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The Formation and Characterization of Multicomponent Crystal Piperin-Urea Using The Solvent Drop Grinding Technique

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A B S T R A C T

Piperine is an alkaloid found in the fruits and roots of *Piper nigrum.* Piperine has an almost insoluble solubility in water. Piperine has pharmacological activities such as antihypertensive andantiplatelet, antioxidant, antitumor, antiasthma, antidepressant, and hepatoprotective. This study aims to increase the solubility of piperine by forming multicomponent crystals using urea as a coformer. Multicomponent crystals were prepared with a mole ratio of 2:1 using the Solvent Drop Grinding method. Multicomponent crystals were characterized by *X-Ray Diffraction* (XRD), *Differential Scanning Calorimetry* (DSC), *Fourier Transformation Infra-Red* (FT-IR), *Scanning Electron Microscopy* (SEM), and dissolution test. The results of the multicomponent crystal characterization showed an improvement in the physicochemical properties of piperine. The x-raydiffraction pattern of the multicomponent crystals shows the same diffractogram as the constituent components, which indicates that no new crystalline phases are formed. The DSC thermogram shows the same endothermic peak for both components. FTIR characterizationshowed no shift in the peak of the piperine group. The SEM results did not reveal any new crystal habit forms. In the dissolution test, it was found that the percentage of dissolution at 60 minutes of piperine had a value of 36.148%, and a multicomponent crystal of 50.530%, this indicates that the formation of multicomponent crystals can increase the dissolution rate of piperine by 1.39 times.

Keywords: Piperin, urea, multicomponent crystal, solvent drop grinding

Introduction

Piperine is an alkaloid found in the fruit and roots of *Piper nigrum* (black pepper) which comes from the Piperaceae family. Piperine in the form of white prisms, almost insoluble inwater, easily soluble in alcohol and ether. Piperine was first isolated by Hans Christian Orsted in 1819 (Vasavirama & Upender, 2014). Piperine exhibits pharmacological activities such as antihypertensive and antiplatelet (Taqvi *et al*., 2008), antioxidant, antitumor (Manoharan *et al*., 2009), anti-asthma (Parganiha *et al*., 2011), antidepressants (Li *et al*., 2007), hepatoprotective (Matsuda *et al*., 2008), and improved cognitive abilities (Wattanathorn *et al*., 2008). Piperine was also found to stimulate pancreatic and intestinal enzymes that aid digestion. Therapeutic activity is associated with the presence of piperine. *Piper nigrum* is used to produce white pepper and black pepper, which are also used as flavoring agents (Ahmad *et al*., 2012). The clinical use of piperine is still limited due to its low solubility in water (Kumar *et al*., 2018).This results in a low dissolution rate, where dissolution is a limiting factor for the rate of drug absorption, thereby reducing bioavailability (Veerareddy *et al*., 2004). Medicinal ingredients musthave good solubility in water in order to have an optimal therapeutic effect. Compounds that are insoluble in water will showimperfect absorption, resulting in a minimum response. Solubility is one of the important physicochemical properties in predicting the degree of drug absorption in the gastrointestinal tract(Ansel, 2005; Martin., 2011).

Multicomponent crystals are a technique of crystal engineering in the formation of new phases, without changing the physicochemical properties of drugs. Multicomponent crystals can be used to increase solubility, dissolution rate, physical and chemical stability, and compressibility. Multicomponent crystals are formed as a result of interactions between molecules and ions contained in covalently active pharmaceutical ingredients and coformers. The multicomponent crystals formed were predicted based on supramolecular Synthon from thedifference in pKa values between the two substances, so that

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the crystals formed could be determined to be salts or cocrystals. Multicomponent crystals consist of solvates/hidrates, salts, and cocrystals (Clarke, 2012; Qiao *et al*., 2011).

The manufacture of multicomponent crystals needed a coformer. The coformers used are materialsthat can be mixed together, easily dissolved in water, and able to bond covalently (Mirza *et al*., 2008). In this study, urea was used as a coformer. Urea is a nitrogen compound containing a carbonyl group attached to an amine group. Urea is used for protein catabolism, is white crystalline, almost odorless, very soluble in water, soluble in alcohol, and practically insoluble in methylene chloride. In a previous study, the solubility of piperine was increased by forming multicomponent crystals of piperine and nicotinic acid 1:1 using the dissolution method. Nicotinic acid is a water-soluble coformer, so it can increase the solubility of water-insoluble drugs. This study was determined based on the difference in the pKa values of piperine and nicotinic acid. The profile of the dissolution rate of multicomponent piperine crystals shows a significant increase comparedto pure piperine, which is around 2.5 times (Sari *et al*., 2019).

Solvent Drop Grinding is a grinding technique followed by the addition of a small amount of solvent. The solvent used is a solvent that can dissolve the two substances and functions as a catalyst to accelerate the cocrystal formation reaction. *The Solvent Drop Grinding technique* has advantages over other methods, such as the ability to control the formation of polymorphs, abetter crystallization process, and increased cocrystallization selectivity (Vitthalrao *et al*., 2013).

In this research, the formation of multicomponent crystals of piperine and urea with a mole ratio of 2: 1 was carried out using *solvent drop grinding*. The multicomponent crystals formed were then characterized by X-ray diffraction analysis, DSC thermal analysis, FT-IRspectroscopy analysis and SEM microscopic analysis, and the amount of dissolved substance was observed.

Methods:

Materials :

The materials used in this study were Piperine (PT BOCSCI), urea (Merck), aquadest (PTNovalindo), and ethanol pro analysis (Merck). Potassium Dihydrogen Phosphate (KH2PO4), Sodium Hydroxide (NaOH

Preparation of Crystal Multicomponent Piperin Urea 2 : 1

Multicomponent piperine–urea is made with a mole ratio of 2:1. The piperine and urea crystals were crushed using a mortar and pestle with the addition of 2 mL of ethanol pa, then crushed for ± 15 minutes until a dry mass was formed. The results obtained were put into a container, stored in a desiccator and characterized

X-ray Diffraction Analysis

analysis was performed using an X-ray diffractometer (Philips X'Pert Powder). The XRD patterns of the powders are traced using X-ray diffraction for the sample. Measurement conditions using Cu target metal, Kα filter, 40 kV voltage, 30 mA radiation current spread in the sample crystal region, as measured by a vertical goniometer. The patterns were obtained using 0.04 ºC step widths with a detector resolution at 2Θ (diffraction angle) between 10 ºC and 80 ºC at room temperature.

Analysis Thermal

Analysis Thermal analysis was observed using Differential Scanning Calorimetry (DSC) Setaram DSC 131 Evo, France. The sample was weighed 5 mg and heated in an aluminum pan at a temperature of 30-300°C with a heating rate of about 20°C per minute.

FT-IR Spectrophotometer Analysis

Tests were carried out on samples using the KBr disc method and analyzed at wave numbers between 400-4000 cm-1 with an FT-IR spectrophotometer. The sample was crushed to apowder state with KBr, then transferred to a die and the sample was then compressed onto a discunder vacuum.

Scanning Electron Microscopy

The sample is placed in a sample holder made of aluminum and coated with 10 nm thick gold. The samples were observed at various magnifications, the voltage was set at 20 kV and the current was 12 mA.

Dissolution Test

Determination of the dissolution profile of piperine and multicomponent crystals was carried out using a type 2 dissolution apparatus. The dissolution medium used 900 ml of phosphate buffer pH 7.4 with the temperature set at 37 °C ± 0.5 °C, at a speed of 100 revolutions per minute . Samples were taken at 5, 10, 15, 30, 45 and 60 minutes. Each pipette is replaced withdissolution medium taken at the same temperature so that the volume of dissolution medium remains constant. The solution that has been pipetted from the dissolution medium is measured at the maximum absorption wavelength and the percent dissolved substance is calculated.

RESULTS AND DISCUSSION

X-Ray Diffraction Analysis (XRD)

X-ray diffraction analysis was used to evaluate the effect of changes in the degree of crystallinityof the solid compound piperine in the binary system. X-ray diffraction analysis is a method for characterizing the solid-state interaction and determining whether a new crystalline phase is formed or not (Zaini *et al*., 2020). The results of XRD analysis of piperine, urea, and 2:1 piperine urea multicomponent crystals using the solventdrop grinding method can be seen in Figure 1. Piperine and urea diffractograms show crystalline solids with specific and sharp interference, in the piperine urea multicomponent crystals no crystalline phase is formed. new. However, the formation of multicomponent crystals showed differences in the degree of crystallinity of

piperine with a decrease in the intensity of the X-ray diffraction pattern.

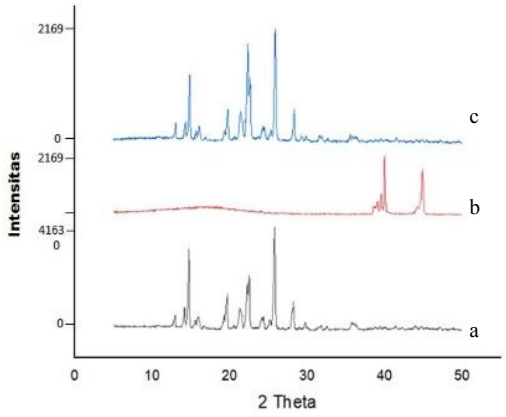


Figure 1. Diffractogram of (a) Piperine, (b) Urea, (c) multicomponent crystals Piperine-Urea (2:1)

Analysis Differential Scanning Calorimetry (DSC) Differential Scanning Calorimeter (DSC)

The analysis is used to determine the heat capacity and enthalpy of a sample. DSC is able to measure the amount of heat absorbed or released during the transition. The results of the DSC analysis of piperine, urea, and 2:1 piperine urea multicomponent crystals can be seen in Figure 2. On the DSC thermogram, the piperine crystals show a single and sharp endothermic peak at an enthalpy of 132.649 (J/g) and a temperature of 132.822°C. The urea thermogram shows an endothermic peak with an enthalpy of 181.491 (J/g) at a temperature of 136.462°C, while in the piperine–urea multicomponent thermogram there is an endothermic peak with an enthalpy of 100.340 (j/g) at a temperature of 128.483°C. The decrease in the melting point and enthalpy of the piperine-urea multicomponent compared to pure piperine and urea shows that there are no intermolecular interactions that can change the physicochemical properties of the compound, where the melting point has a close relationship with solubility, the higher the melting point, the lower the solubility (Sinko, 2011; Jessica *et al*., 2021).

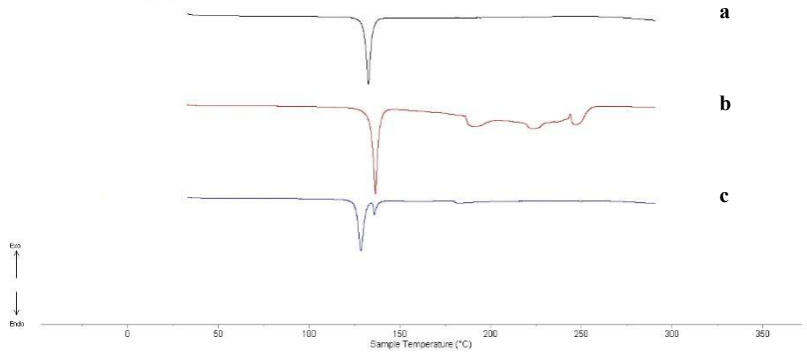


Figure 2. Thermogram of (a) Piperine, (b) Urea, (c) multicomponent crystals Piperine-Urea (2:1)

Fourier Transform Infrared (FT-IR)

Analysis FT-IR (*fourier transformation infrared*) infrared spectrophotometric analysis was used to identify the presence of functional groups in a compound. Each absorption band at a certain wave number describes the presence of a specific functional group. The results of the analysis are signal chromatograms relating the percentage of transmittance to wave number (Sari *et al*., 2019). The results of the FTIR analysis of piperine, urea and the multicomponent 2:1 piperine urea crystals can be seenin Figure 3. In the infrared spectrum of pure piperine, it can be seen that there is a CH functional group in wave number 3010.14 cm^{-1}, ROR functional group at 1250.75 cm^{-1}, NH group at wave number 3435.83 cm^{-1}, functional group C=O at wave number 1634.32 cm^{-1}. Urea's FT-IR spectrum shows the presence of functional groups, ROR has a wavelength of 1152.10 cm^{-1}, the NH functional group is at a wavelength of 3199.50 cm^{-1}. functional group C=O at a wavelength of 1630.87 cm^{-1}. The 2:1 mol piperine-urea multicomponent spectrum (Appendix 1, Figure 20), shows the presence of the CH functional group at wave number 3008.95 cm^{-1}, ROR at wave number 1247.93 cm^{-1}, NH functional group at wave number 3435 .68 cm^{-1}, functional group C=Oat wave number 1633.84 cm^{-1}. In the multicomponent crystal, the characteristics of the infrared spectrum peaks arealmost the same as the infrared spectrum found in piperine and. From the FT IR analysis it can beconcluded that there was no significant chemical interaction between piperine and after the formation of the multicomponents, due to the similarity of the functional groups in the wave numbers.

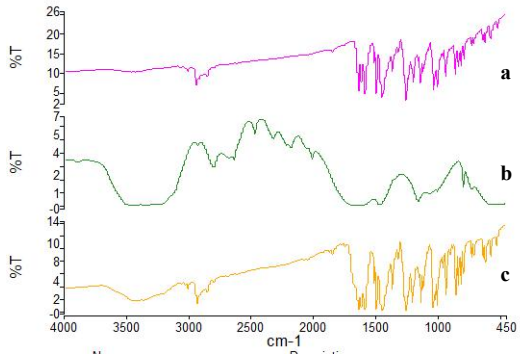
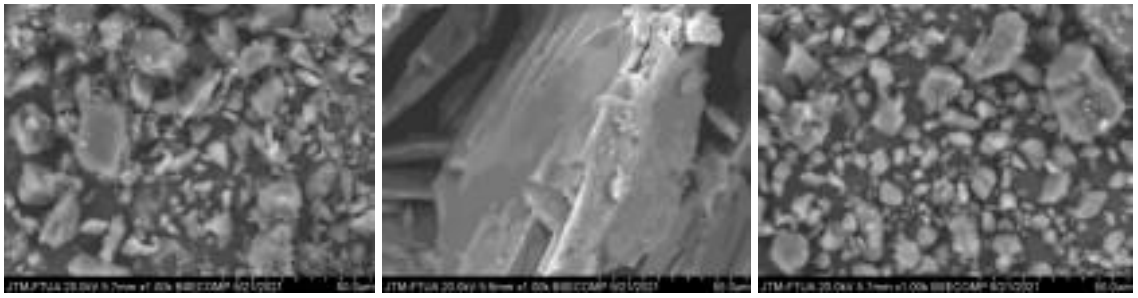


Figure 3. FT-IR Spectrum of (a) Piperine, (b) Urea, (c) multicomponent crystals Piperine-Urea (2:1)

Scanning Electron Microscopy (SEM) Analysis

Purpose of using Scanning Electron Microscopy (SEM) to view the surface morphologyof a sample microscopically and provide information about the surface texture of the sample. Themorphology of a sample can be seen from three sides, namely: the top surface, the side surface, and the inner surface. The results of particle shape analysis using *Scanning Electron Microscopy* (SEM) of piperine, urea and piperine urea multicomponent crystals 2:1 with a magnification of 1000 times can be seen in Figure 4. Micrographs of piperine, urea and multicomponent crystals showdifferent crystal yields. In piperine, the crystal habit is seen to be irregular, in urea the morphology of the crystal habit is rod-shaped and the surface is flat, while the multicomponent piperine-urea 2:1 shows the crystal habit in the form of aggregates. This indicates that there is an interaction between piperine and urea in the form of multicomponent crystals which can affectthe morphology of each substance.



(a) (b) (c)

Figure 4. Micrograph of (a) Piperine, (b) Urea, (c) Multicomponent piperine - Urea 2:1 1000x magnification

In determining the dissolution profile in vitro looking at the amount of dissolved drug versus time from piperine pure, and the 2:1 piperine-urea multicomponent can be seen in Figure 5. The results of the analysis showed that the 2:1 piperine-urea multicomponent increased the dissolution rate. This can be seen from the percent dissolution of piperine in the 60th minute of 36.14% and the multicomponent piperine-urea 2:1 50.530%. These results indicated that there was an increase in the dissolution of the multicomponent crystal piperine urea 2:1 by 1.39 times compared to piperine.

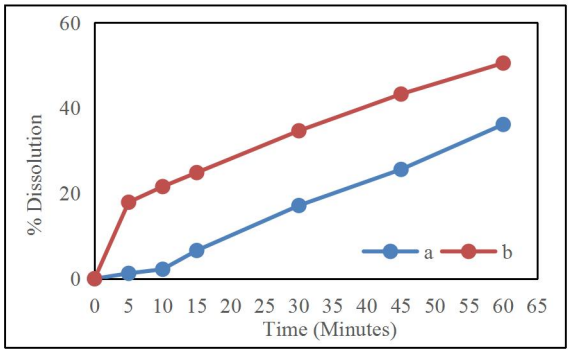


Figure 5. Dissolution profile of (a) Piperine, (b) multicomponent crystals Piperine-Urea (2:1)

CONCLUSION

The results of X-ray diffraction, DSC, FTIR and SEM showed that the multicomponent crystal piperine–urea 2:1 using the *solvent drop grinding* did not affect the physicochemical properties of piperine. Formation of a 2:1 pipein – urea multicomponent crystal gave an increase in the dissolution rate of 2:1 multicomponent piperine urea crystals by 1.39 times compared to pure piperine.

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