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Multicomponent Crystal of Fenofibric Acid- Saccharin: Characterization and Antihyperlipidemic Effectiveness

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ABSTRACT
Fenofibric acid is an active form of fenofibrate which has an antihyperlipidemic effect. Fenofibric acid belongs to BCS class II, which has low solubility and high permeability. The aim of this study was to form multicomponent crystal of fenofibric acid and saccharin that can increase the solubility and dissolution rate, as well as impact on its effectiveness. The ratio of the mixture of fenofibric acid and saccharin was determined using two phase diagram. Characterizations were carried out by Differential Scanning Calorimetry (DSC), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and the evaluations carried out are solubility, dissolution rate and antihyperlipidemic effectiveness. Based on the two phase diagram a mixture of fenofibric acid and saccharin produce eutectic point at 1:1 mole ratio. Multicomponent crystal showed a decrease in melting point with DSC analysis, no new crystalline phase was formed in X-ray diffraction analysis and did not show any chemical interactions in FTIR analysis. Multicomponent crystal showed an increase in the solubility and dissolution rate. The results of the effectiveness of antihyperlipidemic showed that multicomponent crystal was more effective than fenofibric acid.

Keywords: *Fenofibric acid, saccharin, multicomponent crystal, dissolution, antihyperlipidemia*

1. INTRODUCTION

Fenofibric acid is the active metabolite of fenofibrate, which in the body fenofibric is hydrolyzed to fenofibric acid [1]. Fenofibric acid as an antihyperlipidemia works by reduce blood triglyceride levels, lowering total cholesterol levels and decrease low density lipoprotein cholesterol [2]. Based on the Biopharmaceutical Classification System (BCS), fenofibric acid is classified as BCS class II because it has low solubility in aqueous media (0.3 µg/mL at 37 °C) and is highly lipophilic (log P = 5.2) [3]. Drugs with low solubility and high permeability frequently exhibit poor gastrointestinal absorption due to poor solubility of the drug in gastrointestinal fluids leading to low bioavailability of oral drugs [4-5].

Solubility and dissolution rate of active pharmaceutical ingredient are very important physicochemical properties in the development of quality solid dosage form and the effectiveness. The dissolution rate of the active pharmaceutical ingredient in the gastrointestinal medium will affect the absorption rate and bioavailability of the active substance in the systemic circulation (plasma) [6]. Several methods have been reported to increase the solubility of fenofibric acid in previous studies, including the formation of fenofibric acid salt [7], pelleting with the addition of magnesium carbonate [8], formation of ternary solid dispersion with hyaluronic acid and polyethylene glycol [9] and formation of surface solid dispersion with sodium starch glycolate [10]. One of the techniques that recently developed to modify solid state properties was the formation of multicomponent crystals.

Multicomponent crystals can improve the physicochemical and mechanical properties of drug compounds such as solubility, dissolution rate, compressibility and chemical-physical stability by modifying the crystal structure without changing its pharmacological activity [11]. Multicomponent crystals consist of solvates, hydrates, salts, cocrystals, and eutectic mixtures. Multicomponent crystals can be formed by selecting suitable excipients (coformers) that can interact with drug molecules to new crystals form [12]. Cocrystal and eutectic mixture are a material that includes non-covalent derivatives. An eutectic mixture is formed from two solids with the same molecular ratios that produce the minimum thermal point on the phase diagram [13]. At the eutectic point, there is a direct phase transition of all components from the solid to the liquid phase. The main advantage of eutectic mixtures is that can increase the dissolution rate of solid drug particles in a more thermodynamically stable crystalline form [14].

In this study, we were preparing a multicomponent crystals of fenofibric acid with saccharin as coformer. This multicomponent crystals form is aimed to increase

the solubility and dissolution of fenofibric acid so as to increase the effectiveness of fenofibric acid.

2. METHODS

2.1. Materials

Fenofibric acid was purchased from BOC Sciences (USA). Saccharin was obtained from Merck (Germany). Methanol and ethanol were obtained from Merck (Germany). Cholesterol reagent Greiner® was purchased from PT. Rajawali Nusindo (Indonesia). All other chemicals used were of analytical grade.

2.2. Preparation of multicomponent crystal of fenofibric acid-saccharin by two phase diagram

Fenofibric acid and saccharin were mixed homogeneously at a mole ratio of 0.1:0.9 to 0.9:0.1. Each mixture was thermally analyzed using Differential Scanning Calorimetry (DSC). A two-phase diagram is created by plotting the endothermic peaks of a binary mixture against the mole ratio. The resulting eutectic composition is further prepared in the form of multicomponent crystals by solvent evaporation method.

2.3. Physicochemical properties characterization

2.3.1. Differential Scanning Calorimetry analysis

Thermal analysis was performed using a differential scanning calorimetry (Shimadzu DSC 06, Japan). Each samples were sealed in an aluminium pan and heated in the temperature range of 30 °C to 300 °C with a heating rate of 10 °C/min.

2.3.2. X-ray diffraction analysis

X-ray diffraction analysis of the samples was carried out at room temperature using Rigaku diffractometer type RINT-2500 with CuKα monochromatic radiation source at 100 mA current and 40 kV voltage, the analysis was carried out in the range of 2 theta 5º – 35º. The sample was placed in a sample holder (glass) and leveled to prevent particle orientation during sample preparation.

2.3.3. Fourier transform infrared spectroscopy analysis

The sample was measured using an infrared spectrophotometer (Shimadzu IRTracer-100 AH, Japan) by dispersing the sample on a KBr plate which was compressed with high pressure (hydraulic press). The absorption spectra were recorded at a wavenumber of 4000-500 cm^{-1}.

2.4. Solubility Test

An excess amounts of fenofibric acid, multicomponent crystal and the mixture of fenofibric acid and saccharin were each added to 100 mL of distilled water in an erlenmeyer. The samples were shaken using sonicator at ambient temperature for 5 minutes and filtered by a membrane filter (0.45 µm). The concentration of fenofibric acid determined using HPLC (Shimadzu Prominence Analytical HPLC System, Japan) with an injection volume of 20 mL. The mobile phase was the mixture of acetonitrile and aquadest adjust to pH 3 (70:30, v/v) with a flow rate of 1 mL/min and the eluent was detected at 287 nm for the analysis of fenofibric acid.

2.5. Dissolution

Fenofibric acid, multicomponent crystal and the mixture of fenofibric acid and saccharin (equivalent to 105 mg drug) were each filled in hard gelatin capsules. The dissolution test was carried out using the basket method with speed of 50 rpm at 37 ± 0.5 °C in 900 mL of phosphate buffer medium pH 6.8 for 60 minutes. 5 mL solution in the flask was pipetted at 5, 10, 15, 30, 45, and 60 minutes. The samples were filtered by membrane filter (0.45 µm). The concentration of fenofibric acid in the filtrate was analysed by the HPLC method as described above.

2.6. Antihyperlipidemic activity study

The antihiperlipidemic effectiveness of multicomponent crystal was investigated using male Wistar rats (250–300 g). Rats were acclimatized for approximately 7 days, then induced with an atherogenic cocktail for 7 days. Rats were divided into three groups: the control group

(Na CMC suspension 1%), those receiving fenofibric acid (dose equivalent 1.89 mg/200 mg BW) and those receiving multicomponent crystal (dose equivalent 1,89 mg/200 mg BW). The drugs was given for 15 days with blood sampling on the 5th, 10th and 15th days. Total cholesterol levels of rats were measured using cholesterol reagent Greiner® and a photometer 5010 V5+. The experiment had been approved by the Ethics Committee of the Faculty of Medicine, Universitas Andalas No. 61/UN.16.2/Kep-FK/2020.

3. RESULTS AND DISCUSSION

The binary mixtures of fenofibric acid-saccharin in all mole ratios showed endothermic peaks in the temperature range of 170.38 – 220.48 °C. Binary mixture with ratio 0.1, 0.2, 0.3, 0.4 and 0.9 fenofibric acid showed two endothermic peaks. While the binary mixtures with ratio 0.5, 0.6, 0.7 and 0.8 fenofibric acid showed endothermic peaks at 171.92 °C, 171.95°C, 171.93 °C, and 172.21 °C, respectively. Figure 1 shows the binary mixture of fenofibric acid-saccharin 1:1 is the eutectic point with the lowest single endothermic peak.

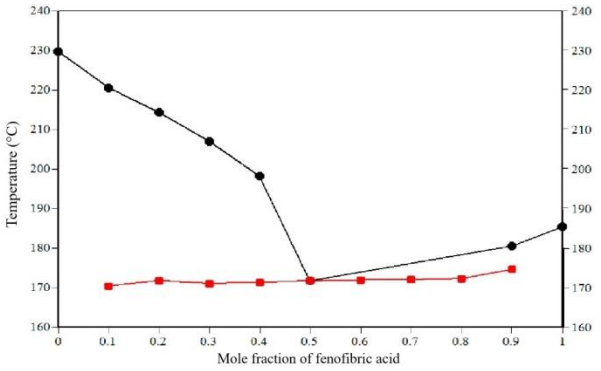

Figure 1 Two-phase diagram of eutectic mixture of fenofibric acid-saccharin

Based on the thermal analysis, there was a decrease in the melting point of the multicomponent crystal from the melting point of fenofibric acid (185.36°C) and saccharin (229.63°C) to 171.92°C as shown in Figure 2. The decrease of the melting point occurs due to the physical interaction between fenofibric acid and saccharin which forms the eutectic system. An eutectic mixture is a mixture consisting of molecules that are weakly bonded to each other, but do not show the formation of chemical interactions, the melting point of the eutectic mixture is lower than the melting point of either component, so that the melting point decreases from both sides of the diagram [15].

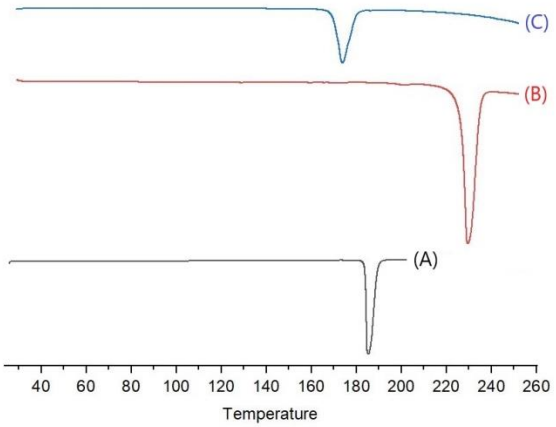

Figure 2 DSC thermogram of fenofibric acid (A), saccharin (B) and multicomponent crystal (C)

XRD is an analytical method to characterize the interaction between two solids and determine the formation of a new crystalline phase [16]. X-ray diffractogram analysis showed that fenofibric acid and saccharin were in crystalline form. It is characterized by the presence of distinctive and sharp peaks as shown in Figure 3. The specific interference peak for fenofibric acid at position 2 theta is 15.871, 18.428, 22.555 and 23.189 while for saccharin it was 15.871, 19.075, 22.555, and 23.189. The results of the diffractogram of multicomponent crystal fenofibric acid-saccharin compared with fenofibric acid and saccharin did not show any new peaks. This shows that the multicomponent crystal of fenofibric acid-saccharin do not form a new crystalline phase, but a conglomerate or a combination of two crystalline phases, each of which is in solid form or is called a simple eutectic mixture [17].

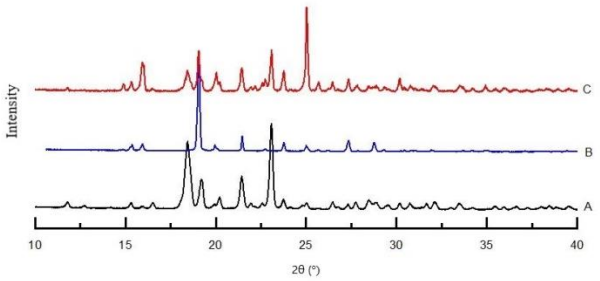

Figure 3 XRD pattern of fenofibric acid (A), saccharin (B) and multicomponent crystal (C)

FTIR analysis was performed to see the shift in the transmission band in the FTIR spectrum. Furthermore, the wavenumber data of the multicomponent crystals were compared with fenofibric acid and saccharin.

Figure 4 shows that the multicomponent crystal wavenumbers for fenofibric acid and saccharin experienced a slight shift but remained within the time range for a functional group. This spectrum shift occurs due to the presence of hydrogen bonds [18].

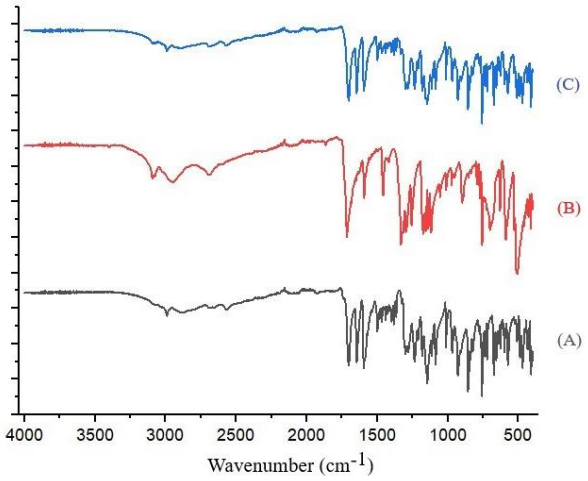

Figure 4 FTIR spectra of fenofibric acid (A), sachharin (B) and multicomponent crystal (C)

Table 1 Solubility data of fenofibric acid, physical mixture and multicomponent crystal

Sample	Solubility (mg/100 mL) ± SD	Solubility enhancing
Fenofibric acid	8.903±0.161	
Physical mixture	11.376±0.156	1.3 fold
Multicomponent crystal	16.110± 0.114	1.8 fold

Solubility data for fenofibric acid, physical mixture and multicomponent crystal are displayed in Table 1. The results showed an increase in the solubility of fenofibric acid by 1.8 fold for multicomponent crystal and 1.3 fold for physical mixtures. This occurs due to the formation of an eutectic mixture that has a minimum melting point which indicates a lower lattice energy and a decrease in the enthalpy of fusion which means a decrease in the degree of crystallinity of the compound. It also indicates a change in the crystal rigidity of the substance which causes the energy required to break it to be lower so that it dissolves easily [19,20].

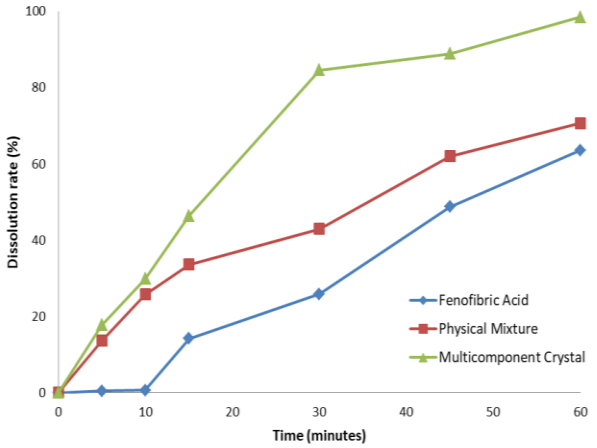

Figure 5 Dissolution rate profile of fenofibric acid, saccharin and multicomponent crystal

The results of the dissolution resemble the solubility test. Dissolution profiles are shown in Figure 5. The results showed that the percent dissolution of fenofibric acid dissolving at 60 minutes in fenofibric acid, physical mixture, and multicomponent crystal were 63.626±0.817%, 70.718±0.103%, and 98.509±0.523%, respectively. Where, multicomponent crystal had the highest percentage of dissolution. Increasing the dissolution rate of drug compounds that are poorly soluble in water is a very appropriate approach to increase the absorption of these drug compounds in the gastrointestinal tract through the oral route [21].

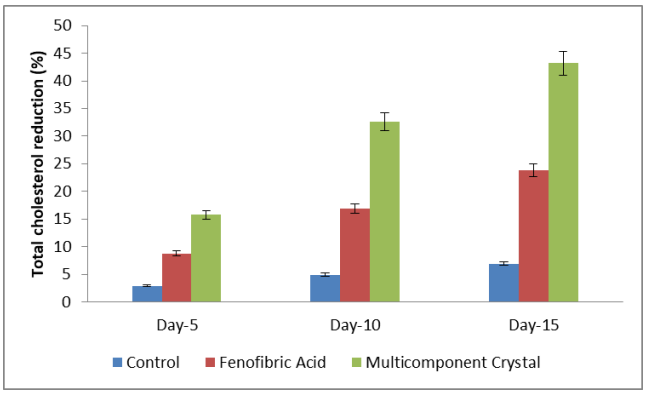

Figure 6 Total cholesterol reduction of rats against time of observation

To determine the correlation between solubility and dissolution rate of multicomponent crystal with antihyperlipidemic effectiveness, we conducted a study of antihyperlipidemic activity in rats. The results of total cholesterol levels in the group receiving multicomponent crystal were significantly reduced compared to the control group and the group receiving fenofibric acid. The results showed that total cholesterol reduction of rats

for 15 days in the group receiving fenofibric acid, physical mixture, and multicomponent crystals were 6.95%, 23.85%, and 43.2%, respectively. Based on the graph (Figure 6), it can be seen that the duration of administration of the test preparation also has an effect on decreasing total cholesterol levels.

The results prove that solubility and dissolution significantly impacted the in vivo antihyperlipidemic activity of fenofibric acid. Increased solubility and dissolution rate is required to achieve optimal bioavailability and effectiveness of poorly soluble drugs.

4. CONCLUSION

In this study, we conclude that the multicomponent crystal formed is an eutectic mixture. Multicomponent crystal fenofibric acid-saccharin increase the solubility and the dissolution of fenofibric acid. The effectiveness of multicomponent crystal increased by reducing total cholesterol levels in rats.

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