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Original Research Article

Characterization of Physicochemical Properties and Dissolution Studies of Multicomponent Crystals of Piperine and Glutamic Acid

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ABSTRACT

Piperine, a secondary metabolite compound, has a low solubility in water. In this study, through the formation of multicomponent crystals, glutamic acid is employed as a coformer to enhance the physicochemical characteristics and improve the dissolution rate of piperine. Solvent drop grinding was used to create multicomponent crystals using molar ratios of piperine and glutamic acid of 1:1 (F1), 1:2 (F2), and 2:1 (F3). Characterization of the solids' properties was performed using X-ray diffraction (XRD) analysis, differential scanning calorimetry (DSC), Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and dissolution profiles in accordance with the United States Pharmacopoeia (USP), facilitating a comparison with the physical mixture and pure piperine. XRD results showed a decrease in intensity, and DSC showed a decrease in the endothermic peak transition temperature and significant decreases in enthalpy value. The FT-IR spectra showed a change in wave numbers, and no new functional groups were formed. SEM revealed changes in particle morphology forming new crystal habits. When contrasted with the physical mixture and pure piperine, the dissolution profiles of multicomponent crystals F1, F2, and F3 demonstrate increased dissolution rates, F2 exhibiting a 1.57-fold increase, followed by 1.44-fold for F1 and 1.49-fold for F3. Multicomponent crystals F1, F2, and F3 show a better dissolution profile than the physical mixture and pure piperine, with dissolved substance percentages of 63.60±6.56, 68.59±2.53, 65.25±12.10, 44.91±10.95, and 43.58±8.92, respectively, after 60 minutes at 37±0.5 ℃. The multicomponent crystals produced using solvent drop grinding exhibited enhanced physicochemical features and an increased dissolution rate.

Keywords: Multicomponent crystals, Piperine, Glutamic acid, Dissolution profile.

Introduction

A medication will have a therapeutic effect when dissolved and can pass through a membrane. A drug's pharmacokinetic profile, which includes absorption, distribution, metabolism, and excretion, will be correlated with its solubility. Because of their low solubility, some medications require specific formulation to improve their bioavailability and therapeutic activity.[1] Around 40% or more of available drug candidates have low solubility in water.[2] One technique to increase solubility is to form multicomponent crystals,[3] which can enhance dissolution rate, stability, hygroscopicity, and crystallinity.[4,5,6] Multicomponent crystals are a product of crystal engineering, a combination of drug molecules with other molecules, known as coformers, in a crystal lattice.[7,8] Multicomponent crystals are formed based on non-covalent interactions, such as hydrogen or ionic bonds, between drug molecules and coformers,[9,10] and are designed with stoichiometric ratios to offer better physicochemical properties than the drug.[11]

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To determine whether a multicomponent crystal is a salt or a cocrystal, its characteristics are predicted using supramolecular synthons and the difference in pKa values between the active ingredient and the coformer.[12]

The Biopharmaceutical Classification System (BCS) groups piperine into BCS class II, defining it as poorly soluble in water.[13,14] Piperine ($C_{17}H_{19}NO_3$) is nearly insoluble in water (40 mg/L at 18 ºC), and has a pKa value of 12.2 and a molecular weight of 285.34 g/mol. The fruit and roots of *Piper nigrum* L. and *Piper longum*, both members of the Piperaceae family, contain the alkaloid compound piperine. Black pepper, or *P. nigrum* L., is a native Indian plant used as a spice and piperine gives black pepper its spicy flavor.[15] In traditional medicine, black pepper fruit is commonly used to treat digestive disorders such as diarrhea, and can also be used to treat respiratory disorders, flu, fever, and asthma.[16]

Glutamic acid is a coformer with a molecular weight of 147.13 g/mol, a melting point of 247−249 ºC, and pKa values of 2.16 and 9.58. Glutamic acid has a low isoelectric point and can readily capture electrons. As an a-amino acid, it bears carboxylic acid and NH_2 groups and has a side chain bearing an additional carboxylic acid group that is polar and readily deprotonated.[17] With these properties, inert glutamic acid can form hydrogen bonds and act as a coformer.

Piperine formulations were prepared in the form of multicomponent crystals, using glutamic acid conformer, in molar ratios of 1:1 (F1), 1:2 (F2), and 2:1 (F3) using a solvent drop grinding technique. It is anticipated that the multicomponent crystals will enhance piperine's physicochemical characteristics and dissolution profile. X-ray diffraction (XRD) analysis, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and dissolution profile analysis were used to

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characterize the piperine-glutamic acid multicomponent crystals produced. These results were compared with those of the physical mixture and pure piperine.

Materials and Methods

Materials

Piperine (Boschi, USA), glutamic acid (Tokyo Chemical Industry, Japan), distilled water (PT Novalindo, Indonesia), ethanol (PT Brataco, Indonesia), potassium dihydrogen phosphate (KH_2PO_4) (Merck, Germany), and sodium hydroxide (NaOH) (Merck, Germany) were utilized in this research.

Preparation of multicomponent crystal

Piperine-glutamic acid mixtures were prepared in molar ratios of 1:1 (F1), 1:2 (F2), and 2:1 (F3) and were ground with a little ethanol solvent in a mortar until a homogeneous and dry mass was obtained. The multi-component crystals formed were stored in a desiccator prior to characterization.[18,19,20]

Preparation of the physical mixture

Piperine and glutamic acid were weighed, combined in a 1:1 molar ratio, mixed in a mortar until homogeneous, and stored in a desiccator prior to characterization.[21]

X-ray diffraction (XRD) analysis

A Philips X'Pert Pro-PAN Analytical X-ray diffractometer (Netherlands) was used with a vertical goniometer, Cu target metal, Kα-filter, 40 KV voltage, and 30 mA current radiation dispersed throughout the sample's crystal region. At room temperature, the patterns were acquired at an angle of 2θ 10º to 80º with a detector resolution of 0.04º.[22]

Differential scanning calorimetry (DSC) analysis

Analysis was carried out with a Setaram DSC 131 Evo (France). Samples of 5 mg were weighed and heated at a rate of 20 ºC/min in an aluminum pan between 30 and 300 ºC. The thermogram curve displays the thermal transitions characteristic of the sample.[20]

Fourier transform infra-red (FTIR) analysis

An FT-IR spectrophotometer (Perkin Elmer, USA) was used. After grinding with KBr powder, the samples were vacuum pressed into a disc in a die mold and analyzed in the wave number range 400–4000 cm^{-1}. The FT-IR spectrum displays absorbance bands characteristic of the sample chemical functionality and molecular interactions.[23]

Scanning electron microscopy (SEM) analysis

Analysis was conducted utilizing a Hitachi S-3400N scanning electron microscope (Japan). The sample was placed on a gold-coated aluminum sample holder 10 nm thick and was examined under 1000x magnification. The current was 12 mA, and the voltage was 20 kV. The microscope images provide visual analysis of the particle surface morphology.[23]

Dissolution profile determination

A Copley Scientific NE4-COPD type II apparatus (England) was used to perform the dissolution tests according to United States Pharmacopoeia (USP) specifications. Phosphate buffer solution (900 mL, pH 7.4) was utilized as the dissolution medium at 37 ± 0.5 °C with stirring at 100 rpm. A sample of 5.0 mg piperine was added to the dissolution container once constant temperature was reached. Samples of the dissolving solution (5 mL) were pipetted at 5, 10, 15, 30, 40, and 60 minutes. A UV-vis spectrophotometer (Shimadzu UV 1800, Japan) was used to determine the dissolved piperine concentration.

Results and Discussion

Multicomponent crystals are classified into solvates/hydrates, salts, and cocrystals.[24] The ΔpKa value (the difference between the base pKa and the acid pKa)[24] can be used to predict the formation of multicomponent crystals by glutamic acid and piperine. With a ΔpKa value of 10.06, the multicomponent crystal formed between piperine and glutamic acid is a salt in which piperine is a weak base and glutamic acid is a weak acid. Drug substances and conformer mixtures with a ΔpKa value ≥1 will form a salt due to complete proton transfer under absolute ionization. Proton transfer can be disregarded when the Δpka is less than 1, when non-ionic interactions cause a cocrystal to form.[24,25] Compared to the physical mixture and pure piperine, the multicomponent crystal characterization data supports these results.

X-ray diffraction

The XRD diffractograms in Figure 1 reveal clear differences in crystalline properties between the multicomponent crystals, the physical mixture, and their components.[26] At an angle of 2θ, the piperine diffractogram displays a crystalline solid and characteristic interference peaks of sharp intensity at 12.9236°, 14.1586°, 14.7176°, 19.6316°, 21.3996°, 22.3096°, 22.5046°, 25.8066°, and 28.2636° with respective intensities of 1367.949, 1505.129, 4414.655, 2046.36, 1793.958, 3273.426, 3262.295, 5214.266, and 1894.802.[11,20,27] Similarly, the diffractogram of glutamic acid shows typical interference peaks of sharp intensity at 2θ angles of 10.2456°, 20.5416°, 21.3866°, 26.4956°, 33.6586°, and 35.6216° with respective intensities of 4095.515, 4915.181, 2309,012, 3494,697, 3106.107, and 2749.111.

The diffractograms of the physical mixture and multicomponent crystal reveal the diffraction patterns of piperine and glutamic acid. However, the multicomponent crystal exhibits reduced peak intensities compared to the individual components and the physical mixture. A reduction in intensity indicates the formation of a multicomponent crystal, with a reduction in pure piperine crystallinity with salt formation. The salt form will dissolve more quickly due to a lower energy requirement than the base form. This data suggests that no new crystal phase is formed.[28]

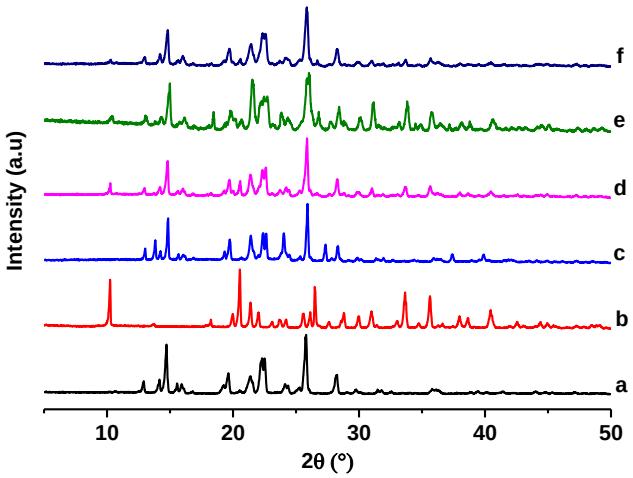

Figure 1: XRD diffratograms of (a) piperine, (b) glutamic acid, (c) their physical mixture, and the multicomponent crystals (d) F1(1:1), (e) F2 (1:2), and (f) F3 (2:1).

Differential scanning calorimetry

DSC reveals crystal physicochemical characteristics, including enthalpy and heat capacity, measuring the heat absorbed or released during thermal phase transitions.[29] Table 1 and Figure 2 display the DSC thermogram curves for the multicomponent crystal, the physical mixture, and their components.

The piperine thermogram displays a distinct and pure endothermic peak with an enthalpy of 80.221 J/g and a melting point of 132.223 °C, suggesting that the piperine sample is in a stable crystalline form.[20,27] The glutamic acid thermogram displays an endothermic peak at 209.812

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°C with an enthalpy of 345.654 J/g. The physical mixture exhibits a broad endothermic peak at 97.463 °C with an enthalpy of 29.009 J/g. Multicomponent crystals F1, F2, and F3 show a sharp decrease in the endothermic peak with low enthalpy values, F1 with an endothermic peak at 131.784 ℃ and enthalpy of 29.927J/g, F2 with an endothermic peak at 131.698 °C with enthalpy of 29.086 J/g, and F3 with an endothermic peak at 131.888 ºC and enthalpy of 55.529 J/g. Comparison of these values indicates that piperine and glutamic acid have formed multicomponent crystals. Due to changes in thermodynamic properties like high free energy, increased molecular mobility, and diminished intermolecular interactions, a lower melting point confers increased solubility to piperine.[26]

Table 1: Thermal analysis results of piperine, glutamic acid, their physical mixture, and multicomponent crystals.

Compound	Melting point (ºC)	Enthalpy (J/g)
Piperine	132.233	80.221
Glutamic acid	209.812	345.654
Physical mixture	97.463	29.009
F1 (1:1)	131.784	29.927
	217.995	64.641
F2 (1:2)	131.689	29.086
	215.010	130.070
F3 (2:1)	131.888	55.529
	218.672	53.796

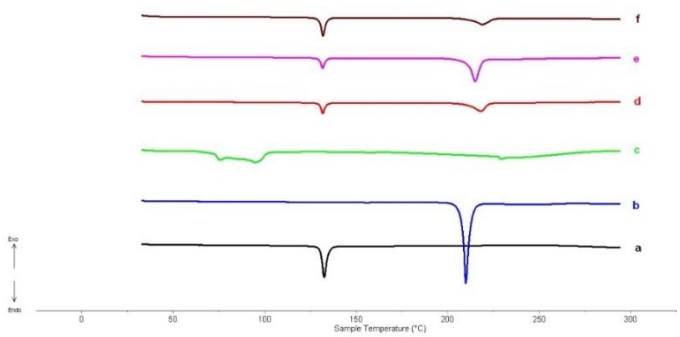

Figure 2: DSC thermograms of (a) piperine, (b) glutamic acid, (c) their physical mixture, and the multicomponent crystals (d) F1(1:1), (e) F2 (1:2), and (f) F3 (2:1).

Fourier transform infrared spectroscopy

FT-IR analysis identifies functional group modifications in a compound by comparing them with the fingerprint spectra of standard compounds. The energy needed for a bond to vibrate is revealed by the power of infrared radiation applied, and the condition of the molecules will be impacted by the amount of energy absorbed.[24,30]

Table 2 and Figure 3 illustrate the FT-IR spectra for the multicomponent crystal, the physical mixture, and their components. The FT-IR spectrum of piperine displays the typical bands of its functional groups, namely aromatic C-H stretching at 2941 cm^{-1}, aliphatic at C-H 2861 cm^{-1}, C=O at 1635 cm^{-1}, aromatic C=C at 1449 cm^{-1}, and C-O at 1256 cm^{-1}.[27,22] Glutamic acid displays typical functional group bands, specifically the carboxylic acid O-H at 3003 cm^{-1}, the N-H group at 3455 cm^{-1}, and C=O at 1681 cm^{-1}.[31,32] The FTIR spectra of the physical mixture and multicomponent crystals show the functional groups of piperine and glutamic acid and that no new functional groups are formed. Following the formation of multicomponent crystals, it can be inferred that there is no substantial chemical interaction between glutamic acid and piperine because their functional group absorbances are similar.[25,33]

Scanning electron microscopy

SEM reveals a sample's surface morphology and texture.[23,28,34] Figure 4 shows the SEM images for the multicomponent crystal, the physical mixture, and their components at the same 1000x magnification. The piperine micrograph reveals a rod-shaped crystalline solid,[20,22] and the glutamic acid micrograph a large block with a flat surface. The physical mixture reveals an irregular crystal block but still shows the characteristics of piperine and glutamic acid. However, the multicomponent crystals F1, F2, and F3 resemble rods which form aggregates, a new crystal habit different from its single compounds. This indicates that multicomponent crystals have formed.

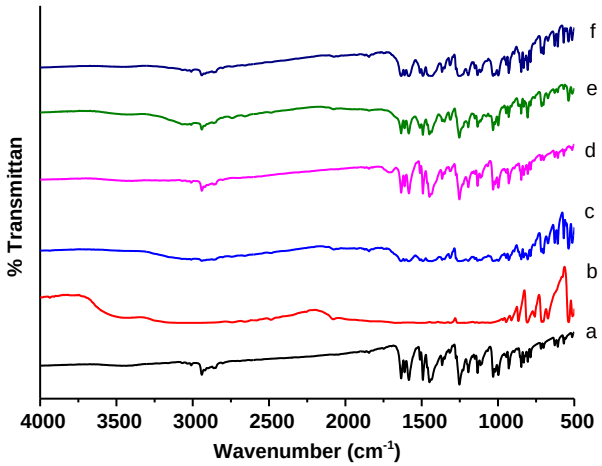

Figure 3: FT-IR spectra of (a) piperine, (b) glutamic acid, (c) their physical mixture, and the multicomponent crystals (d) F1(1:1), (e) F2 (1:2), and (f) F3 (2:1).

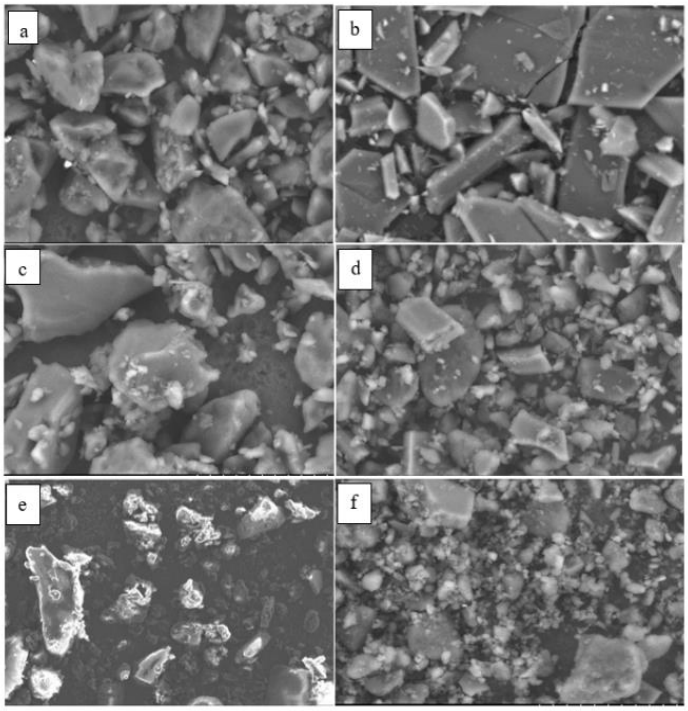

Figure 4: SEM micrographs of (a) piperine, (b) glutamic acid, (c) their physical mixture, and the multicomponent crystals (d) F1(1:1), (e) F2 (1:2), and (f) F3 (2:1) at a 1000x magnification.

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Table 2: FT-IR spectral analysis data of piperine, glutamic acid, their physical mixture, and multicomponent crystals F1 (1:1), F2 (1:2), and F3 (2:1).

Functional Group	Wave number (cm^{-1})					
	Piperine	Cinnamic acid	Physical mixture	F1	F2	F3
NH		3435		3428	3436	3449
OH		3003		3009	3011	309
CH aromatic	2941		2941	2942	2941	2944
CH aliphatic	2861		2965	2966	2966	2967
C=O	1635	1681	1635	1635	1635	1636
C=C aromatic	1449		1449	1449	1448	1448
CO	1256		1263	1254	1255	1261

Dissolution study

Figure 5 shows the solubility curves for the multicomponent crystal, the physical mixture, and piperine. Compared to pure piperine and the physical mixture, the multicomponent crystals exhibit higher solubility and a higher dissolution rate. At 60 minutes, the dissolution results indicate the proportion of dissolved materials of piperine at 43.58±8.92%, the physical mixture at 44.91±10.95%, and the multicomponent crystals F1 at 63.60±6.56%, F2 at 68.59±2.53%, and F3 at 65.25±12.10%. Multicomponent crystal F2 showed the most significant 1.57-fold increase in dissolution rate, followed by F1 at 1.44-fold, and F3 at 1.49-fold. Hence, the multicomponent crystals have a better dissolution profile than pure piperine and the physical mixture. The elevated dissolution rate may result from diminished intensity and alterations in the thermodynamic properties of piperine, in which the multicomponent crystals with lower melting points exhibit weak lattice energy, enhancing solubility and dissolution rate.[21,27]

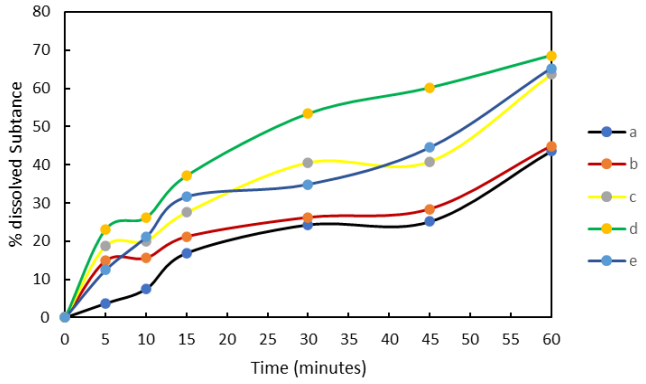

Figure 5: Solubility curves (% substance dissolution) of (a) piperine, (b) the physical mixture of piperine and glutamic acid, and the multicomponent crystals (c) F1(1:1), (d) F2 (1:2), and (e) F3 (2:1).

Conclusion

XRD, DSC, FT-IR, and SEM confirm the successful formation of the multicomponent crystals. Compared to pure piperine and the physical mixture of piperine and glutamic acid, the multicomponent crystals generated by solvent drop grinding demonstrated an increase in the dissolution rate.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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