Formation And Characterization Of Ketoprofen-L-Arginine Co-Amorph Using Neat Grinding Method And Its Effect On Ketoprofen Dissolution Rate

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ABSTRACT

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic properties. Ketoprofen belongs to Biopharmaceutics Classification System (BCS) class II, characterized by high permeability and low solubility. This condition limits the drug absorption process and consequently results in a delayed therapeutic effect. Therefore, efforts are required to improve the solubility and dissolution rate of ketoprofen. This study aimed to enhance the dissolution rate of ketoprofen through the formation of ketoprofen–l-arginine co-amorphous systems at molar ratios of 1:1, 1:2, and 1:3. The co-amorphous systems were prepared using the neat grinding method and characterized by Powder X-Ray Diffractometry (PXRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) spectroscopy, polarized light microscopy analysis, solubility testing, and dissolution rate profiling. The results indicated the successful formation of ketoprofen–l-arginine co-amorphous systems. DSC thermogram analysis showed glass transition temperature in the co-amorphous systems (1:1, 1:2, and 1:3) with glass transition temperatures of 128.96°C, 121.70°C, and 124.24°C, respectively. Spectra of three co-amorphous samples exhibit a combination of functional groups from both ketoprofen and L-arginine. Solubility testing demonstrated an increase of 3.38-, 3.21-, and 2.95-fold for the respective formulations. All ketoprofen–l-arginine co-amorphous samples were able to significantly enhance the dissolution rate compared with pure ketoprofen ($p<0.05$). Co-amorphous ketoprofen-l-arginine at a molar ratio of 1:1 showed the best dissolution rate results at 60 minutes with a value of 92.80%.

Keywords: Ketoprofen, L-arginine, co-amorphous system, dissolution rate

INTRODUCTION

Ketoprofen belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs) and is widely used as an anti-inflammatory, analgesic, and antipyretic agent (Miles et al., 2018). Ketoprofen is classified under Biopharmaceutics Classification System (BCS) class II due to its high permeability and low solubility. Its very low aqueous solubility (1:100,000) (USP, 2007) results in slow absorption, which consequently delays the onset of its therapeutic effect.

Crystal engineering plays an important role in improving various physicochemical properties of pharmaceutical solids. A variety of new single-component or multicomponent solid forms can be developed using crystal engineering approaches, including the formation of polymorphs, solvates/hydrates, salts, cocrystals, and eutectics, to modify physicochemical properties (Thakur et al., 2020).

Another technique to enhance the dissolution rate is the formation of co-amorphous systems. Co-amorphous systems consist of a combination of two or more low molecular weight active pharmaceutical ingredients that form a homogeneous single-phase amorphous system. These systems exhibit a three-dimensional disordered structure that can potentially improve the dissolution rate. Combined with higher internal energy and reactivity, drugs in co-amorphous systems may exhibit increased solubility, which in turn enhances drug absorption (Beyer et al., 2016). Over the past few decades, an increasing number of studies have reported on co-amorphous drug delivery systems, demonstrating promising and satisfactory results in improving both in vitro and in vivo performance of poorly water-soluble drugs (Jiawei et al., 2020).

In this study, a co-amorphous system of ketoprofen was prepared using L-arginine as a coformer. L-arginine is considered inert, meaning

it is chemically non-reactive and stable under normal temperature conditions, and it has low toxicity. The use of L-arginine in a co-amorphous system is expected to enhance the solubility of ketoprofen. The resulting co-amorphous system will be characterized using Differential Scanning Calorimetry (DSC), X-ray diffraction analysis, FT-IR spectroscopy, polarized light microscopy, particle size analysis, recovery studies, solubility testing, and dissolution rate evaluation.

METHODS

Materials

The equipment used in the formulation includes Digital balance (Shimadzu AUX-220, Japan), hot plate, magnetic stirrer, Differential Scanning Calorimetry (Shimadzu DSC-60 Plus, Japan), powder X-ray diffractometer (Rigaku RINT-2500), Scanning Electron Microscope (JEOL JSM-6360LA, Japan), dissolution rate testing apparatus (SR8 Plus Dissolution Test Station, Hanson Virtual Instrument), UV-Visible spectrophotometer (Shimadzu, Japan), orbital shaker, nylon filter paper (Agilent, 0.2 µm), desiccator, aluminum foil, volumetric pipettes, beakers, Erlenmeyer flasks, syringes, and other standard laboratory glassware. The materials used in this research are ketoprofen (Boc Science), l-arginine (Sigma Aldrich), acetonitrile pro analysis hplc grade (Merck), CO_2-free distilled water, methanol (pro analysis grade, Merck, Germany), double-distilled water (Ikapharmindo Putramas, Indonesia), and phosphate buffer (Merck).

Method

Preparation of Co-Amorphous Ketoprofen

Ketoprofen and L-arginine were prepared in equimolar ratios of 1:1, 1:2, and 1:3. The initial step involved weighing 0.254 g of ketoprofen and 0.174 g, 0.348 g, and 0.522 g of L-arginine, respectively. The weighed materials were then transferred into a mortar and ground for 15 minutes. After grinding, the resulting mass was placed into a closed container and stored in a desiccator.

Differential Scanning Calorimetry (DSC) Analysis

The DSC analysis was initiated by weighing approximately 5 mg of the sample and placing it into a sealed aluminum pan. The DSC instrument was programmed over a temperature range of 30–250°C with a heating rate of 10°C/min under a nitrogen flow of 20 mL/min. Endothermic and exothermic events were recorded. The analysis was conducted on pure ketoprofen, pure L-arginine, and the ketoprofen–L-arginine co-amorphous system. This analysis aimed to determine the melting point and identify the glass transition temperature (Tg), which is characteristic of co-amorphous systems.

X-ray Diffraction (XRD) Analysis

X-ray diffraction analysis was performed on pure ketoprofen, pure L-arginine, and the ketoprofen–L-arginine co-amorphous system using a PANalytical MPD diffractometer. The measurement conditions were as follows: Cu metal target, Kα filter, voltage 45 kV, current 40 mA, and a scanning range of 2θ from 5° to 45°. XRD analysis was conducted to determine the material properties of the co-amorphous samples, including structure, size, and strain.

Fourier Transform Infrared (FT-IR) Spectroscopy

Samples of pure ketoprofen, pure L-arginine, and the ketoprofen–L-arginine co-amorphous system were analyzed using an infrared spectrophotometer. Approximately 1–2 mg of sample powder was mixed with 10 mg of KBr in a mortar and ground until homogeneous. The mixture was then transferred into a mold and compressed under a pressure of 800 kPa to form an airtight disc. This analysis produced spectra representing the functional groups of each sample. FT-IR analysis aimed to obtain spectra resulting from the absorption of infrared radiation, expressed as peaks at specific wavenumbers that identify functional groups in the co-amorphous system.

Solubility Test

The solubility test was conducted on pure ketoprofen and the ketoprofen–L-arginine co-amorphous system by preparing saturated solutions. Each sample, equivalent to 50 mg of ketoprofen, was placed into a 100 mL Erlenmeyer flask and made up to volume with 100 mL of CO_2-free distilled water. The test was performed for 24 hours using an orbital shaker at 25°C and 100 rpm. The samples were then filtered using a 0.2 µm nylon membrane filter and analyzed using a UV-Visible spectrophotometer at the maximum wavelength of ketoprofen. The experiment was conducted in triplicate.

Dissolution Profile Determination

Dissolution profile testing was carried out for pure ketoprofen and the ketoprofen–L-arginine co-amorphous system. The dissolution vessel was filled with 900 mL of phosphate buffer (pH 6.8). The temperature was maintained at 37 ± 0.5°C with a rotation speed of 50 rpm. The samples were introduced into the dissolution medium. Aliquots of 5 mL were withdrawn at 5, 10, 15, 30, 45, and 60 minutes. At each sampling point, the withdrawn volume was replaced with fresh dissolution medium of the same volume and temperature. The absorbance of the samples was measured using a UV-Visible spectrophotometer at the maximum wavelength of ketoprofen. All experiments were performed in triplicate.

Data Analysis

The dissolution rate data were analyzed using a two-way ANOVA test. Recovery, solubility, and dissolution efficiency data were analyzed using a one-way ANOVA test.

RESULTS AND DISCUSSION

The prepared ketoprofen–L-arginine co-amorphous systems were characterized using Differential Scanning Calorimetry (DSC), X-ray diffraction, FT-IR spectroscopy, particle size analysis, solubility testing, and dissolution profiling using a UV-Visible spectrophotometer.

Differential Scanning Calorimetry (DSC) Analysis

DSC was used to observe changes in the thermodynamic properties of the samples upon the application of thermal energy, including melting, recrystallization, desolvation, and solid-state phase transitions. Based on the observations in Figure 1, changes in melting points were observed in the co-amorphous systems with ratios of 1:1, 1:2, and 1:3 of ketoprofen and L-arginine, each showing glass transition temperature (Tg). These shifts in melting points indicate the presence of interactions between ketoprofen and L-arginine. The decrease in melting point suggests a reduction in lattice energy, which may enhance the dissolution profile of the compound (Dwichandra et al., 2016).

In addition to the melting point changes, a decrease in the enthalpy of fusion (heat of fusion) was observed in the co-amorphous systems (1:1, 1:2, and 1:3). Enthalpy represents the amount of energy required for a substance to undergo melting. The enthalpy of fusion of ketoprofen and L-arginine were 74.96 mJ/g and 539.65 mJ/g, respectively, whereas the co-amorphous systems (1:1, 1:2, and 1:3) showed values of 48.05 mJ/g, 82.39 mJ/g, and 148.44 mJ/g, respectively. These results indicate that less energy is required to melt the co-amorphous systems compared to the pure active substances.

Based on the thermograms, the three ketoprofen–L-arginine formulations generally did not exhibit sharp endothermic peaks, indicating that the systems prepared using the neat grinding method at ratios of 1:1, 1:2, and 1:3 had transformed into co-amorphous forms. This observation is further supported by the presence of a glass transition (Tg) in the co-amorphous systems (1:1, 1:2, and 1:3), with transition temperatures of 128.96°C, 121.70°C, and 124.24°C, respectively, which are characteristic of amorphous materials. Glass transition refers to the temperature at which an amorphous material changes from a glassy state to a liquid-like state upon heating (Hancoock & Zografi, 1997; Alatas et al., 2020).

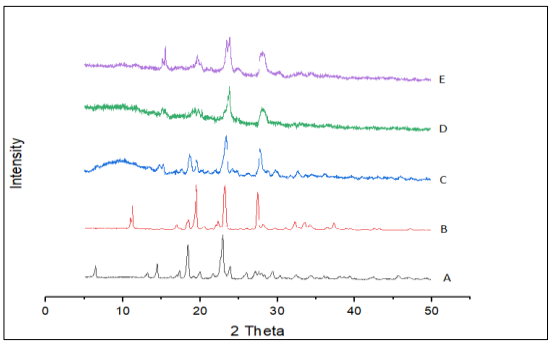

Figure 1. DSC thermograms of (A) ketoprofen; (B) L-arginine; (C) Keto–Arg 1:1; (D) Keto–Arg 1:2; and (F) Keto–Arg 1:3

X-ray Diffraction (XRD) Analysis

X-ray diffraction is a method used to characterize solid-state interactions between two components and to determine changes in the degree of crystallinity (Zaini et al., 2010). Based on Figure 2, a comparison of the diffractograms of ketoprofen and L-arginine with the co-amorphous ketoprofen–L-arginine systems at ratios of 1:1, 1:2, and 1:3 can be analyzed. The results showed no new diffraction patterns in the co-amorphous ketoprofen–L-arginine samples (1:1, 1:2, and 1:3). However, all three co-amorphous formulations exhibited a decrease in the intensity of X-ray diffraction peaks compared to pure ketoprofen and L-arginine.

The characteristic crystalline peaks observed in the three co-amorphous formulations indicate the formation of amorphous solids. This is evidenced by the reduction of sharp peaks in the X-ray diffraction patterns, which is a typical feature of amorphous materials. The decrease in sharp peaks in the diffraction patterns of the co-amorphous ketoprofen–L-arginine samples is attributed to intermolecular interactions between ketoprofen and L-arginine during the grinding process. This interaction prevents the molecules from rearranging into their respective crystal lattices, ultimately resulting in the formation of co-amorphous systems (Yamamura et al., 2010; Skieneh et al., 2017).

This phenomenon alters the crystal structure conformation of the samples, making it more disordered and reducing the rigidity of the solid state. The decreased rigidity leads to lower stability and contributes to enhanced solubility of the co-amorphous system due to the increased internal energy of the amorphous form (12).

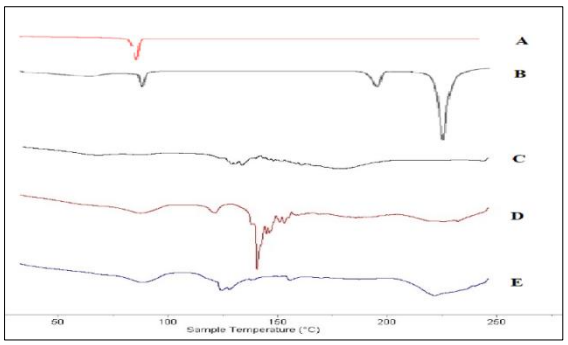

Figure 2. X-ray diffractograms of (A) ketoprofen; (B) L-arginine; (C) Keto–Arg 1:1; (D) Keto–Arg 1:2; and (E) Keto–Arg 1:3.

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

FT-IR is used to analyze chemical interactions between molecules in the solid state. Based on Figure 3, the FT-IR spectra allow comparison of functional groups among ketoprofen, L-arginine, and the three ketoprofen–L-arginine formulations (1:1, 1:2, and 1:3).

The spectra of the three co-amorphous ketoprofen–L-arginine samples show similar functional groups compared to those of pure ketoprofen and L-arginine. All three co-amorphous samples exhibit a combination of functional groups from both ketoprofen and L-arginine. This indicates the absence of new chemical interactions between ketoprofen and L-arginine in the co-amorphous systems.

The interaction between ketoprofen and L-arginine is most likely non-covalent, specifically hydrogen bonding, due to interactions between hydrogen atoms and electronegative atoms. These interactions may occur between the carboxylic acid group of ketoprofen and the amine group of L-arginine, or between the carboxylic acid groups of both ketoprofen and L-arginine (Jeffres & Saenger, 1996).

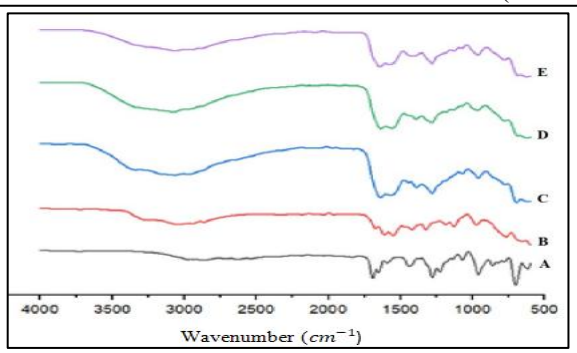

Figure 3. FT-IR spectra of (A) ketoprofen; (B) L-arginine; (C) Keto–Arg 1:1; (D) Keto–Arg 1:2; and (E) Keto–Arg 1:3.

Solubility Test

Solubility is an important property in the design of solid dosage forms. In this study, the three ketoprofen–L-arginine co-amorphous formulations were able to enhance the solubility of ketoprofen. The solubility data are presented in Table 1.

Table 1. Solubility data of ketoprofen, Keto–Arg 1:1, Keto–Arg 1:2, and Keto–Arg 1:3

Sample	Solubility ± SD (mg/100 mL)	Enhanced Solubility
Ketoprofen	$10,80^{a}$ ± 0,37	
Keto – L-arginine 1:1	$36,58^{c}$ ± 0,34	3.38
Keto – L-arginine 1:2	$34,72^{b,c}$ ± 2,11	3.21
Keto – L-arginine 1:3	$31,87^{b}$ ± 2,35	2.95

a, b, c : superscript showed a significant difference ($p<0.05$)

Based on the solubility test results, an increase in ketoprofen solubility was observed in the co-amorphous systems for all three formulations (1:1, 1:2, and 1:3), with respective increases of 3.38, 3.21, and 2.95-fold. The enhanced solubility of ketoprofen in the co-amorphous ketoprofen–L-arginine systems is supported by DSC and XRD analysis, which showed lower melting points and reduced crystallinity indices compared to pure ketoprofen, as presented in Tables 5 and 6.

The decrease in the degree of crystallinity in the co-amorphous ketoprofen–L-arginine systems indicates a less ordered molecular arrangement, resulting in lower energy requirements to break intermolecular bonds during the dissolution process compared to crystalline ketoprofen (Löbmann et al., 2012). Furthermore, the reduction in melting point in the co-amorphous systems relative to the pure compound leads to increased solubility but decreased stability, due to the reduction in intermolecular bonding energy, making the particles more readily soluble in water (Babu et al., 2011).

Dissolution Rate

Enhancing the dissolution rate of poorly water-soluble drugs is an effective approach to improve drug absorption in the gastrointestinal tract via oral administration. The dissolution profile is presented in Figure 4.

Based on the data in Table 2, the percentage of drug dissolved at 60 minutes for ketoprofen, ketoprofen–L-arginine 1:1, 1:2, and 1:3 were 29.91 ± 19.56, 88.62 ± 12.19, 71.90 ± 4.68, and 68.06 ± 0.53, respectively. The co-amorphous ketoprofen–L-arginine samples exhibited faster dissolution rates due to their higher internal energy, which facilitates interactions between particles and water molecules, thereby enhancing solubility (Jeffres & Sanger, 1997).

Dosage forms with higher solubility generally exhibit faster dissolution rates of the active pharmaceutical ingredient, whereas those with lower solubility show slower dissolution rates (Babu et al., 2011). In addition, co-amorphous systems can inhibit recrystallization of the pure drug upon contact with the dissolution medium due to molecular interactions between ketoprofen and L-arginine, preventing precipitation or incomplete dissolution of the drug (Jeffres & Sanger, 1997). The use of amino acids such as L-arginine as co-formers, which possess high solubility, can improve drug dissolution and stabilize the unfavorable properties of amorphous systems through intermolecular interactions between the drug and the co-former (Abdou, 1989).

The dissolution test results showed that the ketoprofen–L-arginine co-amorphous sample at a 1:1 molar ratio exhibited the highest dissolution rate compared with the other three samples. In several co-amorphous systems, improved physical stability has been attributed to the intimate molecular mixing between the active

pharmaceutical ingredient (API) and the co-former. At the 1:1 ratio, the intermolecular interactions between the API and the co-former are stronger and more stable, thereby facilitating an increase in the solubility of the API and, consequently, enhancing its dissolution rate (Löbmann et al., 2012).

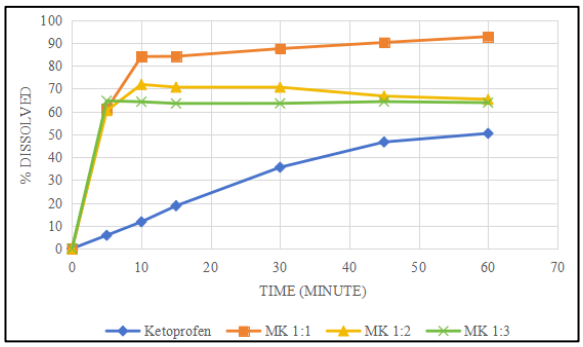

Figure 4. Dissolution rate profiles of ketoprofen, Keto–Arg 1:1, Keto–Arg 1:2, and Keto–Arg 1:3

CONCLUSION

In this study, it can be concluded that the co-amorphous system was successfully prepared using the neat grinding method. This finding was confirmed by the results of X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), and Fourier Transform Infrared (FT-IR) analyses. The ketoprofen–L-arginine co-amorphous system was shown to enhance both the solubility and dissolution of ketoprofen.

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