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Original Research Article

Improving the Solubility of Fenofibric Acid via Multicomponent Crystal Formation with Theobromine Coformer

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ABSTRACT

Fenofibric acid (FA) is a class II biopharmaceutical system drug, a potential drug for antihyperlipidemic. FA lowers low-density lipoprotein (LDL) and triglyceride levels and increases high-density lipoprotein (HDL). Due to its low solubility, the bioavailability of FA is also low. To overcome the undesirable effects of these biopharmaceutical properties, this study focused on improving the solubility of FA in the form of FA multicomponent crystals with theobromine (TH) coformer using solvent drop grinding as the crystallisation method. Multicomponent crystals of FA with TH coformer named FA-TH were successfully prepared. Detailed structural studies of this new solid form were carried out using powder X-ray diffraction (PXRD), Fourier Transform Infrared (FT-IR), Differential Scanning Calorimetry (DSC), Scanning Electron Microscope (SEM), and hot-stage microscopy. The thermogram of the DSC test showed that the melting point of FA-TH multicomponent crystals was lower than the melting point of the forming compound. The X-ray diffraction exhibits diffraction peaks of FA-TH multicomponent crystal and superimposition between the diffraction peaks of FA and TH. Solubility of FA-TH multicomponent crystals showed improvement up 4.4-fold compared to pure FA. These results demonstrate the potential of this new solid form to improve the solubility of FA.

***Keywords*:** Fenofibric acid, Theobromine, Multicomponent Crystals .

Introduction

Fenofibric acid is a new antihyperlipidemic drug that can reduce Low-Density Lipoprotein (LDL) and triglycerides and increase High-Density Lipoprotein (HDL). Fenofibric acid is the active form of fenofibrate and is more effective than fenofibrate because the absorption of fenofibric acid is better than fenofibrate.[1] Although the absorption of fenofibric acid is better, the solubility of fenofibric acid is minimal at 162.5 µg/mL. The largest dose of fenofibric acid is 105 mg, and 646 mL of water is needed to dissolve it. The drug substance is said to be soluble when the highest dose strength can dissolve in < 250 mL of water in the pH range of 1-7.5.[2]

The solubility of active pharmaceutical ingredients (APIs) is a problem when active pharmaceutical ingredients are developed because APIs with low solubility have slow dissolution and absorption rates. Currently, 90% of drug compounds in the research stage and 40% of drugs already in the market have poor water solubility.[3] So, it is necessary to increase the solubility of poorly soluble APIs.

Various efforts have been made to increase the solubility of FA, such as the addition of $MgCO_3$ and carrageenan catalysts,[4] formations of ternary solid dispersions with hyaluronic acid and polyethyleneglycol,[5] salt formation with choline bases, diethanolamine, tromethamine, calcium, ethanolamine, and piperazine,[6] formations of surface solid dispersions using sodium croscarmellose,[7] formations of self nanoemulsions,[8] multicomponent crystals of eutectic mixtures with nicotinamide and nicotinic acid coformers,[9,10] and formation of multicomponent crystals with saccharin coformers.[11]

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Increasing the solubility of active pharmaceutical ingredients by forming multicomponent crystals is a popular method nowadays because the APIs are produced in the form of crystalline phases. Efforts to increase the solubility of FA using theobromine coformer have never been reported. In this study, theobromine (TH) coformer was used. In addition to functioning as a coformer, theobromine can also reduce LDL cholesterol levels and can increase HDL levels,[12] so that it functions in line with the antihyperlipidemic properties of FA.

Theobromine has a hydrogen bond donor and is an important acceptor group for multicomponent crystal formation. Theobromine has been used as a coformer in crystal engineering of several medicinal substances such as sulfasalazine,[13] glimepiride,[14] daidzein,[15] and has proven to increase solubility. This research aims to study the effect of multicomponent crystal formation of fenofibric acid and theobromine as coformer (Figure 1) on physicochemical properties and its effect on solubility.

Materials and Methods

Materials

Fenofibric acid was purchased from BOC Sciences (New York, USA), and theobromine from Tokyo Chemical Industry (Tokyo, Japan). Acetonitrile and ethanol of HPLC grade (Merck -Darmstadt, Germany).

Preparation of FA-ACE Multicomponent Crystals

FA-TH multicomponent crystals were prepared using the solvent drop grinding method in a mole ratio of FA and TH 1:1. The mole ratio of 1:1 is a standard ratio used to prepare multicomponent crystals. 390 mg FA (1 mmol) and 180 mg TH (1 mmol) were ground in a mortar and pestle for 15 minutes in the presence of 3 drops of ethanol. The multicomponent crystals obtained were stored in a dessicator.

Differential Scanning Calorimetric Analysis

A sample of 5-7 mg (FA, TH, and FA-TH multicomponent crystals.) was placed in a crucible 10 µL, using DSC (Shimadzu DSC-60 plus Japan). The sample was heated and measured in the temperature range

of 30 - 250ºC, heating rate of 10ºC per minute. Nitrogen was used as a purge gas at a flow rate of 20 mL/min.

Powder X-ray Diffraction Analysis

X-ray diffraction analysis was performed at room temperature on FA, TH, and FA-TH multicomponent crystals. Using Panalytical PW 30/40 X-ray diffractometer (The Netherlands) measuring angle 2 teta 5º - 40º. The X-ray diffractometer was programmed as follows: target metal, Cu; filter, Kα; voltage, 45 kV; and current, 40 mA.

Fourier Transform-Infrared Spectroscopic Analysis

Samples of 3-10 mg were mixed with KBr and then compressed into pellets. The absorbance of the pellet was measured at wave numbers 4000 cm^{-1} - 600 cm^{-1}. Analysis was carried out on FA acid, TH, and FA-TH multicomponent crystals.

Scanning Electron Microscopic Analysis

The morphology of the crystal habit was studied using SEM analysis. The crystal was characterized using a Scanning Electron Microscope (HITACHI FLEXSEM 1000, Japan) at an accelerating voltage of 10 kV. The samples were placed in the sample holder and sprayed with a thin gold-palladium film. The measurement conditions were set to 10 kV and 12 mA.

Polarizing Microscope Analysis

The contact method was performed under a polarizing microscope equipped with a hot stage. A quantity of FA powder (melting point 185.8ºC) was placed on the cover glass and heated until it melts and re-crystallizes. TH powder (melting point 143.7ºC) was placed on the side border of the cover glass. Reheat until all the TH melts and comes into contact with the FA crystal surface. The contact zone between the FA solid and TH was observed under a polarizing microscope with 200 times magnification and recorded with a digital camera.

Solubility Test

FA and FA-TH multicomponent crystals were weighed in excess amounts (equivalent to 25 mg FA) and dissolved in 100 mL distilled water. It was stirred for 24 hours using an orbital shaker at room temperature.[6] The amount of soluble FA was analyzed using HPLC (Shimadzu, Japan) with a DAD UV-Vis detector. HPLC system; Pursuit XRS C18 4.6×150 column. Mobile phase acetonitrile: water pH 3 (70:30). FA retention time 6.187 min. The experiment was performed in triplicate.

Statistical Analysis

Data were expressed as mean+SD analysed with an independent student t-test with 95 confidence interval, n = 6, using Microsoft Excel package version 2021.

Results and Discussion

Multicomponent crystals are crystalline phases consisting of two or more molecules bound together in the crystal lattice through noncovalent interactions in a stoichiometric mole ratio.[16] Preparation of FA-TH multicomponent crystals using solvent drop grinding (SDG) method. FA and TH with a mole ratio of 1:1 were ground for 15 minutes with a mortar and pestle in the presence of a few drops of ethanol as a solvent. The mixture formed was analyzed using DSC, PXRD, SEM, FTIR, hot stage microscope, and solubility test using an orbital shaker. Thermal analysis using DSC (Differential Scanning Calorimetry) is a fast and simple analytical method to evaluate material properties when heated (melting, boiling, sublimation, evaporation, desolvation, solid transition, chemical decomposition). Changes in melting point indicate interactions between samples.[17] The thermogram overlay of the DSC test results (Figure 2) shows 3 endothermic peaks, respectively, the melting point endotherms of FA, TH, and FA-TH multicomponent crystals. The melting points of FA and TH were 185.86°C and 380.18°C, respectively, while the melting point of FA-TH multicomponent crystal was 182.11°C. The melting point of the FA-TH multicomponent crystal was lower than the melting point of the forming compound. This is an indication of the formation of a eutectic mixture.[18] The eutectic point was determined by a binary phase diagram. FA and TH were mixed in various mole ratios. The melting point of the binary mixture decreased gradually at the lowest point of 180 ^{O}C there was a FA TH ratio of 5:5, and this is the eutectic temperature of the binary mixture. Thermogram data of FA and TH binary mixtures can be seen in Table 1. The binary mixture diagram exhibited a V-shaped form that demonstrated the formation of a simple FA–TH eutectic mixture (Figure 7).

The DSC analysis results were confirmed by X-Ray Diffraction analysis. XRD analysis is one of the analyses used in the characterisation of a material. The result of this analysis is a diffractogram that shows the intensity of the peaks that are typical for each substance. Diffraction patterns are then compared to analyse the formation of a new crystalline phase.[19] The X-ray diffraction patterns for FA, TH, and multicomponent crystals can be seen in (Figure 3). The diffractogram of FA shows a solid material with sharp diffraction peaks and a high degree of crystallinity. The diffraction peaks of FA at angle 2Θ were 18.64, 23.40, and 23.50, the diffraction peaks of theobromine were 27.24 and 13.59, while the diffraction peaks of FA-TH multicomponent crystal are superimposition between the diffraction peaks of FA and TH (18.6; 23.40; 27.24 and 13.59) which appear with lower intensity values. Comparison of diffraction peaks and intensity values can be seen in Table 3. The PXRD pattern of the FA-TH multicomponent crystal shows no new diffraction peaks and indicates that it does not form a new crystalline phase, so it can be said that the FA and TH mixture is a eutectic mixture.[20]

FT-IR spectroscopic analysis is a method used to identify functional groups and determine the interaction of active pharmaceutical ingredients with excipients. This technique can show the functional groups responsible for chemical changes or noncovalent supramolecular interactions. This can be seen from the variation in peak shape and intensity of the resulting absorption spectrum.[21]

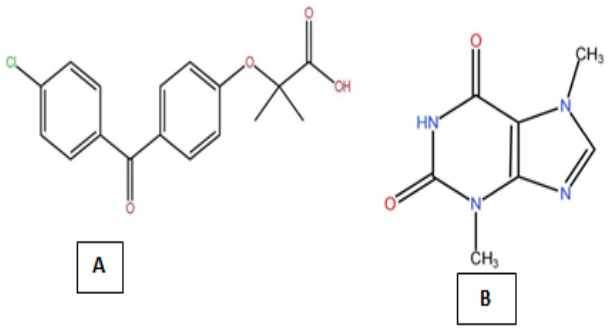

Figure 1: Molecular structure of (A) fenofibric acid (B) theobromine

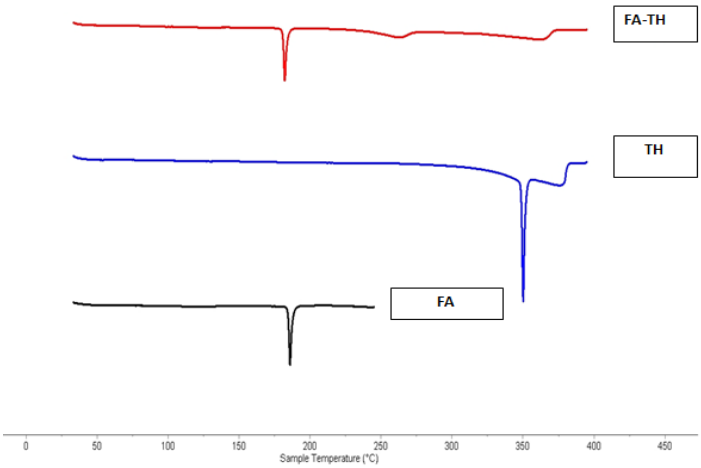

Figure 2: DSC thermogram FA, TH dan FA-TH multicomponent crystals

Each bond in a compound will absorb infrared, and the bond can experience stretching or bending vibrations. The results of the analysis are in the form of a spectrum of the relationship of the percentage of transmittance to the wavelength.[21] Figure 4 shows the wave number data of the FA-TH multicomponent crystal compared to the wave number data of its constituent components. FTIR spectra of FA-TH multicomponent crystals have no new peaks or missing peaks. This indicates that FA and TH do not interact chemically. Some studies have shown that slight changes in wave numbers suggest the presence of weak hydrogen bonding.[21]

To determine the shape and structure of particles of FA, TH, and FA-TH multicomponent crystals, a scanning electron microscope (SEM) analysis method was used. Scanning electron microscopy (SEM) was used to observe the morphology and topography of the multicomponent crystals formed microscopically.[22] The SEM test results can be seen in Figure 5. The habit of FA crystals is irregular cubic agglomerates with sharp edge structures. The habit of TH crystals is like aggregates, while the habit of FA-TH multicomponent crystals is cubic crystals scattered among aggregates. From the images of the test results with SEM, it can be seen that there has been a change in the crystal habit of the FA-TH multicomponent crystal due to the mixing process of the two solid materials, which causes physical changes in the material.[23]

Characterisation with a polarising microscope on a hot stage was used to evaluate the crystal habit in the zone between FA and TH (Figure 6). The sample was heated, and the solid phase FA melted at 185 °C, the contact zone at 182 °C, and TH solid at 380 °C. The recrystallised FA fused on side C, while TH fused at side A, while both sides show typical crystal habit. Side B is the contact zone between FA and TH, which has an empty black zone. This indicates no new crystalline formation, which is typical of eutectic mixtures.[24]

Solubility tests were performed for FA and FA-TH multicomponent crystals. The solubility test results in Table 2 show that the increase in solubility of FA-TH multicomponent crystals is 4.4 times more than the solubility of FA. The increase in solubility of FA-TH multicomponent crystals may be due to a decrease in crystallinity, which lowers the crystal lattice energy. This is evidenced by the melting point of FA-TH multicomponent crystals, which is lower than that of the original compound. A decrease in melting point indicates weaker lattice energy. Crystals with strong lattices have higher melting points and thus require greater heat. Solubility involves disruption of the crystal lattice so that the weaker the crystal lattice, the better the solubility.[25]

Table 1: Thermogram data of FA and TH binary mixtures

Mole ratio FA and TH	T_1 °C	T_2 °C
1:9	178	320
2:8	177	305
3:7	185	348
4:6	183	335
5:5	180	
6:4	186	
7:3	185	275
8:2	185	283
9:1	185	

Table 2: Solubility of FA and FA-TH multicomponent crystals

Compound	Solubility (µg/mL)	Increase in solubility
FA	88.16 ± 4,86	-
FA-TH	390.21 ± 5.23	4.4 fold

Solubility data are expressed as mean SD and analyzed with an independent t-test with a 95 confidence interval, n = 6, P< 0.05.

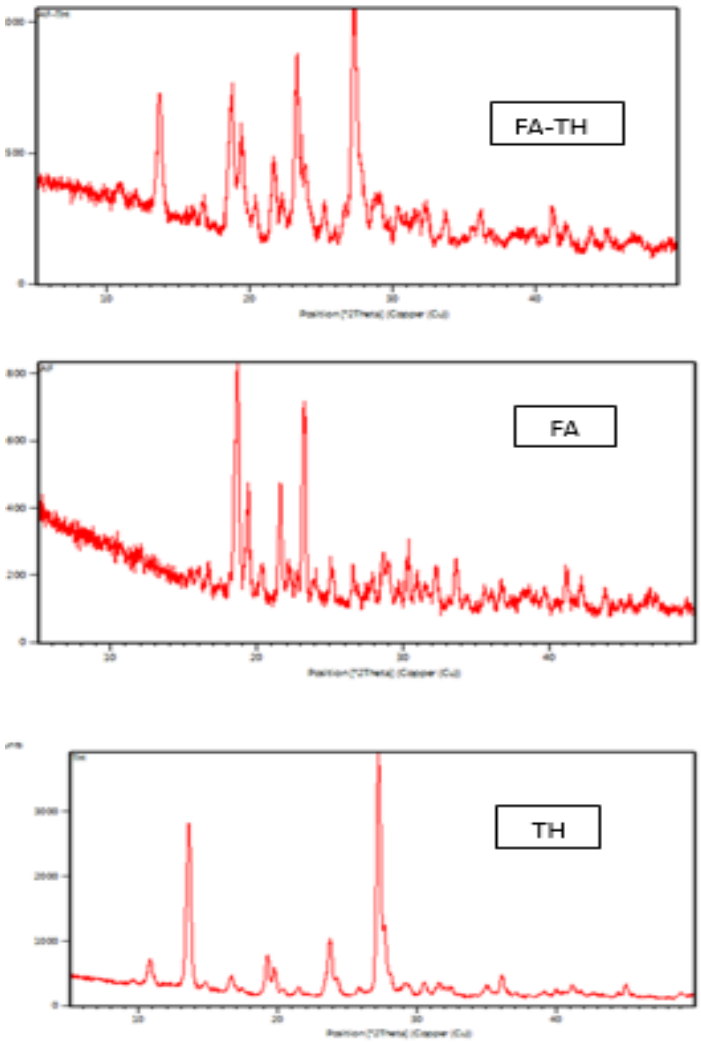

Figure 3: Diffractogram FA, TH, and FA-TH multicomponent crystals

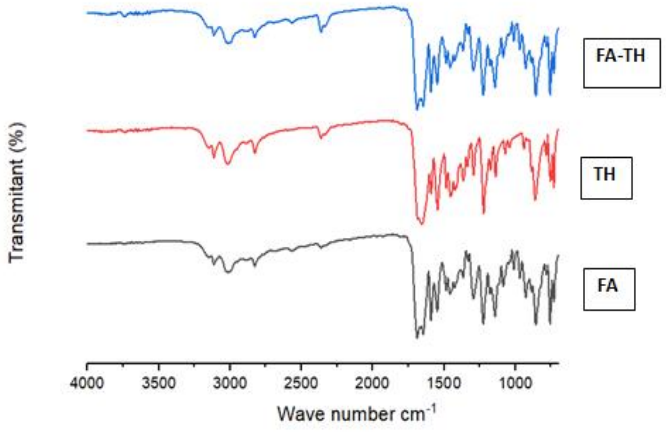

Figure 4: FT-IR spectra of FA, TH, and FA-TH multicomponent crystals

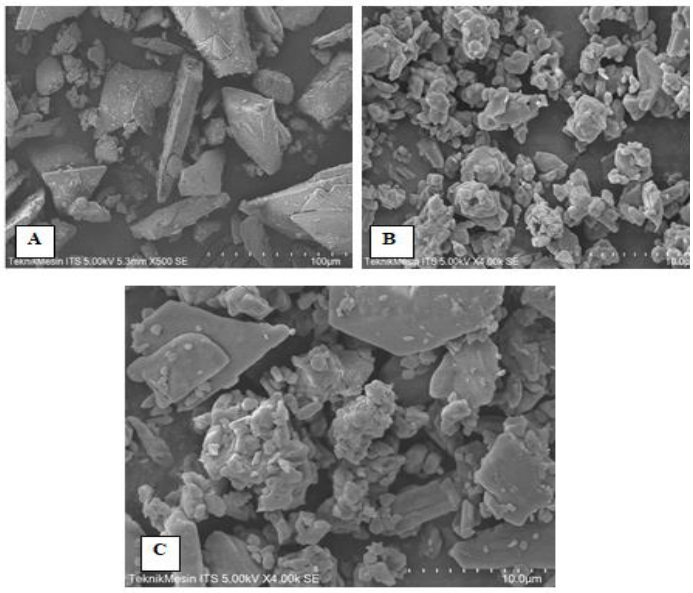

Figure 5: Scanning electron microphotograph (A) FA (B) TH (C) FA-TH multicomponent crystals

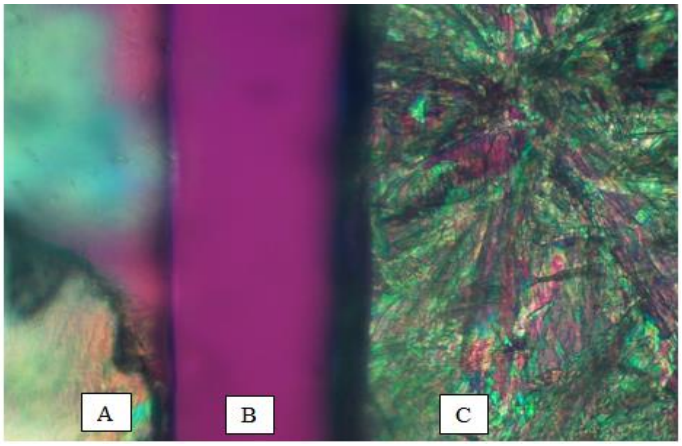

Figure 6: Polarizing Microphoto (A)FA (B)Contact Zone Between FA-TH and (C) TH

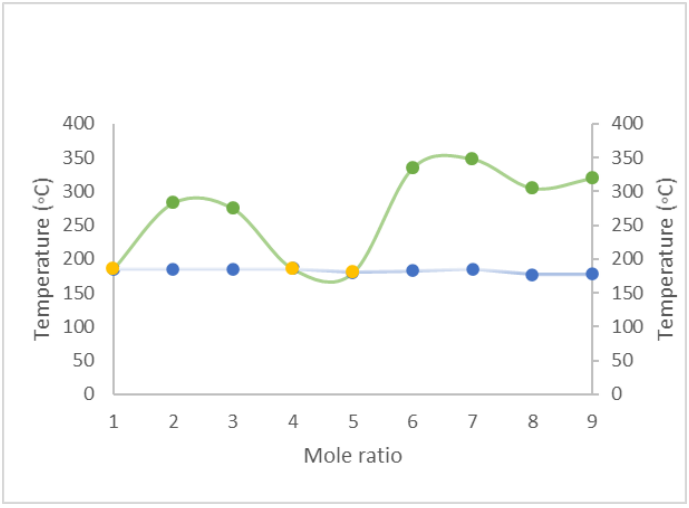

Figure 7: Binary Phase Diagram FA-TH

Table 3: Diffractogram data of diffraction peaks at 2θ and Intensity

	Intensity		
2 θ	**FA**	**TH**	**FA-TH**
18.64	809	-	576
23.40	708	-	578
23.50	675	-	578
27.24	-	502	586
13.59	-	395	605

Conclusion

This study revealed that the solubility enhancement of fenofibric acid multicomponent crystals with theobromine coformer was 4.4 times greater than that of pure unprocessed fenofibric acid. Fenofibric acid multicomponent crystals with theobromine coformer in a mole ratio of 1:1 formed a eutectic mixture. This method could be used in the formulation of FA to improve its pharmacokinetic properties.

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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