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Preparation and Characterization of Multicomponent Crystal Piperine-Caffeine

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ABSTRACT: Piperine ($C_{17}H_{19}NO_3$) belongs to the alkaloid group mainly found in the Piperaceae family, especially in black pepper (Piper nigrum L.). Piperine is practically insoluble in water. This study aims to improve the solubility of piperine by preparing it into a multicomponent crystal using caffeine as a coformer. The formation of piperine-caffeine multicomponent was prepared by solvent evaporation and solvent drop grinding. Each sample was characterized using Powder X-ray diffraction (XRD), Differential Scanning Calorimetry (DSC), and FT-IR spectroscopy. The solubility test was carried out by sonication for 30 minutes at room temperature (30 °C), and the dissolved piperine was analyzed by High-Performance Liquid Chromatography (HPLC) using methanol: water (80:20) as the mobile phase. This study shows a decrease in peak diffraction intensity on PXRD analysis and a reduction in endothermic peak on the DSC thermogram of multicomponent crystals. In the FTIR analysis, multicomponent crystals slightly shift, indicating the physical interaction. The multicomponent crystal of piperine-caffeine prepared by solvent evaporation method has an increase in solubility of 2.56 times, and the multicomponent crystal with solvent drop grinding method has an increase in solubility of 1.61 times, respectively. In conclusion, the multicomponent crystal piperine–caffeine can enhance the solubility of piperine.

Keywords: **piperine; caffeine; multicomponent crystal; solvent evaporation; solvent drop grinding.**

Introduction

Piperine is an alkaloid compound primarily found in the Piperaceae family, especially in black pepper (Piper nigrum L.). Black pepper is widely consumed as a cooking spice and is increasingly used to stimulate metabolism and nutrient absorption and for medicinal efficacy [1]. The use of piperine in pharmaceutical preparations is still constrained due to its physicochemical properties.

Piperine is a pale yellow crystal with a melting point of 135°C [2] and practically insoluble in water (40 mg/L at 18°C) but easily soluble in alcohol (1 g/15 mL) and ether (1 g/1.7 mL) [3]. Piperine is a weak base, with a pKa of 12.22 [4]. The low solubility of piperine in water is a challenge in preparing piperine into pharmaceutical dosage forms. Therefore, several efforts have been done to increase the solubility of piperine, including the formation of solid dispersions and inclusion complexes and nanoparticles [5].

Multicomponent crystal designs, such as cocrystals or salts, can modify the crystal's physicochemical properties without changing the chemical properties and are, therefore, very useful in the case of active pharmaceutical ingredients [6]. Recent research has investigated the potential of multicomponent crystals containing drug combinations, providing technological advantages and improving pharmacological benefits and patient compliance. This phenomenon is probably the most critical advantage of multicomponent drug-drug crystals compared with multicomponent crystals of single drugs and non-drugs [7]. Previous studies have been conducted in preparing multicomponent crystals of piperine, including piperine–saccharin [8], piperine–succinic acid [9], and piperine–nicotinic acid [10].

Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine2,6-dione) is known as a central nervous system stimulant existing in two polymorphic forms, anhydrous and hydrate forms, is an alkaloid present in more than 60 plant species [11]. Caffeine (molecular weight = 194 Da) has a pKa of 14 and a lipid partition coefficient of 0.85 [12]. Caffeine has a pKa of 10.4 at 40 °C [13], where, as a weak electrolyte, it does not dissociate in gastric fluid (pH 2 to 3). The melting point of caffeine is 238 °C, and it has a nearly neutral pH of 6.9 and water solubility of 2.17 g/100 ml at 25

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°C [14]. From the physicochemical data on piperine and caffeine, it is known that piperine has solubility problems in the water, so there is a possibility of the formation of the multicomponent crystals of piperine with caffeine. Moreover, caffeine also has the potency to form self- and hetero-complexes [15]. Multicomponent studies of caffeine crystals have been widely carried out, such as the formation of caffeine cocrystals with hydroxy-2-naphthoic acid, which shows hydrogen-bonded carboxylic acid dimers in the presence of hydroxyl-caffeine heterosynthone [16], caffeine - methyl gallate cocrystals show the formation of hydrogen bonding and a dramatic increase in the compressibility properties of the structure [17].

Based on the considerations above, in this research, the formation of multicomponent caffeine-piperine crystals will be carried out, which is expected to increase the solubility of piperine. The multicomponent crystals formed were then characterized using X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), FTIR spectroscopy, and solubility testing using High-Performance Liquid Chromatography (HPLC).

Methods

Materials

Piperine (Tokyo Chemical Institute, Japan), Caffeine (Konimex, Indonesia), KOH, methanol pro analysis (Merck, Germany), acetonitrile pro HPLC (Merck, Germany), aqua pro injection (PT. Ikapharmindo Putramas, Indonesia).

Preparation of Multicomponent Crystal Piperine–Caffeine by Solvent evaporation (MC SE)

Piperine and caffeine were mixed in a 1:1 mole ratio. This mixture was then dissolved with methanol pro analysis on a magnetic stirrer at a temperature of 50°C while stirring. The solution is then left until all the solvent has evaporated. Then, the MC was stored in a tightly closed container in a desiccator.

Preparation of Multicomponent Crystal Piperine–Caffeine by Solvent drop grinding (MC SDG)

Multicomponent crystals were prepared at a mol ratio of 1:1, and then the piperine and caffeine were ground for about 10 minutes while 0.05 mL of methanol was added. The MCs then were stored in a desiccator.

Physicochemical Characterization

X-ray Diffraction Analysis

X-ray diffraction analysis of the samples was carried out at room temperature using a RINT-2500 diffractometer (Rigaku, Japan). The measurement conditions are as follows: Cu metal target, Kα filter, voltage 40 kV, current 40 mA, analysis carried out in the range 2 theta 50 – 500. The sample was placed in a sample holder (glass) and leveled to prevent particle orientation during sample preparation. Analysis was carried out on samples of piperine, caffeine, and multicomponent crystals piperine-caffeine.

Differential Scanning Calorimetry (DSC) Analysis

Thermal analysis of the samples was carried out using a DSC instrument (DSC-60 Shimadzu, Japan),

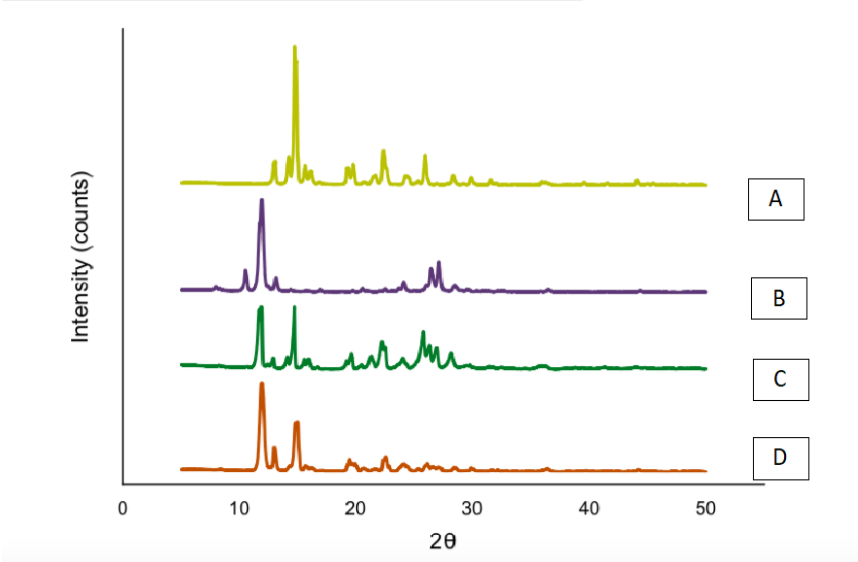

Figure 1. The diffractogram of (A) Piperine, (B) Caffeine, (C) MC SDG, and (D) MC SE

Table 1. Specific peak intensity of piperine and MC piperine - caffein

Position 2θ	Peak Intensity		
	Piperine	MC SE	MC SDG
14.765	28322.75	9034.502	10439.71
15.635	4110.421	1379.116	1393.163
16.075	3016.969	917.5172	1179.061
19.745	4293.641	1640.407	976.5115
21.625	2202.193	761.8904	958.1079
22.345	7359.312	2477.06	4345.208
24.285	2086.018	1233.798	1440.777
25.915	6389.621	1471.206	3366.708

which had been calibrated using Indium. Samples were placed on a closed aluminum plate. The DSC instrument was programmed in a temperature range of 25 – 270 °C. Analysis was carried out on samples of piperine, caffeine, and multicomponent crystals piperine-caffeine.

IR Spectrophotometry Analysis

IR spectrophotometer (Shimadzu, Japan) analysis was carried out with the sample placed on top of the ATR crystal so that it covered all the crystal surfaces. The sample was closed by applying a little pressure, and an IR absorption spectrum was taken on the sample. Analysis was carried out on samples of piperine, caffeine, and multicomponent crystals piperine-caffeine.

Solubility Test

Solubility tests were carried out for piperine and multicomponent crystals piperine-caffeine by preparing them into a saturated solution. The solubility test was carried out for 30 minutes by sonication at room temperature (30 °C). After that, the sample was filtered using 0.45 µm Whatman filter paper. The filtrate obtained was then analyzed using HPLC (Shimadzu, Japan) with a mobile phase of methanol: water (80: 20) and a C_{18} silica stationary phase. The amount of dissolved piperine was detected at a UV wavelength of 343 nm. Solubility testing was carried out triplicated.

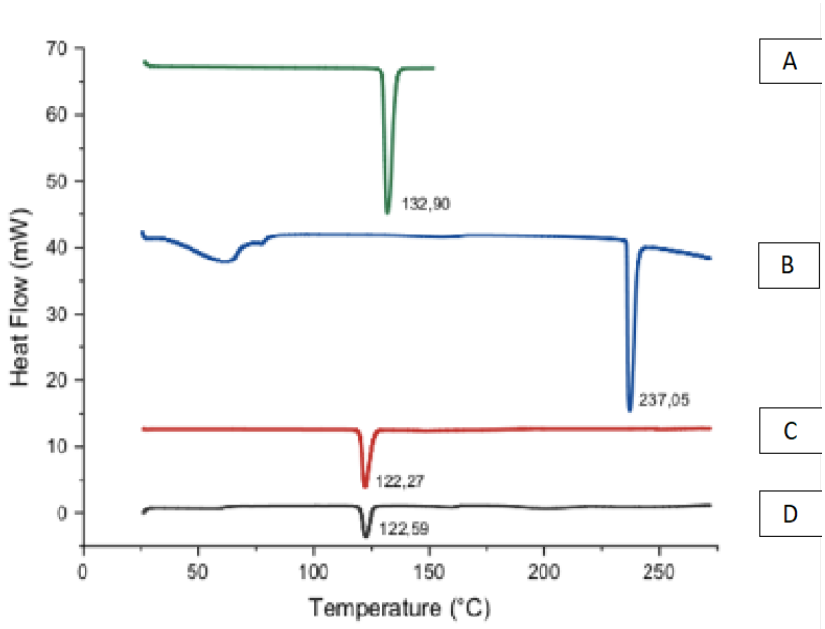

Figure 2. The thermogram of (A) Piperine, (B) Caffeine, (C) MC SDG, and (D) MC SE

Table 2. Result of thermal analysis of piperine, caffeine and MCs

Samples	Melting point (°C)	ΔH fusion (J/g)
Piperine	132.90	135.32
Caffeine	237.05	95.10
MC SDG	122.27	58.63
MC SE	122.59	31.45

Result and Discussion

Powder X-Ray Diffractometry (PXRD) is a characterization technique that is commonly used to identify and analyze the crystallinity of a substance. Through PXRD, the formation of cocrystals can be determined by the appearance of new diffraction peaks in the diffractogram compared to the intact materials [14]. The X-ray diffractogram of piperine and multicomponent piperine-caffeine crystals is seen in Figure 1, which revealed the crystalline phase that proved by the existence of peaks with high intensity at diffraction angles of 2θ at 12.935°, 13.035°, 14.765°, 15.635°, 16.075°, 19.745°, 21.625°, 22.345°, 24.285°, 25.915°, 28.325°, 29.835 °, 31.555°, 44.125°.

The results of X-ray diffraction analysis of multicomponent piperine-caffeine crystals from both methods did not show any new diffraction peaks. However, in general, there is a decrease in intensity in the multicomponent X-ray diffraction pattern of the crystal compared to the intact materials. The peak intensity of multicomponent piperine-caffeine crystals prepared by the solvent evaporation method shows lower intensity compared to solvent evaporation method, as seen in Table 1.

The decreasing peak intensity of multicomponent crystals of piperine -caffeine indicates the formation of a multicomponent crystalline solid, also known as a eutectic mixture [18,19]. From a structural perspective, eutectics are defined as "conglomerates of solid solutions" that form between materials that don't have an excellent geometric match or can create heteromolecular interactions [19].

Differential Scanning Calorimetry (DSC) is a thermal analysis technique that is often used to characterize solid materials. DSC characterization provides information regarding changes in thermal properties which are shown by the appearance of endothermic and exothermic peaks on the thermogram due to melting, phase transition, recrystallization, and dehydration [15].

Based on the results of DSC analysis as seen in Figure 2 and Table 2, the piperine thermogram shows a sharp endothermic peak with a melting point of 132.90 °C and an

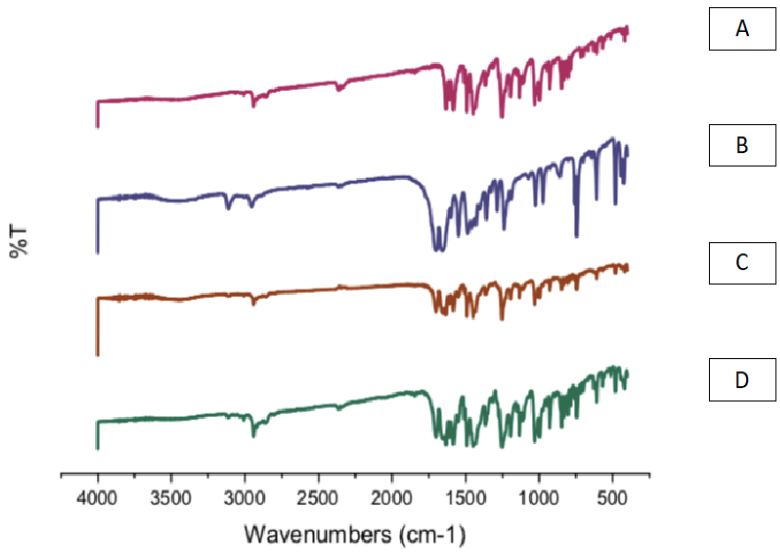

Figure 3. FTIR spectrum of (A) Piperine, (B) Caffeine, (C) MC SDG, and (D) MC SE

Table 3. Result of solubility test for piperine MC piperine - cafffein

Samples	Average AUC ± SD	Solubility enhancement
Piperine	1083288.866 ± 106395.495	-
MC SDG	1717609.333 ± 119360.630	1.61 times
MC SE	2700070.333 ± 231590.580	2.56 times

enthalpy value of 135.32 J/g. The caffeine compound also shows a sharp endothermic peak with a melting point of 237.05 °C. Compared to the intact material, the formation of multicomponent piperine-caffeine crystals has a single endothermic peak, which is different with a lower melting point compared to its intact materials at 122.27 °C (solvent drop grinding), and 122 .59 °C (solvent evaporation).

In addition, there is also a decrease in enthalpy for each multicomponent crystal. Enthalpy is the energy needed for a substance's fusion to occur. The decrease in the enthalpy value corresponds to the reduction in the degree of crystallinity found in the diffractogram [18,20]. Thus, the formation of multicomponent crystals using solvent drop grinding and solvent evaporation methods can increase the solubility of piperine [18,20,21].

Infrared spectrophotometry analysis was carried out to observe the occurrence of chemical interactions between piperine and caffeine in the formation of multicomponents [9]. In this study, this analysis is helpful in identifying multicomponent piperine-caffeine crystals by observing the spectrum shift that occurs by comparing the multicomponent spectrum of piperine-caffeine crystals with the spectrum of piperine and caffeine. The results of the piperine-caffeine FTIR analysis can be seen in Figure 3.

The infrared spectrum of inatct piperine contains characteristic peaks of piperine at wave numbers 1583, 1634, and 2940 cm^{-1} which are the C=O, C-O, and C-H groups [10]. The infrared spectrum of caffeine has characteristic peaks for caffeine at wave numbers 1659, 2954, 3112, and 3448 $cm^{-1,}$ which are the C-O, C-H, and N-H groups [22]. In multicomponent piperine-caffeine crystals, no new wave numbers indicate the no new functional groups formed and confirmed the physical interaction between piperine and caffeine as shown in the previous study [23].

Solubility is an important parameter that needs to be considered in drug discovery and development because it influences the drug concentration in the systemic circulation to achieve the desired pharmacological effect [24]. Therefore, solubility test need to be carried out to predict the bioavailability of drugs in the body. The solubility test of piperine and multicomponent piperine-caffeine crystals is shown in Table 3.

Based on the solubility test data above, it is known that there was a significant increase in the solubility of piperine, where the increase was 1.61 times and 2.56 times, respectively, for MC prepared by solvent drop grinding and solvent evaporation. This increase in solubility was caused by a decrease in the degree of crystallinity of piperine in the multicomponent crystals prepared by the solvent drop grinding and solvent evaporation methods, which were also investigated in the previous study [23].

Conclusion

Multicomponent piperine-caffeine crystals were successfully prepared by solvent drop grinding and solvent evaporation methods which characterized by prominent solid-state characterization including X-ray diffraction, DSC, and FTIR. Moreover, multicomponent crystals of piperine-caffeine crystals provide a significant increase in solubility compared to intact piperine.

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