IR absorption bands similar to the characteristics of the reference material. These results confirmed the purity of all the starting materials and suitability for co-crystal preparation. The preparation of ferulic acid physical mixture was conducted by simply mixing the ferulic acid and each co-former using a spatula. Hence, the preparation of the co-crystal was performed by the addition of 10% b/b water to produce a slurry, then the slurry was exposed to the microwave radiation that was set at 450 W for 20 minutes. The co-crystal of ferulic acid prepared by these two co-formers exhibited a white color, and there was no alteration of the powder color after microwave radiation or melting tendency. As observed in the co-crystal, the white powder was also depicted by the physical mixture. The yield of co-crystal preparation was 97.95% for ferulic acid-malonic acid co-crystal and 97.75% for ferulic acid-nicotinamide co-crystal. The results indicated the high yield of co-crystal formation and an efficient process for co-crystal preparation through the microwave irradiation method (Vemuri and Lankalapalli, 2021).

3.2 Fourier-Transform Infrared (FT-IR) Spectroscopy Characterization

FT-IR spectroscopy is a characterization technique that provides information regarding co-crystal formation mechanisms. FT-IR characterization is essential for determining the conformation of co-crystal structures. In the confirmation of co-crystal structures, the formation of the hydrogen bond is regularly observed, represented by a band shift to lower wavenumber regions (Chaves Júnior et al., 2020). FT-IR can also distinguish co-crystals from salts when carboxylic acids play a part in hydrogen bond formation. The nature of the hydrogen bond itself will affect the vibrational frequency and help visualize which functional groups are involved in the formation of supramolecular synthons (Chadha et al., 2017a). The type of chemical bond present in a sample can be identified by observing the absorption spectrum at infrared radiation wavelengths that cause vibrational transitions of certain functional groups. This can be observed from variations in peak shape and intensity of the absorption spectrum (Garbacz and Wesolowski, 2018). Co-crystal formation influences the shift peaks, decreases peak intensity, reduces peak size, and produces new peaks in the FTIR spectrum (Setyawan et al., 2018a).

FT-IR analysis provides specific information regarding the spectrum shifting constructed due to the interaction of a multicomponent system consisting of ferulic acid-malonic acid and ferulic acid-nicotinamide. The bonding that occurs between these components can exhibit stretching and bending phenomena, which affect the physicochemical characteristics of substances, primarily the solubility. The FT-IR spectra of ferulic acid, co-former, physical mixtures, and co-crystals are presented in Figure 1.

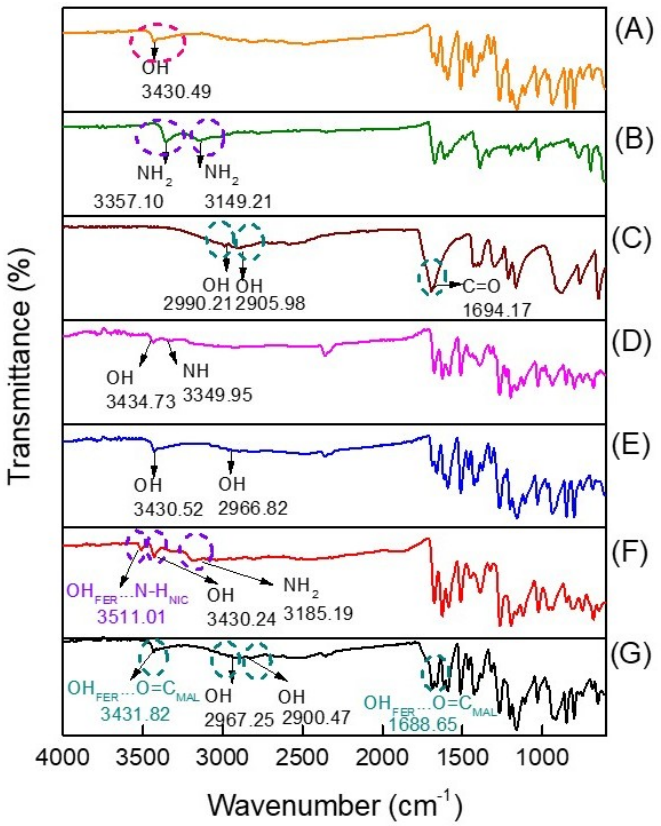

Figure 1. FT-IR Spectrum of (A) Ferulic Acid, (B) Nicotinamide, (C) Malonic Acid, (D) Physical Mixture Ferulic Acid-Nicotinamide, (E) Physical Mixture Ferulic Acid -Malonic Acid, (F) Co-Crystal Ferulic Acid -Nicotinamide, (G) Co-Crystal Ferulic Acid-Malonic Acid

FT-IR spectrum of ferulic acid–nicotinamide co-crystal exhibited specific peaks indicating C−H aromatic (679.68, 748.68, 813.27, and 847.81 cm^{-1}), C−O ether (1023.85 and 1263.55 cm^{-1}), C−N aromatic (1194.59, 1325.41, and 1389.18 cm^{-1}), C=C aromatic (1510.23 and 1586.35 cm^{-1}), C=O amide (1675.61 cm^{-1}), primary N−H amide (3185.19 and 3312.33 cm^{-1}), and O−H carboxylic acid (3188.31, 3430.24, and 3511.01 cm^{-1}). These peaks showed significant shifts compared to the constituent components, ferulic acid and nicotinamide. This shifting implied that these functional groups contributed to the formation of ferulic acid–nicotinamide co-crystal (Wicaksono et al., 2017). The possible interactions predicted from the FT-IR spectrum were hydrogen bonds and π bonds between ferulic acid and nicotinamide. Hydrogen bonds were observed as a heterosynthon within co-crystal formation. The undefined band at 3511.01 cm^{-1} in the co-crystal may be involved in the formation of new hydrogen bonds, but

the supramolecular arrangement or structure of the co-crystal was undetermined due to the presence of several functional groups in ferulic acid and nicotinamide. Thus, various possible hydrogen bonds in the co-crystal were implied (Chaves Júnior et al., 2020). The FT-IR spectrum of the physical mixture and co-crystal of ferulic acid–nicotinamide was different from their constituent materials. This indicated that non-covalent interactions (hydrogen bonds and π bonds) are formed intermolecularly between ferulic acid and nicotinamide molecules.

The formation of a co-crystal was also observed from the ferulic acid-malonic acid co-crystal spectrum. The FT-IR spectrum displayed a significant shift of the −OH functional group, indicating that the -OH carboxylic acid functional group of ferulic acid and malonic acid contributed to the construction of the co-crystal. The interaction between ferulic acid and malonic acid was predicted as a hydrogen bond between the −OH functional group of ferulic acid and malonic acid. Hence, the hydrogen bond observed between these molecules was an acid-acid homosynthon between the carboxylic acid group in ferulic acid and the carboxylic acid group in malonic acid (Shimpi et al., 2018). The FT-IR spectrum of the physical mixture and co-crystal of ferulic acid-malonic acid was different from the pure drug and co-former, suggesting the occurrence of a non-covalent interaction, presumably an intermolecular hydrogen bond between ferulic acid and malonic acid.

3.3 Differential Scanning Calorimetry (DSC) Characterization

DSC characterization can determine several phases, such as the melting process, crystallization, and thermal transition. This method can also be employed to determine the purity of the crystalline phase (Liu et al., 2016). The formation of a co-crystal is indicated by the melting point of the phase, which differs from that of the initial component due to alterations in the crystal lattice and conformation (Wicaksono et al., 2019). The difference in melting point is caused by alterations in lattice energy and crystal conformation. Moreover, the co-crystallization process can enhance or change the thermal properties of a substance (Liu et al., 2016). The DSC characterization in this study was performed in the temperature range between 50°C to 300°C with a heating rate controlled at 10°C/minute. The DSC thermogram of ferulic acid, co-former, physical mixture, and co-crystal is displayed in Figure 2.

DSC thermogram showed that the melting point of ferulic acid was 178.83°C. From this result, the purity of ferulic acid used in this study was confirmed, since the literature revealed that ferulic acid exhibited a sharp endothermic peak around 176°C (Sharif et al., 2018). The melting point of nicotinamide was observed at 133.6°C, in line with the previous literature, which confirmed its melting point ranges between 130-133°C (Moreschi et al., 2009). Meanwhile, the melting point of malonic acid was obtained at 141.66°C. This result was near to the melting point reported from the previous literature, herein 135-137°C. The DSC thermogram of malonic acid showed three endothermic peaks. The first endothermic

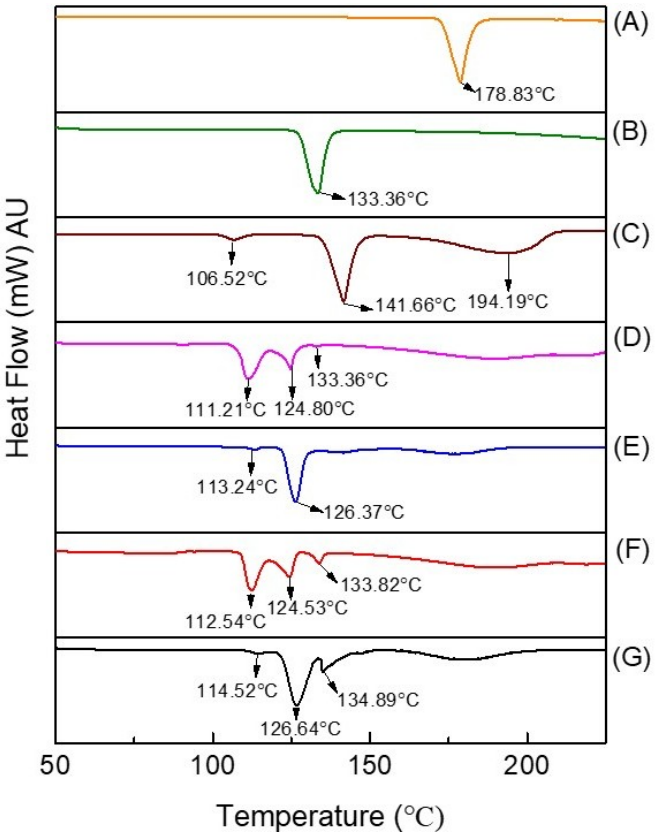

Figure 2. DSC Thermogram of (A) Ferulic Acid, (B) Nicotinamide, (C) Malonic Acid, (D) Physical Mixture Ferulic Acid-Nicotinamide, (E) Physical Mixture Ferulic Acid-Malonic Acid, (F) Co-Crystal Ferulic Acid-Nicotinamide, (G) Co-Crystal Ferulic Acid-Malonic Acid

peak was at 106.52°C, which is a solid phase transition, then 141.66°C is the melting point, and the third endothermic peak at around 194.19°C indicates that malonic acid is decomposed. This result referred to the research conducted by Shimpi et al. (2018) which also detected three endothermic peaks of malonic acid. Peak at 96.2°C indicating a solid phase transition, around 134.4°C indicating a melting point, and the third endothermic peak at 178.8°C indicating thermal decomposition. According to the literature, it was also noticed that malonic acid crystals undergo a solid-state phase transition from the β-form to the α-form, a melting stage, and a decomposition stage upon heating (Yu et al., 2018).

DSC thermogram of the physical mixture and co-crystal of ferulic acid-nicotinamide revealed that the melting point of these systems was lower than the constituent materials. The difference in co-crystal melting point indicated the alteration of molecular structure within the ferulic acid crystal lattice by the strong supramolecular homomer interaction with heteromer synthon (Chadha et al., 2017b). The alteration of the crystal lattice influenced the physicochemical characteristics of a substance; consequently, the solubility enhancement of

ferulic acid within the co-crystal will be accomplished (Sopyan et al., 2017). The thermogram of ferulic acid-nicotinamide co-crystal exhibited three endothermic peaks, presumably due to the formation of a new crystal phase, the occurrence of another hydrogen bond, and π-stacking interaction between ferulic acid and nicotinamide. The addition of water during the synthesis process of the co-crystal supported the probability of hydrogen bond formation between ferulic acid and nicotinamide (Sathisaran and Dalvi, 2021). Another new crystal lattice will also appear as an endothermic peak in the DSC results, resulting in more than one endothermic peak.

The other possibility that supports this result is the formation of a binary phase in the co-crystal. The DSC thermogram of ferulic acid-nicotinamide co-crystal was observed to be similar to the binary phase system with incongruent melting points, which have been reported previously (Yamashita et al., 2013). In this system, when a 1:1 molar ratio of ferulic acid and nicotinamide is heated, all components of ferulic acid and some part of nicotinamide melt at a metastable eutectic temperature, which is observed as an endothermic peak at 112.54°C. Subsequently, the co-crystal melt and nicotinamide recrystallize at an incongruent melting point or also known as the peritectic point, at 124.53°C. Afterwards, nicotinamide melts gradually at temperatures higher than the peritectic point, which was displayed as a third endothermic peak at 133.82°C (Yamashita et al., 2013). Moreover, the binary phase diagram can be interpreted as a co-crystal habit pattern (Sopyan et al., 2017). The formation of hydrates in the co-crystal structure is also probably causing this phenomenon, as reported in previous research. The endothermic behavior of ferulic acid-nicotinamide co-crystal explained the dehydration of the co-crystal at a temperature of 112.54°C, followed by the melting process of the co-crystal at 124.53°C. Subsequently, the decomposition of the co-crystal and the unreacted part of nicotinamide occurred at 133.82°C. This phenomenon was due to water molecules entrapped in the crystal lattice, resulting in an endothermic peak. However, this hypothesis must be further studied through TGA analysis to analyze the disappearance of the hydrate phase mass of the co-crystal during heating (Aitipamula and Das, 2020; Sathisaran and Dalvi, 2021).

Meanwhile, the physical mixture and co-crystal of ferulic acid-malonic acid thermogram indicated a lower melting point of these systems compared to the pure ferulic acid and malonic acid. The alteration of molecular structure within the crystal lattice due to substitution by strong supramolecular homomer interaction and heteromer synthon contributed to the change of the melting point. The difference in co-crystal melting point indicated the direct effect of co-formers in adjusting the character of the solid state, thus influencing the physicochemical properties (Chadha et al., 2017a). The lower melting point observed from the co-crystal, the higher solubility of ferulic acid will be observed (Sopyan et al., 2017). The emergence of a new single peak illustrates the formation of new crystals with high crystallinity and purity, but the ferulic acid-malonic acid co-crystal shows two endothermic peaks at 126.64°C and 134.89°C. The reasoning of this observation was probably due to the melting point of the other crystal system within the structure of ferulic acid and malonic acid, which presented as an endothermic peak at 134.89°C (Chadha et al., 2017b). The other hypothesis describes that a new crystal phase is constructed in the ferulic acid-malonic acid co-crystal. However, some phase of intact malonic acid was also present in the co-crystal system, generating two endothermic peaks. The very weak endothermic peaks observed at 113.24°C, 114.52°C, and 134.89°C are expected as monomeric malonic acid (Setyawan et al., 2018a).

3.4 X-ray Diffraction (XRD) Characterization

XRD is a versatile and fast analysis method for identifying the crystal phase of a substance (Shimpi et al., 2018). The XRD pattern of the crystalline sample has been employed to identify the fingerprint part of crystalline materials, because each materials produce a distinct characteristic XRD pattern (Wicaksono et al., 2017). All peaks in the XRD pattern are characterized due to the reflectance of a specific atomic plane; moreover, each alteration of these reflectances represents the variation of the crystal lattice. The formation of a co-crystal or a new crystal phase is identified when the XRD pattern of the sample differs from the constituent materials (Chadha et al., 2017a). The alteration in the XRD diffraction pattern, such as peaks disappearing, shifting, or the formation of new peaks, indicates the formation of a new crystal phase (Bhatia et al., 2021). The XRD diffractogram of ferulic acid, nicotinamide, malonic acid, physical mixture, and co-crystal is presented in Figure 3.

The XRD diffractogram of ferulic acid exhibited specific peaks at 2θ = 9.0500, 10.5100, 12.8400, 15.6499, 20.4799, 21.1599, 21.6399, 29.0499, 29.4899, and 29.9500. Nicotinamide exhibits specific peaks at 2θ = 11.3299, 14.8299, 19.6000, 19.9500, 27.3299, 28.4300, and 32.5299. Meanwhile, Malonic acid showed specific peaks at 2θ = 11.6700, 17.8500, 18.0200, 19.0699, 23.4300, 23.9400, 27.3099, and 33.3499. The diffractogram of both physical mixtures was a superposition of the two constituent materials, which have the same diffraction pattern but only differ in intensity (Setyawan et al., 2014). The co-crystal of ferulic acid-malonic acid exhibited specific peaks at 2θ = 18.8099, 23.7299, and 35.4199. These peaks were different compared to both constituents, ferulic acid and malonic acid. This result indicated the formation of a new crystalline phase (Chadha et al., 2017a). The identical pattern was also ascertained from the XRD diffractogram of ferulic acid-nicotinamide co-crystal. Specific peaks at 2θ = 11.0299, 13.9399, 14.3500, 15.2100, 16.5699, 16.9799, 18.9500, 19.3400, and 27.9099 were viewed at this co-crystal diffractogram, indicating a new crystalline phase formed due to the different character of those peaks in comparison to the constituent materials. The reduction of crystallinity arose due to the existence of a co-former in the crystal structure, which influenced the regularity of the co-crystal lattice (Wicaksono et al., 2019).

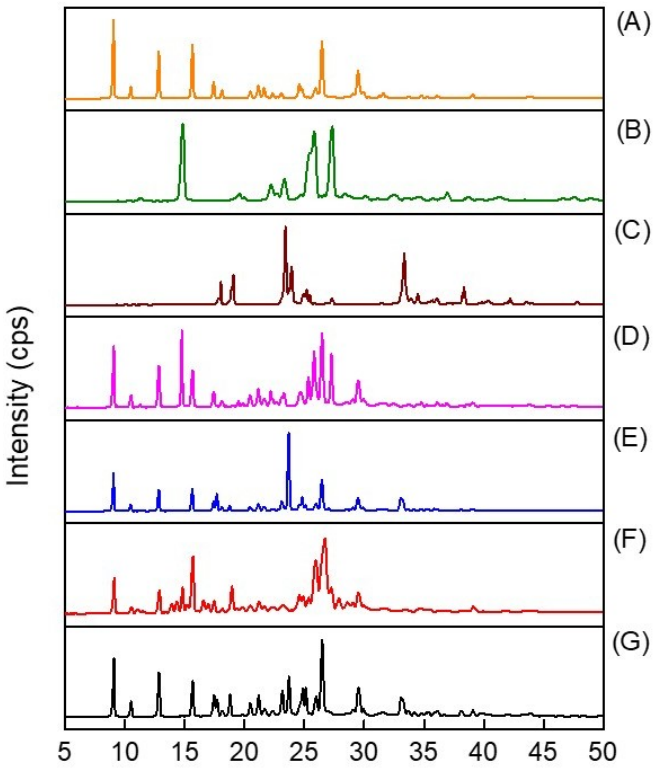

Figure 3. XRD Diffractogram of (A) Ferulic Acid, (B) Nicotinamide, (C) Malonic Acid, (D) Physical Mixture Ferulic Acid-Nicotinamide, (E) Physical Mixture Ferulic Acid-Malonic Acid, (F) Co-crystal Ferulic Acid-Nicotinamide, (G) Co-crystal Ferulic Acid-Malonic Acid

3.5 Scanning Electron Microscopy (SEM) Characterization

SEM is a characterization technique that is used to examine the surface morphology of particles until 1 nm. Not only the surface morphology, but also the structure and chemical composition of the substance can be provided by this method (Xiao et al., 2022). The SEM micrograph of ferulic acid, nicotinamide, malonic acid, and the co-crystals system is presented in Figure 4. Ferulic acid was presented mostly as rectangular and/or needle-shaped particles (Chaves Júnior et al., 2020). Nicotinamide was observed as rectangular particles that form aggregates. Nicotinamide was employed as a co-former because of its polarity, possibility of forming synthon bonds, and stability. The micrograph of a ferulic acid-nicotinamide co-crystal showed that the morphology of the co-crystal was different from each initial components. The micrograph also revealed that ferulic acid lost its crystal habit, thus implying the formation of a new crystalline phase (Wicaksono et al., 2019). Furthermore, malonic acid presented as a thin material or pebble characteristics (Setyawan et al., 2018a). The other literature stated that malonic acid is characterized as a round material, and the size was approximately 500 μm (Wicaksono et al., 2017). The SEM micrograph of ferulic acid-malonic acid co-crystal was consistent with the findings in ferulic acid-nicotinamide co-crystal. Ferulic acid was observed to have lost its crystal habit, indicating the formation of a new crystalline phase (cocrystal).

The SEM analysis of those two co-crystals indicated that the co-crystals produce a more compact structure and higher density. The hydrogen bond interaction between ferulic acid and co-former resulted in this structure reinforcement. The hydrogen bond plays an important role in the molecular interaction of co-crystals. Apart from the reduction of crystal lattice energy through co-crystal formation, the presence of the co-former also contributed to increasing the solubility and dissolution rate of ferulic acid. Co-former strongly influenced the affinity of the solvent toward the active ingredient, upon this system's contact with the dissolution medium (Sopyan et al., 2017). The analysis of particle size from SEM images using ImageJ exhibited the reduction of co-crystal particle size compared to the pure ferulic acid, as tabulated in Figure 4. Co-crystal of ferulic acid-nicotinamide presented a significant reduction of particle size and homogenous particle size distribution, implying the improvement of particle solubility and dissolution due to the higher surface area contacted with the aqueous medium (Huang et al., 2022). The improvement of kinetic solubility due to particle size reduction was also considered in enhancing ferulic acid dissolution, apart from the reduction of crystal lattice energy and hydrogen bonding formation.

3.6 Analysis of Ferulic Acid Content in Physical Mixture and Co-Crystal

The determination of ferulic acid content in the physical mixture and co-crystal was performed using a UV-Vis spectrophotometer. The absorbance of all samples was analyzed through the derivative method. The observation of absorbance was conducted at a wavelength (λ) of 262 nm and 270 nm for ferulic acid-nicotinamide and ferulic acid-malonic acid mixture, respectively. The results of ferulic acid content in either the physical mixture or the co-crystal are tabulated in Table 1. The co-crystal systems, as well as the physical mixture, exhibited adequate content of ferulic acid, ranging from 100.00±0.459 to 100.72 ± 0.367. The ferulic acid content in all samples was within the limit of recovery percentage as specified by the ICH, herein, 95%-105%. Moreover, the coefficient of variation value was <2%, showing the reproducibility of each replication procedure and fulfilling the requirement described by ICH (European Medicine Agency, 2022). From this result, a captivating homogeneity of the sample, both in physical mixture and co-crystal, was provided by the preparation procedure employed.

3.7 Solubility Study

The solubility study was conducted to predict the maximum amount of ferulic acid soluble in a phosphate buffer medium (pH 6.8) at 25 ± 0.5°C which was determined in the saturation state. The solubility of ferulic acid from the co-crystal using nicotinamide as co-former was 10.39% higher compared

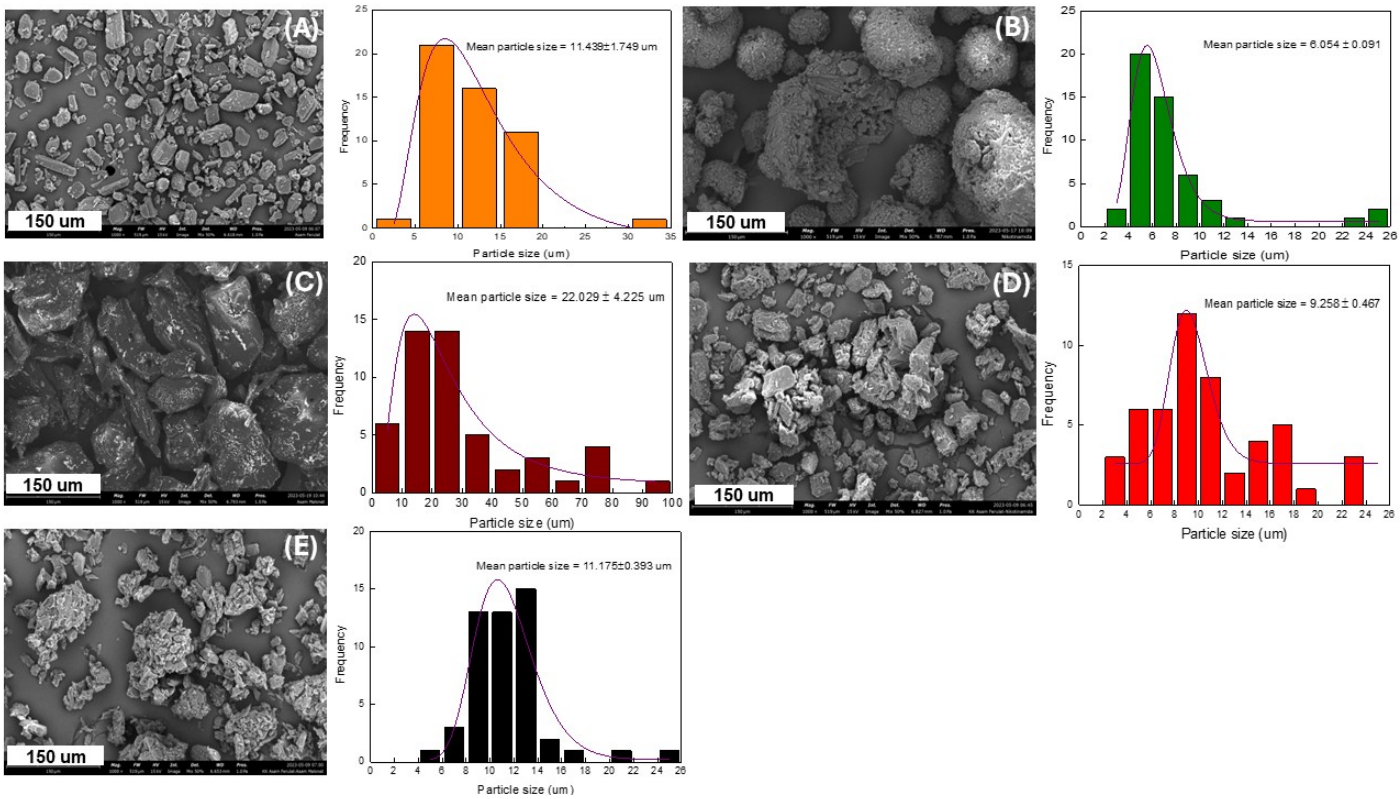

Figure 4. SEM Micrograph and Particle Size Distribution of (A) Ferulic Acid, (B) Nicotinamide, (C) Malonic Acid, (D) Co-Crystal of ferulic Acid-Nicotinamide, (E) Co-Crystal of Ferulic Acid-Malonic Acid. Magnification 1000×

Table 1. Ferulic Acid Content in The Physical Mixture and Co-Crystal of Ferulic Acid-Nicotinamide and Ferulic Acid-Malonic Acid

Sample	Ferulic Acid Content (% ± SD)	Coefficient Variation (CV) (%)
Physical mixture of ferulic acid–nicotinamide	100.00±0.459	0.459
Physical mixture of ferulic acid–malonic acid	100.72±0.367	0.364
Co-crystal ferulic acid–nicotinamide	100.42±0.482	0.480
Co-crystal ferulic acid–malonic acid	100.67±0.323	0.321

**Data was average from three replicates (n=3)*

to the pure ferulic acid. Meanwhile, the physical mixture of those components only showed a 0.86% increment in solubility. Compared to its physical mixture, the co-crystal systems performed an enhancement of solubility, approximately 9.45%. Therefore, the formation of ferulic acid-nicotinamide co-crystal significantly improved the solubility of ferulic acid compared to the pure drug and its physical mixture (p <0.05). The result of ferulic acid-malonic acid solubility also exhibited a similar profile. Co-crystal of ferulic acid-malonic acid enhanced the solubility of ferulic acid by about 11.85% and 9.44% respectively, compared to the pure drug and its physical mixture. Whereas, the physical mixture only increased ferulic acid solubility by 2.20%. The solubility results of the physical mixture and co-crystal of ferulic acid from this study are tabulated in Figure 5.

The mechanism of solubility enhancement through co-crystal formation is caused by the reduction of crystal lattice energy and greater hydrophilicity of the co-former. The crystal lattice controlled the solubility of ferulic acid in media through a solvation capacity scheme. Hence, co-formers such as nicotinamide and malonic acid increased the solubility of ferulic acid by reducing solvation hindrance and enhancing the dissolution rate of the co-crystal (Sopyan et al., 2019). The interaction between ferulic acid and co-former also contributed to the physicochemical properties of the co-crystal, including solubility. Non-covalent interactions such as hydrogen bond, π-π interaction, halogen interaction, and Van Der Waals force contributed as the possible interactions between the drug and co-former in the co-crystal conformation (Emami et al., 2019).

The interaction between ferulic acid and nicotinamide was the hydrogen bond and π-π interaction. The hydroxyl group of ferulic acid forms hydrogen bonds with the N-pyridine of

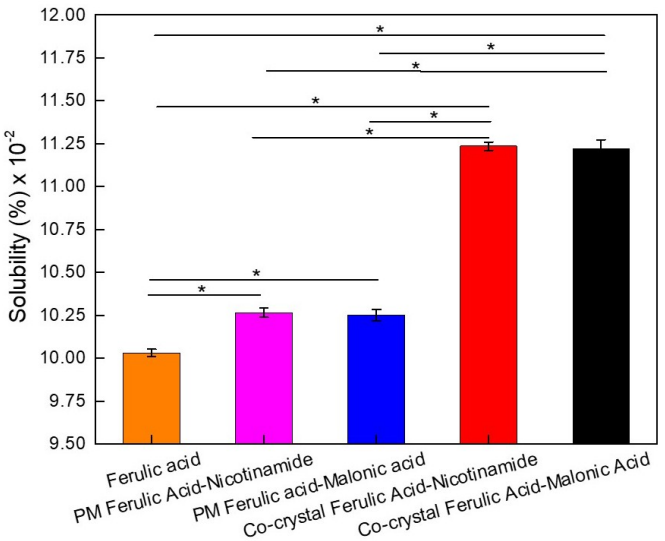

Figure 5. Solubility of Ferulic Acid, Physical Mixture (PM), and Co-Crystal of Ferulic Acid Using Nicotinamide and Malonic Acid Co-Former

nicotinamide (hydroxyl-pyridine heterosynthons). Furthermore, this interaction connects the hydrogen bond chains formed by nicotinamide molecules to produce a hydrogen-bonded sheet structure. The crystal structure is also supported by acid-amide heterosynthons between the carboxylic acid group of ferulic acid and the amide group of nicotinamide (Aitipamula and Das, 2020). The nicotinamide molecule, which is more soluble than the co-crystal, leaves an empty structure in the co-crystal. When the strong intermolecular interaction between ferulic acid and nicotinamide disappears, it results in lower co-crystal stability. Hence, the remaining ferulic acid structure with weaker intermolecular interactions will easily break. This phenomenon will certainly affect the dissolution and increase the solubility of ferulic acid (Zaini et al., 2020). The possible interactions between ferulic acid and nicotinamide were predicted by the Gaussian program, displayed in Figure 6. The hydrogen bond between the carbonyl (C=O) group of ferulic acid and the primary amine ($-NH_2$) group of nicotinamide, hydroxyl group (−OH) of ferulic acid and carbonyl (C=O) group of nicotinamide, hydroxyl group (−OH) of ferulic acid and tertiary amine (−N) of nicotinamide have been predicted as the main interaction between ferulic acid and nicotinamide. Moreover, nicotinamide also improved the solubility of ferulic acid through the stacking complex interaction, characterized as the interaction between π donor and π electron acceptor. The complex is constructed between the hydrophobic part of nicotinamide, herein aromatic ring, and ferulic acid. The stacked arrangement protects the hydrophobic part of ferulic acid from the aqueous medium, contributing to the significant solubility improvement (Sopyan et al., 2019).

The hydrogen interaction was also characterized as a possible interaction between ferulic acid and malonic acid. Malonic acid has two carboxylic acid groups, which can form hydrogen bonds with drug molecules. The possible interactions between these two compounds were predicted by the Gaussian program as presented in Figure 7. The hydrogen bond was observed from the predicted interaction between ferulic acid and malonic acid using the Gaussian program. The hydrogen bonds are constructed between the hydroxyl group (−OH) of malonic acid and the carbonyl group (C=O) of ferulic acid, the carbonyl group (C=O) of malonic acid and the hydroxyl (−OH) group of ferulic acid, and the hydroxyl group (−OH) of malonic acid and the methoxy ($-OCH_3$) group of ferulic acid. The weak interaction, such as a hydrogen bond, which binds the drug and co-former, will easily dissociate in a biological medium. Afterward, malonic acid, which is more soluble than the drug component, was pulled out from the crystal lattice. Ferulic acid, which is the hydrophobic component in the co-crystal system, was saturated with the aqueous medium. This structure was called a spring, a distinctive formation that immediately settles to form a loose aggregate. Therefore, some excipients are required to control nucleation and crystal formation, typically referred to as a nucleation control agent, or a "parachute." These mechanisms will inhibit the regular crystal growth due to the slow transformation process from amorphous structures to the stable crystal phase. This high-energy amorphous phase solution will transform into a metastable polymorph phase that produces higher solubility and consequently becomes a stable form (Bavishi and Borkhataria, 2016). The higher solubility and optimal concentration in aqueous medium represented the parachute effect (Sathisaran and Dalvi, 2018). However, the absence of an aromatic ring in the co-former, such as malonic acid, attenuated the interaction between the drug and co-former compared to the co-former that employed an aromatic ring (Dalpiaz et al., 2018).

The reduction of crystallinity in co-crystal formation due to the existence of a co-former reduced the crystal lattice energy and increased the solubility of ferulic acid, as presented in the XRD diffractogram of the co-crystal (Wicaksono et al., 2019). The enhancement of ferulic acid solubility was also influenced by alterations in solid-phase thermodynamic characteristics, particularly the melting point. The DSC thermogram of the ferulic acid co-crystal revealed that its melting point was lower than ferulic acid and the co-former. The lower melting point indicated that the crystal lattice energy of the co-crystal was reduced than the former constituents. The lower melting point produced by the co-crystal correlated with the higher solubility and dissolution (Zaini et al., 2020).

3.8 Dissolution Study

The dissolution study was emphasized by analyzing the dissolution profile, slope of the dissolution profile, and the percentage of ferulic acid dissolved in 60 minutes from all the samples, including ferulic acid, physical mixture, and co-crystal. The dissolution profile of all the samples is presented in Figure 8. The analysis of the slope of the dissolution profile was conducted between 0 and 15 minutes, due to the significant drug

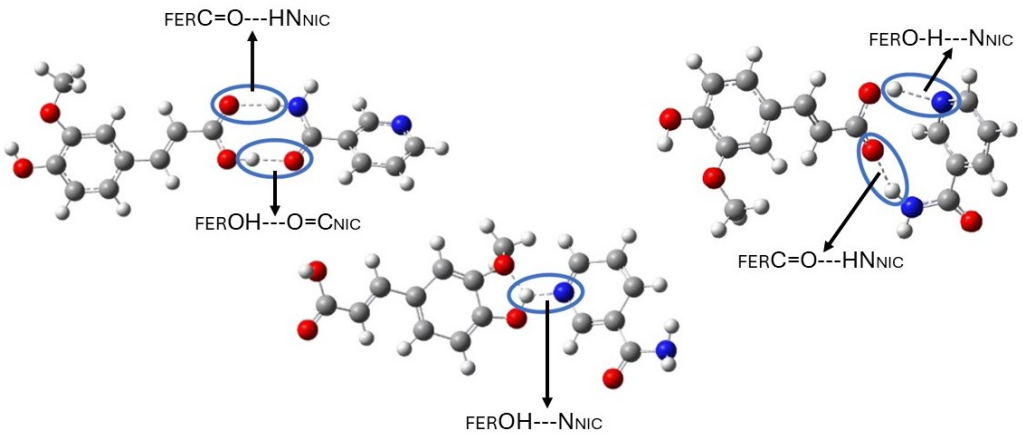

Figure 6. The Possible Interactions Between Ferulic Acid and Nicotinamide (FER=Ferulic Acid, NIC=Nicotinamide)

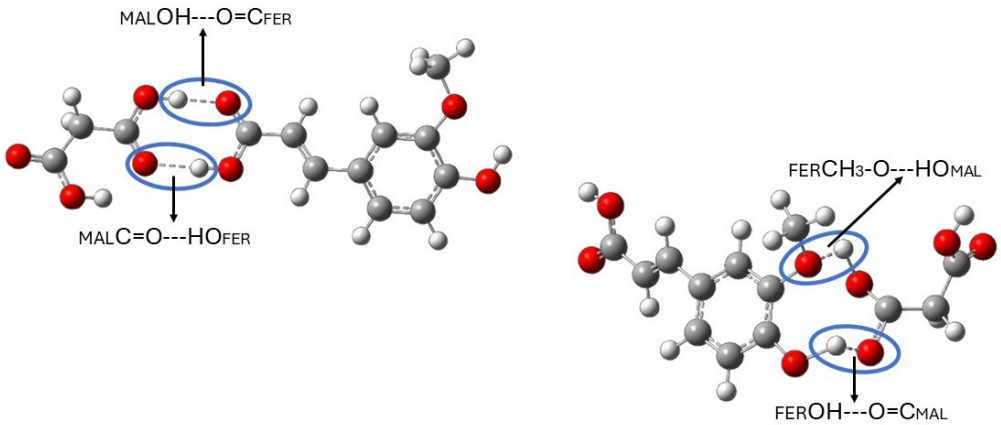

Figure 7. The Possible Interaction Between Ferulic Acid and Malonic Acid (FER=Ferulic Acid, MAL=Malonic Acid)

dissolved observed from this range. Hence, it can be predicted as an indicator to describe the difference in drug dissolution rate between the samples. The calculation is carried out using the Hixson-Crowell equation, and this equation can be used if the graph plot of the cubic root of the initial amount of substance minus the cubic root of the amount of undissolved substance (Y-axis) against time (X-axis) produces a straight line (Johnson et al., 2018). The results of the slope calculation are tabulated in Table 2. The results exhibited significantly different slopes between ferulic acid, physical mixture, and co-crystal for both co-formers observed from the dissolution profile (p<0.05). The higher slope value from the co-crystal dissolution profile indicated the higher dissolution rate of ferulic acid achieved by co-crystal formation.

The analysis of the drug dissolved percentage in dissolution medium also revealed the same pattern as the slope value. The percentage of ferulic acid dissolved in 60 minutes was 49.659± 0.192%. Meanwhile, the physical mixture showed an enhancement of ferulic acid dissolution by 1.85 and 1.98-fold compared to pure ferulic acid, for the physical mixture prepared using nicotinamide and malonic acid. The significant enhancement of ferulic acid dissolution was achieved by the co-crystal of ferulic acid, since the dissolution of ferulic acid increased by 3.61 and 3.50-fold for ferulic acid-nicotinamide and ferulic acid-malonic acid co-crystal, respectively. From these results, it can be emphasized that co-crystal formation of ferulic acid in this study significantly enhanced the dissolution of ferulic acid in aqueous medium compared to the pure drug and its physical mixture (p<0.05). The enhancement of ferulic acid dissolution efficiency presented in this study was higher than the previous study that prepared ferulic acid-nicotinamide using the solvent evaporation technique. The enhancement of dissolution efficiency was 1.15 in the previous study; meanwhile, the dissolution efficiency of ferulic acid-nicotinamide prepared in this study was 3.50-fold compared to the pure ferulic acid (Chaves Júnior et al., 2020). Nicotinamide and malonic acid also presented excellent properties to enhance the dissolution rate of felodipine, as these co-formers improved the dissolution rate by 2.5 and 2-fold compared to the pure drug (Chadha et al., 2017a). The comparison of dissolution

Table 2. Slope Value Calculated from The Dissolution Profiles

Sample	Slope
Ferulic acid	0.0307 ± 0.0003[a]
Physical mixture of ferulic acid-nicotinamide	0.0587 ± 0.0004[b]
Physical mixture of ferulic acid-malonic acid	0.0609 ± 0.0004[b]
Co-crystal ferulic acid-nicotinamide	0.1149 ± 0.0003[c]
Co-crystal ferulic acid-malonic acid	0.1076 ± 0.0012[c]

**Data was average from three replicates ± SD (n=3)*

Table 3. Comparison of Co-Crystal Dissolution Efficiency to the Pure Drug from Several Studies

Drug	Co-former	Enhancement of Dissolution Efficiency Compared to the Pure Drug	Reference
Ferulic acid	Nicotinamide	3.61	Current study
Ferulic acid	Malonic acid	3.50	Current study
Felodipine	Nicotinamide	2.50	(Chadha et al., 2017a)
Felodipine	Malonic acid	2.00	(Chadha et al., 2017b)
Tadalafil	Malonic acid	9.00	(Shimpi et al., 2018)
Zaltoprofen	Nicotinamide	3.44	(Panzade and Shendarkar, 2019)
Mefenamic acid	Nicotinamide	13.50	(Wichianphong and Charoenchaitrakool, 2018)
Quercetin	Malonic acid	1.28	(Setyawan et al., 2018a)
Glibenclamide	Malonic acid	1.81	(Srivastava et al., 2022)
Carbamazepine	Malonic acid	1.50	(Wasim et al., 2021)
Azilsartan	Nicotinamide	5.50	(Xiao et al., 2022)
Ferulic acid	Nicotinamide	1.15	(Chaves Júnior et al., 2020)

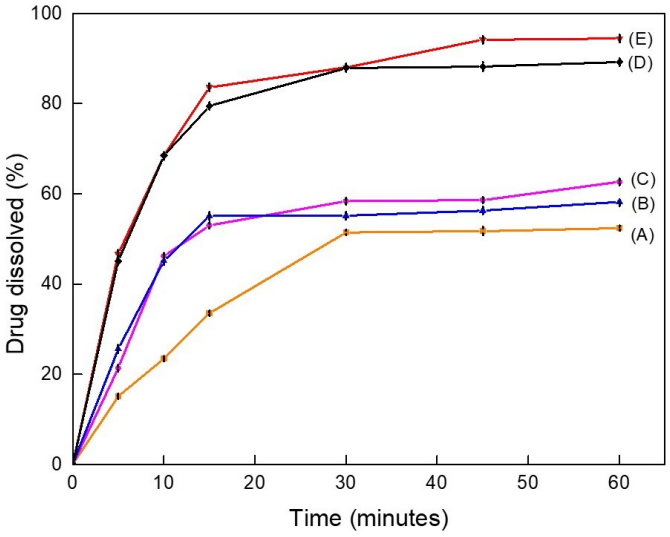

Figure 8. Dissolution Profile of (A) Ferulic Acid, (B) Physical Mixture of Ferulic Acid-Malonic Acid, (C) Physical Mixture of Ferulic Acid-Nicotinamide, (D) Co-Crystal of Ferulic Acid-Malonic Acid, (E) Co-crystal of Ferulic Acid-Nicotinamide

efficiency of the co-crystal using nicotinamide and malonic acid from several studies compared to the pure drug was tabulated in Table 3. Therefore, this work was beneficial for evaluating the capability of nicotinamide and malonic acid as co-formers to improve the dissolution of phenolic acid compound, herein ferulic acid, compared to the other drugs from the previous study.

The dissolution enhancement of the co-crystal was primarily influenced by the solubility increment of ferulic acid in aqueous medium. This phenomenon was supported by the Noyes-Whitney Equation, which revealed that the dissolution of solid particles was proportional to their solubility. The hydrogen bond between ferulic acid and both co-formers induced the reduction of crystal lattice energy. Hence, the bond between ferulic acid and nicotinamide or malonic acid was easier to disrupt than the pure ferulic acid (Panzade and Shendarkar, 2019). The dissolution of ferulic acid-nicotinamide physical mixture was slightly increased due to the dissolution effect of nicotinamide. This process increased the wettability of ferulic acid in aqueous medium. Malonic acid also exhibited a similar phenomenon, due to its ability to increase the water contact on the surface of the physical mixture.

The dissolution enhancement of ferulic acid was ascertained to be higher in the ferulic acid-nicotinamide co-crystal than in the ferulic acid-malonic acid co-crystal. Ferulic acid exhibited low solubility in aqueous and acidic media. As a co-former, nicotinamide produced a basic pH environment, promoted the higher solubility of ferulic acid. The analysis using the Gaussian method also supported these findings, since the Gibbs

free energy of ferulic acid-nicotinamide co-crystal was more negative (-3.3252 kcal/mol) than ferulic acid-malonic acid co-crystal (-2.4485 kcal/mol). The more negative Gibbs free energy indicated that the formation of a co-crystal between these two components was a spontaneous process. The more negative Gibbs free energy revealed that the substance is more polar and will make the electronegativity value of a compound greater. This condition impacts the ability of this compound to bind to the solvent and affect its solubility (Qiao et al., 2011). In vitro dissolution enhancement of ferulic acid-nicotinamide co-crystal exhibited in this study was highly correlated with the reduction of Gibbs free energy and the solubilization process (Bhatia et al., 2021). The results from XRD and DSC characterization were also in agreement with the Gaussian analysis. The higher reduction of ferulic acid crystallinity, as well as the higher propensity of interaction between ferulic acid and nicotinamide, led to the in vitro dissolution enhancement. The hydrogen bonding and π-stacking interactions were emphasized as the main mechanism of ferulic acid and nicotinamide interactions. Therefore, the higher amount of ferulic acid molecules interacted with the co-former, resulting in a higher percentage of drug release at the same interval. The higher solubility and drug release in dissolution medium can be predicted to be correlated with drug activity. The study conducted by Wicaksono et al. (2023) revealed that the preparation of curcumin-isonicotinamide co-crystal improved the in vitro dissolution and anti-inflammatory activity of curcumin about 1.3 times compared to curcumin. This was presumably due to the enhancement of curcumin dissolved in the digestive tract, followed by the higher absorption of curcumin into the systemic circulation.

4. CONCLUSIONS

The formation of ferulic acid-nicotinamide and ferulic acid-malonic acid as co-formers by the microwave irradiation metho d significantly enhances the solubility and dissolution of ferulic acid. The co-crystal of ferulic acid-nicotinamide increases the solubility of ferulic acid approximately 10.39% and the dissolution rate by 3.61-fold. Meanwhile, ferulic acid-malonic acid co-crystal increased the solubility and the dissolution rate of ferulic acid by 11.85% and 3.50-fold. Either nicotinamide or malonic acid is potentially applicable in co-crystal formation and pharmaceutical formulation development.

5. ACKNOWLEDGMENT

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