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Preparation and Physicochemical Characterizations of *p*-Methoxycinnamic acid – Succinic Acid Cocrystal by Solvent Evaporation Technique

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Abstract

BACKGROUND: *p*-Methoxycinnamic acid (PMCA) is an active pharmaceutical ingredient (API) derived from *Kaempferia galangal* L (known as *kencur* in Indonesia), which has the potential as an antinociceptive, analgesic, and antihyperglycemic. Unfortunately, its physical property is poorly soluble in water, so can cause problems in the development of pharmaceutical dosage forms. Several methods have been carried out to increase the solubility of PMCA such as complex formation with β-cyclodextrin, or solid dispersion. The cocrystal formation method is a solubility enhancement method that has been developed recently.

AIM: The aim of the study was the preparation and physicochemical characterization of PMCA cocrystal with succinic acid (SA) as its conformer by solvent evaporation technique.

MATERIALS AND METHODS: PMCA-SA cocrystal was made by the solvent evaporation method with a 1:1 molar ratio. Physicochemical characterization of PMCA and SA cocrystal was performed by differential scanning calorimetry (DSC), powder X-ray diffraction, and scanning electron microscope (SEM).

RESULTS: The DSC thermogram showed a decrease in the melting point of cocrystal compared to PMCA (173.55°C), SA (187.55°C), and its physical mixture (159.53°C). The cocrystal thermogram displayed an endothermic peak at 158.46°C. Diffractogram of PMCA-SA cocrystal exhibited new diffraction peaks at an angle of 2θ = 21.92; 25.91; and 39.25° which was not found in the diffractogram of every single component nor its physical mixture. SEM photomicrograph showed PMCA-SA cocrystal as a rod-shaped crystal that had a different surface morphology and smaller size than the constituent materials.

CONCLUSIONS: Based on the physicochemical characterization data above, it could be ascertained that PMCA-SA cocrystals had formed, these cocrystals were expected to increase the solubility of PMCA in water.

Introduction

p-Methoxycinnamic acid (PMCA) is a cinnamic acid derivative, obtained from the hydrolysis of ethyl *p*-methoxycinnamate. PMCA has antinociceptive, analgesic, and antihyperglycemic activity by increasing insulin secretion, glycolysis, and reducing gluconeogenesis. However, PMCA is an active ingredient that is very difficult to dissolve with a solubility of 0.71 mg/mL at 25°C [1], [2]. Low solubility is a major problem in the development of new drugs because it affects the rate of drug dissolution and limited the drug's absorption [3], [4]. Therefore, it urges to find a method to increase the solubility of PMCA. Various methods can be used to increase the solubility of active pharmaceutical ingredient (API), including solid dispersion, hydrotropic agent, surfactant, and salt formation. The cocrystal formation is one method of formulation approach that solve the drug's solubility problem [4].

Cocrystals were solid materials formed from two or more different components with certain stoichiometric ratios at room temperature that were bonded through non-covalent bonds, usually hydrogen bonds. Cocrystals could increase solubility, by lowering lattice energy and increasing solvent affinity [4]. The cocrystal form had several other advantages such as eliminating the need to make or break covalent bonds, forming stable crystals, improving the physicochemical and pharmacokinetic properties of an API without reducing its pharmacological activity [4], [5]. Increased stability in the form of cocrystals could extend the shelf life of API in pharmaceutical products [5].

Cocrystals consisted of two compounds, namely, API and coformers (formers of cocrystals). Succinic acid (SA) was one of the commonly used

coformers, which belonged to the carboxylic acid group. The presence of a carboxylic group in the structure of SA could form intermolecular bonds, such as hydrogen bonds with API in forming cocrystals [6], [7]. SA was easily soluble in water and had been proven in the formation of sildenafil citrate-SA cocrystals with a molar ratio of 1:1. It also increased the solubility of sildenafil citrate cocrystal by 5 times and in the formation of carbamazepine-SA cocrystals with a molar ratio of 2:1 [8], [9], [10].

Solvent evaporation is the most frequently used technique in the formation of cocrystals. In this method, API and coformer are mixed in a certain stoichiometric ratio and then dissolved in a suitable solvent. The mixture then allowed to evaporate. The advantage of the thermodynamic solvent evaporation method is that the obtained cocrystals are more preferred [6]. The cocrystals prepared by the solvent evaporation method provide lower energy and are more homogeneous in terms of crystal composition [8], [9].

The purpose of this study was to perform the formation of PMCA-SA cocrystals using the solvent evaporation method. Then, it evaluated the physicochemical characteristics (such as thermal properties, crystal pattern, functional groups, and surface morphology) of the cocrystals using differential scanning calorimetry (DSC), Powder X-Ray Diffraction (PXRD), Fourier transform infrared (FTIR) spectrophotometer, and scanning electron microscope (SEM).

Materials and Methods

Materials

The chemicals used in this study were PMCA (TCI, Japan, Product Number M0576), SA (E. Merck, Germany, Lot: K48212482 708), ethanol pa (E. Merck, Indonesia, Lot: M1009832500), and KBr powder (E. Merck, pro spectroscopy).

Screening of p-Methoxycinnamic acid-succinic acid cocrystal formation with a binary phase diagram

Screening of the cocrystal formation was accomplished by composing a binary phase diagram. The purpose of this screening was to identify the ability of a compound that formed cocrystals due to the interaction of API with certain coformers [11], [14]. The molar ratios of the physical mixtures used in this study were 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0: 10. The physical mixture of PMCA-SA was prepared by mixing the two materials according to their molar ratios using a porcelain dish and a stirring rod for 5 min, after which the DSC test was performed on each sample.

Methods of preparation of cocrystal p-Methoxycinnamic acid-succinic acid

PMCA and SA were weighed carefully according to a 1:1 molar ratio, where both components were dissolved in ethanol separately. The two solutions were mixed into a porcelain bowl and stirred until the solvent evaporated. The crystalline solids formed were placed at room temperature for 48 h and then stored in a desiccator [9].

Characterization of thermal property with differential scanning calorimetry

This test was carried out on PMCA, SA, physical mixture of PMCA-SA, and PMCA-SA cocrystal. Temperature calibration DSC (Mettler Toledo3, Swiss) was carried out with indium before carrying out the evaluation. Samples of 2–3 mg were put into an airtight aluminum pan and then analyzed at a temperature of 50°C–250°C with a heating speed of 10°C per min. The thermograms obtained were compared with the literature [9], [10], [12]. The formed cocrystal was sieved by sieve no 200–100 mesh before performing the followed procedure.

Analysis of cocrystal by powder X-ray diffraction test

PXRD tests (PANalytical X'Pert 3 Powder, Netherland) were carried out on PMCA, SA, physical mixture, and PMCA-SA cocrystals. The sample was placed in the sample holder and flattened to prevent agglomeration of the powder during preparation. The test conditions were set as follows: Cu Kα source, voltage 40 kV, 40 mA. Data collection was carried out at room temperature in a step range of 2θ = 5° to 50° [9], [10].

Characterization of hydrogen bond interaction with Fourier transform infrared spectrophotometer

The spectra of PMCA, SA, and PMCA-SA cocrystal were prepared with KBr pellets by FTIR Spectrophotometer (Shimadzu IR Tracer-100, Japan). The sample powder of 10 mg was homogenized with KBr, with a ratio of sample and KBr of 1:5. Then, it was put into a vacuum dryer and then molded with a hydraulic press to form pellets. The pellet is placed in a sample holder and fired with infrared light. The next absorption band was observed at wavenumbers 400–4000 cm^{-1} [9], [10].

Scanning electron microscope study

SEM microscopy tests (Hitachi TM3000, Japan) were carried out on samples of PMCA, SA, and PMCA-SA cocrystal. A sample of 10 mg was placed in a sample holder and coated with gold with a thickness of 10 nm. Sample observations were carried out with voltages of 20kV and 12mA [9], [10].

Cocrystal bond prediction test by in silico study

Cocrystal bond predictions were made using 2D and 3D Hyperchem ver.15.0. The structure of PMCA and SA was made using Hyperchem 2D ver.15.0 and then copied to Hyperchem 3D ver.15.0. Prediction of hydrogen bonds formed between the two structures to form cocrystals was analyzed.

Results

Screening of p-Methoxycinnamic acid-succinic acid cocrystal formation with a binary phase diagram

The thermogram of the physical mixture of PMCA-SA at various molar ratios (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10) is shown in Figure 1. The result of the binary system of a phase diagram of PMCA-SA with various molar ratios of the physical mixture showed a congruent melting system**.** From the DSC result of various molar ratio of PMCA-SA, we can generate the binary phase diagram.

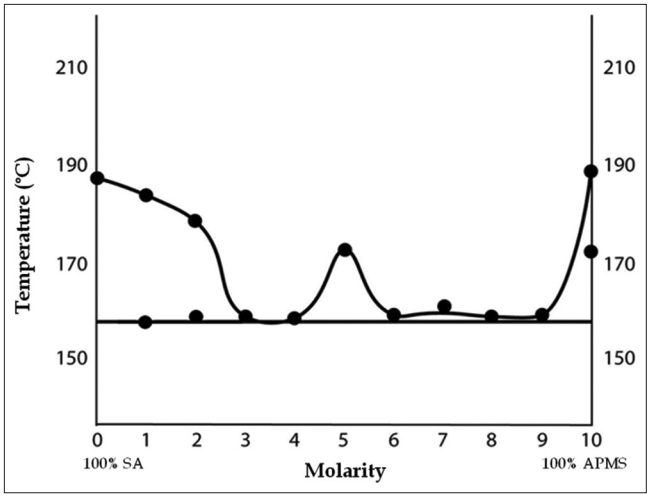

Figure 1: Phase Diagram of the p-Methoxycinnamic acid-succinic acid's binary system

Characterization of thermal property with differential scanning calorimetry

Thermal analysis using DSC showed a decrease in the melting point of the cocrystal compared to the melting point of PMCA (173.55°C), SA (187.55°C), and physical mixture (159.53°C). The PMCA-SA cocrystal thermogram showed one endothermic peak at 158.46°C as depicted in Figure 2.

Analysis of crystal pattern by powder X-ray diffraction test

Characterization by PXRD of PMCA-SA cocrystals showed some new specific diffraction peaks at an angle of 2θ = 21.92°; 25.91°; and 39.25° which were not found in the diffractogram of the respective constituent materials, PMCA and SA, and also its physical mixture. X-ray diagram of the tested compounds is exhibited in Figure 3.

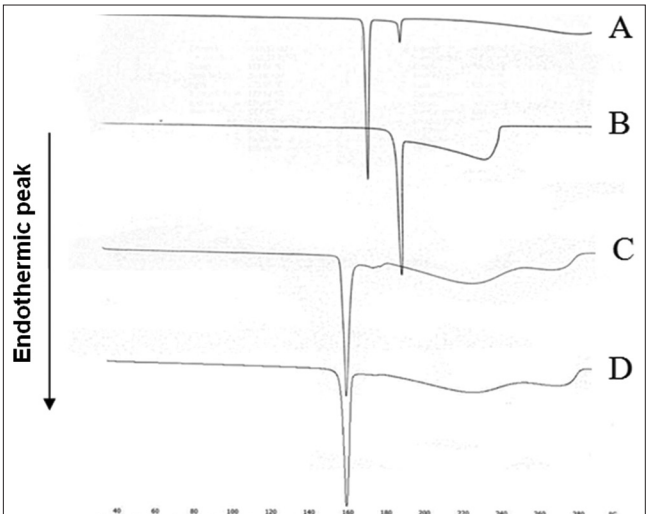

Figure 2: Thermogram of (A) p-Methoxycinnamic acid, (B) succinic acid, (C) physical mixture, and (D) p-Methoxycinnamic acid-succinic acid cocrystal

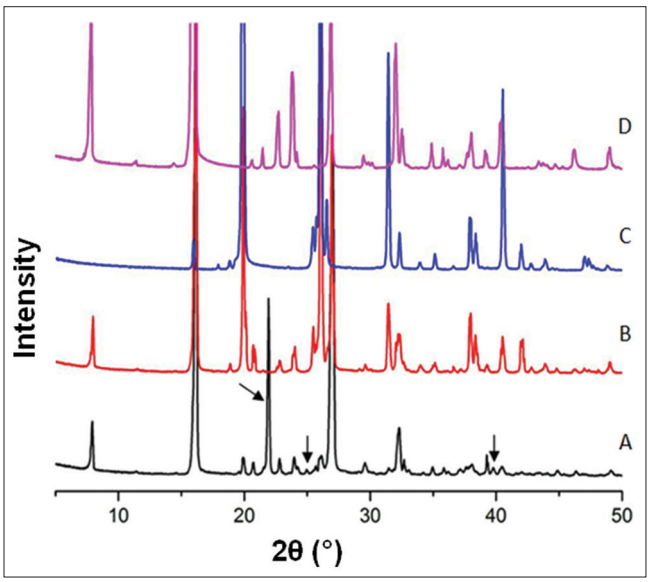

Figure 3: X-ray diffractogram of (A) p-Methoxycinnamic acid-succinic acid cocrystal, (B) physical mixture, (C) succinic acid, and (D) p-Methoxycinnamic acid. Description: ↓ showing a new diffraction peak

Characterization of hydrogen bond interaction with Fourier transform infrared spectrophotometer

Infrared spectrophotometry was often used to determine the interaction between APIs and coformers in the cocrystals formed. The change in the shape of the absorption spectrum was seen by comparing the absorption spectrum of each drug and coformer with

the resulting cocrystal [9], [10], [13]. In this study, infrared spectroscopy was performed on PMCA, SA, and PMCA-SA cocrystal. The FTIR spectra of PMCA, SA, and PMCA-SA cocrystal are shown in Figure 4.

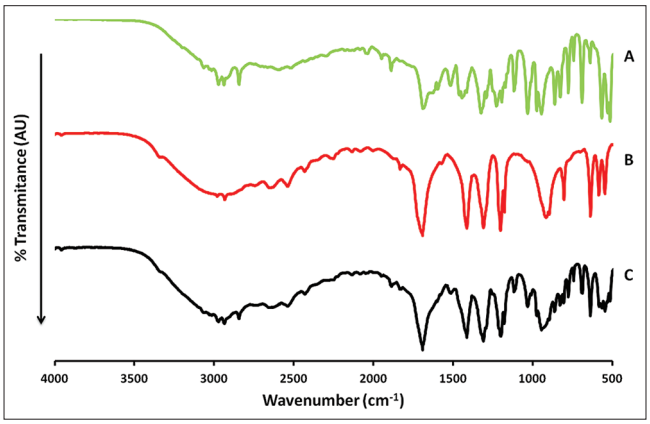

Figure 4: IR spectrum of (A) p-Methoxycinnamic acid, (B) succinic acid, and (C) p-Methoxycinnamic acid-succinic acid cocrystal

Scanning electron microscope study

SEM photomicrographs showed that PMCA-SA cocrystal had different surface morphology and smaller crystal sizes than PMCA and SA.

Cocrystal bond prediction test (in silico study)

In this study, an *in silico* evaluation was carried out, to predict the bond that occurs between PMCA and SA to form cocrystals. The PMCA-SA cocrystals can be formed when the two compounds are bonded through non-covalent bonds, such as hydrogen bonds [7]. Prediction of cocrystal bonds could be done by calculating the total energy value. The total energy showed the bond energy that occurred in the structure. Higher energies represented more stable bonds. Prediction of PMCA-SA cocrystal bond is described in Figure 5.

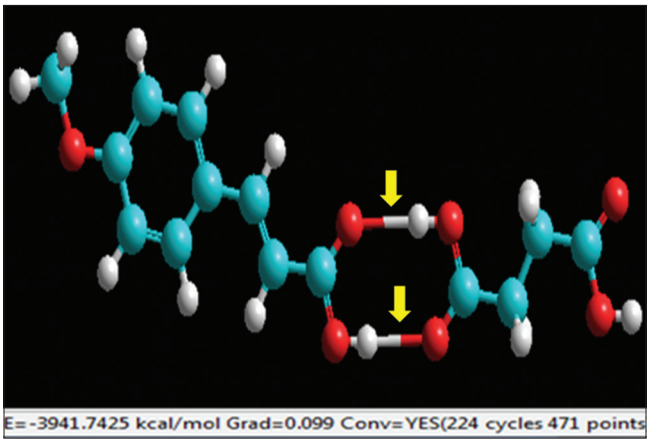

Figure 5: Prediction of the bond between the C=O group of succinic acid with the O-H group of p-Methoxycinnamic acid to form the cocrystal. Description: yellow arrow showing hydrogen bonding

Discussion

A congruent fusion system was a system that has two eutectic points where between the two eutectic points, there was one congruent melting point, namely, the cocrystal melting point, and this congruent system indicated the formation of cocrystals [11]. When a physical mixture with a molar ratio of 5:5 was heated, each component melted into one component to form a cocrystal and a eutectic melting point occurred in a physical mixture of a molar ratio of 4:6 and 6:4. The phase diagram obtained was a congruent binary phase diagram and is shown in Figure 1.

A method to determine the crystal properties was thermal analysis with DSC. Using this test, the change in the melting temperature of a crystal was analyzed. Thermal analysis using this DSC was carried out by starting at a temperature of 50°C–250°C with a heating rate of 5°C/min [12], [14]. The results of the PMCA thermogram, SA, physical mixture, and the cocrystal are shown in Figure 2. The melting temperature of each sample is shown in Table 1. The results showed that the PMCA's thermogram had an endothermic peak at 173.55°C which represented the melting point of PMCA. The SA's thermogram showed an endothermic peak of 187.55°C which represented the melting point of SA. The physical mixture's thermogram showed one endothermic peak at 159.53°C. The PMCA-SA cocrystal's thermogram also showed a sharp endothermic peak at 158.46°C, which represented the melting point of the cocrystal [9], [10], [13]. The change in the melting point indicated that the PMCA-SA cocrystal was formed. These results required other characterization data such as crystallinity with PXRD and surface morphology with SEM.

Table 1: Endothermic peaks of samples

Code	Sample	Endothermic peak (°C)
A	PMCA	173.55; 189.86
B	SA	187.55
C	Physical mixture	159.53
D	Cocrystal	158.46

PMCA: *p*-Methoxycinnamic acid, SA: Succinic acid.

PXRD was a method that was used to identify crystalline materials and provided information on the cell dimensions [9], [10]. Crystal identification was carried out by comparing the location and intensity of the lines on the diffractogram to the lines on the known sample's images. The X-ray diffractogram of PMCA, SA, and the cocrystal is shown in Figure 3. The physicochemical characterization by the PXRD instrument was performed at an angle of 2θ = 5°–50° and produced diffractograms of PMCA and SA, respectively, with specific and sharp peaks indicating the crystalline phase. The specific peak of PMCA appeared at an angle of 2θ = 7.85; 16.00; 16.11; 23.74; 26.89; 32.09; and 40.43°, while the specific peak of SA appeared at an angle of 2θ = 15.97; 19.89; 26.08; 31.46; and 40.52°, in accordance with the previous studies which stated the specific peak of SA at an angle of 2θ = 16.02; 19.99; 26.10; and 31.41°.

The results of characterization by X-ray diffraction of PMCA-SA cocrystal showed some new peaks compared to the single component and the physical mixture of the two components at 2θ = 21.92; 25.91; and 39.25°. This diffractogram pattern indicated the formation of a new crystal lattice. The new crystalline phase formed from the interaction between the two constituent materials would be observed concretely from the X-ray diffractogram and it would be different from the physical mixture of the two components. The X-ray diffractogram of the physical mixture was a superimposition between the two constituent components, which had the same diffraction pattern and differed only in peak intensity [9], [10], [13].

FTIR spectra of PMCA and SA showed absorption bands for each compound. PMCA and SA both had carboxylic groups so they showed almost the same absorption values in Figure 4. PMCA's IR spectra showed O-H absorption at 3064–2843 cm^{-1}, C=O absorption at 1689 cm^{-1}, and C-O absorption at 1226–1170 cm^{-1}. The FTIR spectra of SA showed O-H absorption at 2933–2980 cm^{-1}, C=O absorption at 1691 cm^{-1}, and C-O absorption at 1201 cm^{-1}. The FTIR spectra of the PMCA-SA cocrystal showed a shift and loss of absorption peaks when compared to the single constituent components. The shift or loss of absorption peaks occurred in the carboxylic OH group and C=O which were predicted to form hydrogen bonds between PMCA and SA. The appearance of the C=O absorption peak in the cocrystal was also possible due to the C=O group of unbound SA. The loss and shift of the absorption peak on the FTIR spectrum indicated the occurrence of intermolecular interactions in the APMS-SA cocrystal.

From *in silico* study, it was predicted that hydrogen bonds formed due to the presence of a carbonyl group from PMCA which bonded to a hydroxyl group in SA, as shown in Figure 5. The bond prediction was carried out using the Hyperchem ver. 15. The results showed that PMCA and SA formed hydrogen bond and had a total bond energy of −3941.7425 kcal/mol. The interaction occurred between the carboxylic group of PMCA and SA forming hydrogen bonds. The bond formed between the (C=O) group of PMCA binds to the (O-H) group of SA [15], [16].

In addition to thermal and crystal pattern analysis, morphological characterization of the sample material was carried out to determine the texture or properties, size, and arrangement of the particles that make up the object on the surface using SEM. Observations by SEM were providing visual information about the differences in cocrystal morphology compared to its constituent materials [9], [10], [11]. The results of the SEM photomicrograph observations on each sample are shown in Figure 6. It showed the differences in the surface morphology of PMCA-SA cocrystals compared to its constituent materials, PMCA and SA. PMCA had a large bladed surface morphology, and SA had a plumose surface morphology. The PMCA-SA cocrystal's photo micrograph had a surface morphology of a rectangular prism or rod with a smaller particle size than PMCA and was viewed at a magnification of 5000 times. The cocrystal had a more uniform and regular size. The smaller the particle size in the cocrystal, the larger the surface area, making the cocrystal more soluble in the solvent.

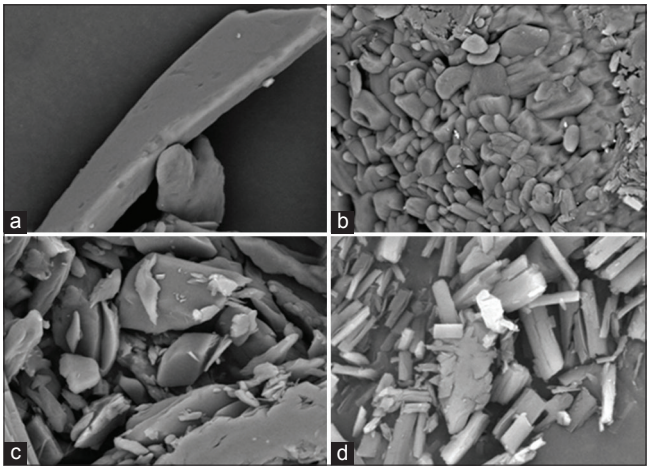

Figure 6: Scanning electron microscope photomicrograph of (a) p-Methoxycinnamic acid, (b) succinic acid, (c) p-Methoxycinnamic acid-succinic acid cocrystal (×2000 magnification), and (d) p-Methoxycinnamic acid-succinic acid cocrystal (×5000 magnification)

Based on the results of the physicochemical characterizations of PMCA-SA cocrystals prepared by the solvent evaporation method, it could be concluded that the APMS-SA cocrystals had been successfully formed. Further research on making the cocrystals of API is needed to minimize the use of organic solvents.

Conclusion

The results of characterization using DSC, PXRD, FTIR, and SEM on PMCA-SA cocrystals made by solvent evaporation method resulted in data showing that there were changes in physicochemical characteristics compared to the constituent materials and their physical mixtures that were included changes in thermal properties, crystal pattern, and surface morphology. This indicated that PMCA and SA were able to interact to form PMCA-SA cocrystals prepared by the solvent evaporation method.

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