Original Article

SOLID DOSAGE FORM DEVELOPMENT OF GLIBENCLAMIDE-ASPARTAME COCRYSTAL USING THE SOLVENT EVAPORATION METHOD TO INCREASE THE SOLUBILITY OF GLIBENCLAMIDE

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ABSTRACT

Objective: The solubility of a drug in water plays an important role in the absorption of the drug after oral administration. Cocrystal is one method that improves the solubility of the active pharmaceutical ingredient (API). The aim of this study was to investigate the formation of a glibenclamide (GCM)-aspartame (APM) cocrystal using the solvent evaporation method and to evaluate its solubility and dissolution rate.

Methods: Molecular docking of the GCM-APM cocrystal was observed using an *in silico* method. The GCM-APM cocrystal (1:2) was prepared by using the solvent evaporation method. The cocrystal of GCM-APM was evaluated by the saturated solubility test and the dissolution rate test (USP type 2 apparatus). The solvent evaporation product of GCM-APM was characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), and powder X-ray diffraction (PXRD).

Results: *In silico* study showed that the interaction of GCM-APM has hydrogen bonding and the potential to improve the solubility of GCM. Evaluation of the cocrystal of GCM-APM showed that the solubility and dissolution rate of the cocrystal are significantly increased. Characterization of FT-IR showed that no chemical reaction occurred in the GCM-APM cocrystal. The DSC analysis showed the changes in the melting point of GCM. Measurement of PXRD showed the formation of a new solid crystal phase that is different from GCM and APM.

Conclusion: GCM-APM has hydrogen bonding can improve the solubility and dissolution rate of GCM.

Keywords: Cocrystal, Glibenclamide, Aspartame, Solvent Evaporation

INTRODUCTION

Solubility is an important parameter of the absorption process of a drug in the gastrointestinal tract that affects the drug concentration that can reach the blood circulation. This is important because the effectiveness of a drug treatment depends on the level of drug in the blood. Drugs with poor solubility in water exhibit poor bioavailability and low dissolution rates in the drug absorption [1]. Almost 70% of new active pharmaceutical ingredients (API) show poor water solubility, which can cause poor dissolution in the GI fluids. Therefore, increasing the dissolution rate of poorly water-soluble drugs is important in enhancing their bioavailability [2].

Glibenclamide (GCM) (5-chloro-N-[2-[4-(cyclohexyl carbamoyl-sulfamoyl) phenyl] ethyl]-2-methoxybenzamide) is a type 2 of antidiabetic drug used orally for controlling glycemia to treat non-insulin dependent [3]. In the biopharmaceutical classification system (BCS), GCM is included in class II, which means it has a high permeability and a low solubility. The properties of GCM can produce poor dissolution rate and can result in low bioavailability. Several studies have been conducted to increase the solubility of GCM such as solid dispersion [4], surface solid dispersion, nanoparticles [5], and nanoemulsion [6]. However, none of these methods stably yield a solid preparation.

One method that can increase the solubility of the API is cocrystal formation. Structurally, components of a cocrystal contain crystalline material present in a definite stoichiometric amount [7]. A cocrystal can improve solubility, dissolution rate, bioavailability, and stability because it has two or more neutral molecular constituents bonded together in the crystal lattice through hydrogen bonding [8]. Complex crystal formations were connected by a synthon that in pharmaceutical crystal engineering is called "a non-covalent interaction involving hydrogen bonds, Van der Waals, and π-π electrons" [9]. For designing cocrystals, choosing a synthon in a cocrystal is important for the crystallization process. Using the *in silico* method for designing cocrystals can help predict the interaction of synthon between the API and the conformer [8].

Improving bioavailability is desirable for an antidiabetic drug, especially GCM. Hence there is strong scientific and clinical need to prepare novel forms of GCM possessing modified solubility and dissolution rate, which can be formulated in tablet dossage form. Accordingly, the aim of the present study was to prepare a glibenclamide cocrystal by using the solvent evaporation method with aspartame as a coformer. Previous studies have proved that glibenclamide-oxalic acid cocrystals increase GCM solubility by up to sixfold [10]. In addition, the use of aspartame (APM) as a coformer was shown to increase atorvastatin solubility at the rate of 136.77% compared to atorvastatin [11].

MATERIALS AND METHODS

Material

GCM was obtained from Indofarma (Indonesia) with a purity>99% and APM pro analysis was obtained from Merck (Germany).

Methods

In silico molecular docking

Two-dimensional structures of GCM (Chem Spider ID: 54809) and coformers in. mol format were downloaded from www. chemspider. com. All. mol files of the molecules were converted into. pdb files by employing OpenBabelGUI 2.2.3. The files were then opened in AutoDock 4.2.3 and converted into. pdbq files by adding polar hydrogen and Kollman charges. The. pdbq files were converted into. pdbqt files by calculating their torsion angles, and were then ready to be used for docking. Docking was repeated five times for each coformer. Parameters observed were the type and energy (Ei) of interactions [11, 12]

Preparation of cocrystal using solvent evaporation method

GCM and APM were weighed in equimolar ratios of 1:1 and 1:2. Each compound was dissolved in methanol and mixed for a few minutes. An equimolar solution was evaporated using a water bath at 40 °C for 48 h. The obtained product was dried at room temperature overnight and further analyzed for its characterization [13].

Determination of solubility

Cocrystal GCM equivalent to 50 mg was placed in an Erlenmeyer flask that contained water. Cocrystals were agitated in a mechanical shaker for 24 and 48 h at room temperature. The saturated solutions were filtered through a 0.45 µm membrane filter, and the amount of the drug dissolved was analyzed spectrophotometrically at 266 nm [1].

Optimization of pH of GCM dissolution medium

Phosphate buffer media was prepared at 900 ml with pH variations of 6.4, 7.6 and 8. The dissolution test was then performed on standard GCM using a dissolution tester type 2 (paddle) at 75 rpm for 60 min at 37±0.2 °C. At each time point (5, 10, 15, 30, 45 and 60 min), samples of 5 ml were taken and then 5 ml of phosphate buffer media was re-added. The 5 ml samples taken were then measured by UV spectrophotometry (Analytical Jena, Germany) so that the amount of GCM concentration and the optimal pH of the medium could both be determined [14].

Dissolution test

Cocrystals of GCM were measured with a dissolution test (USP type 2 apparatus). The sample of cocrystal was put into 900 ml of a buffered phosphate solution with a pH of 8 and stirred at 75 rpm. The dissolution samples were filtered through a syringe filter of 0.45 µm pore size and were measured periodically (at 0, 10, 15, 30, 45, and 60 min) and were analyzed spectrophotometrically at 266 nm [15].

Characterization of cocrystal

Fourier transform infrared spectroscopy (FT-IR) analysis

Samples were mixed with potassium bromide crystal and crushed until homogeneous, and then compressed to a pressure of 20 psi. The infrared spectrum was obtained using an infrared spectrophotometer in a range of wavenumbers (400-4000 cm-1) using FT-IR [8, 9, 16]

Powder X-ray diffraction (PXRD) analysis

Powder X-ray diffraction (Phillips *PW1835®* diffractometer) analysis was performed at room temperature . The condition of measurement was set using Cu Ka radiation (l = 1.54 Å), a tube stage of 40 kV, and a tube current of 40 mA. Data were collected on the range of 2 theta of 5–40 ° at a scan rate of 1–2 °/min [8, 9, 17].

Differential scanning calorimetry (DSC) analysis

Thermal analyses of cocrystal were performed on a DSC. A thermograph was recorded under a gas flow of 50 ml/min. Samples were analyzed from 30 to 200 °C with a heating rate of 10 °C/min [17, 18].

Statistical analysis

The data of the solubility test and dissolution rate test were presented as a mean of samples±standard deviation (SD) and were analyzed using the one-way analysis of variance (ANOVA) at the level of (P<0.05) to determine if the changes in the applied factors were statistically significant at the level of (p<0.05) and non-significant at the level of (p>0.05)) [19].

RESULTS

Selection of coformers by an *in silico* method was used to predict the interaction between glibenclamide and a coformer molecularly. The results of an *in silico* method between GCM-APM and the prediction of interaction between GCM-APM can be seen in fig. 1 and fig. 2 respectively.
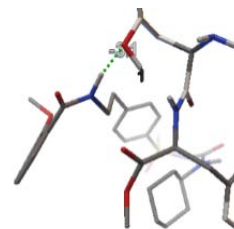

Fig. 1: *In silico* molecule modeling the simulation of GCM and APM
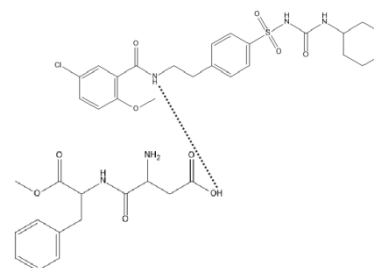

Fig. 2: The interaction between GCM and APM

The coformer used has no pharmacological activity and has an acceptor group and a hydrogen donor that is between GCM and coformer which allows the formation of a hydrogen bond [12].

The interaction of hydrogen bonds formed between GCM and APM has an effect on the hydrophilicity of GCM [20]. The hydrogen atom in the secondary amine group of GCM functions as a hydrogen bonding donor, whereas the oxygen atoms in the APM hydroxyl group function as the hydrogen bond acceptor [10].

The results of the solubility study of the GCM cocrystal can be seen in table 1.

Table 1: The result of the solubility study of the GCM cocrystal (All the values were calculated as mean±standard deviation) (n=3)

Sample	Concentration (µg/ml)	
	24 h	48 h
Pure GCM	2.11+0.21	4.40+0.12
Physical mixture of GCM-APM	13.51+0.76	18.72+0.54
The Solvent evaporation Product of GCM-APM (1:1)	18.74+0.81	30.48+0.78
The Solvent evaporation Product of GCM-APM (1:2)	31.71+1.15	60.63+1.21

The result of the solubility test was analyzed statistically and showed that ρ value was less than 0,05; therefore, H$_0$ was rejected and H$_a$ was accepted, which means that there were significant differences among each treatment (pure GCM versus cocrystal of GCM).

The formation of hydrogen bonds between GCM and APM causes increased solubility of GCM. The water-soluble aspartame properties also affect the increased solubility of GCM cocrystal in water [21]. Using a coformer will decrease the barrier on the crystal so that the dissolution process of GCM becomes easier. According to the previous study when the cocrystals are ionized, the pH changes because the ion of the cocrystal component changes the pH of the environment [22].

The dissolution test was performed on the cocrystal with the best equimolar ratio based on the solubility test, i.e., GCM-APM 1:2 compared with pure GCM. Optimization of pH of dissolution medium was performed at pH 6.4, 7.6, and 8.0. In table 4, the concentration of standard GCM at 60 min was 9.91, 16.43, and 45.31 ppm for pH 6.4, 7.6, and 8.0, respectively. Based on the results, 8.0 was the optimum pH for the dissolution test of GCM.
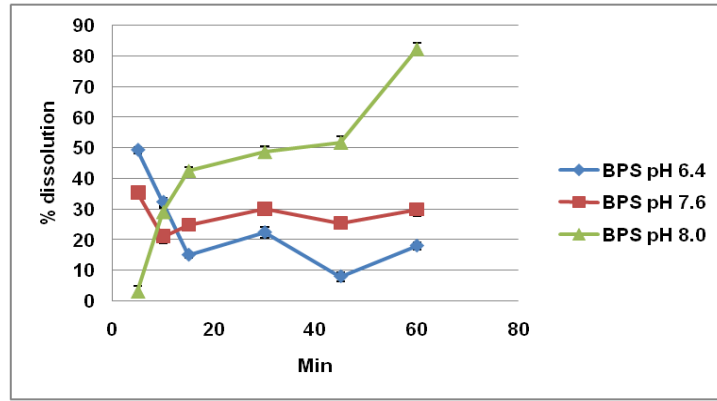
Fig. 3: Optimization of pH of dissolution medium (All the values were calculated as mean±standard deviation) (n=3), the results of the dissolution test for GCM can be seen in fig. 4
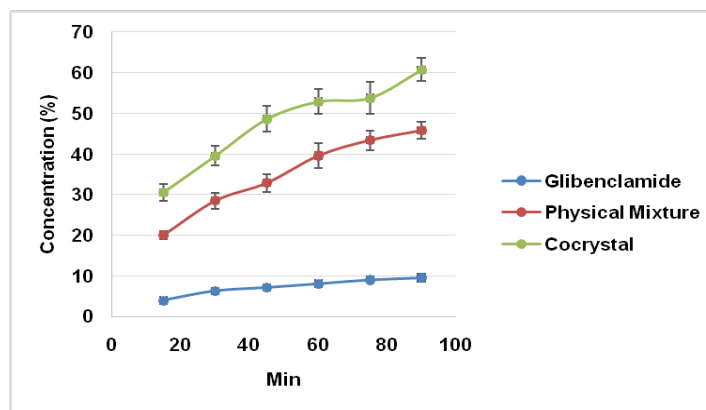

Fig. 4: The results of the dissolution test for GCM cocrystal (All the values were calculated as a mean±standard deviation) (n=3)

The result of the dissolution test was analyzed statistically and showed that the ρ value was less than 0.05; therefore, H_0 was rejected and H_a was accepted, which means that there were significant differences among pure GCM compared to cocrystal of GCM and physical mixture.

The increase of the dissolution rate of the glibenclamide-aspartame cocrystal is caused by the hydrogen bonding interaction in the co-crystalline thus increasing the polarity of GCM In addition, the solubility of good APM in the water can affect the dissolution of GCM

cocrystal [11]. Molecules in the physical mixture could become non-random and organized, upon heating results in lowering of melting points. It indicated that the eutectic formation was accelerated by grinding. Increased surface contact between components after grinding induces a weaker eutectic interaction which requires lower activation energy to reach the melting point [23].

The results of the FTIR spectrum from GCM, APM and cocrystal of GCM-APM can be seen in fig. 5.
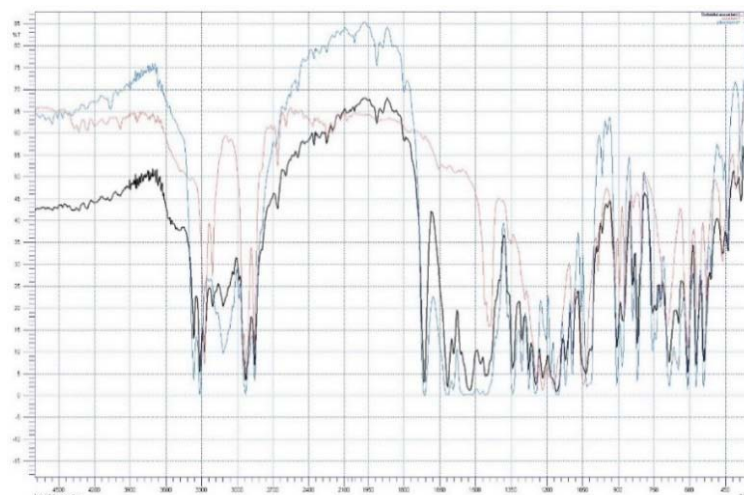

Fig. 5: The results of the FTIR spectrum of GCM (blue), APM (red), GCM-APM cocrystal (black), analysis of functional groups from the FTIR results of the GCM cocrystal can be seen in table 2

Table 2: Analysis of functional groups from the FTIR results of the GCM cocrystal (n=3)

No	GCM		APM		GCM-APM cocrystal	
	Peak (cm-1)	Stretch	Peak (cm-1)	Stretch	Peak (cm-1)	Stretch
1	3313.17	N-H Stretch	3275.13	N-H Stretch	3367.71	N-H Stretch
2	3116.97	O-H Stretch	2931.8	C-H Stretch	3205.69	O-H Stretch
3	2931.8	C-H Stretch	1696.35	C=O Stretch	2854.65	C-H Stretch
4	1712,79	C=O Stretch			1593.20	C=O Stretch
5	1612,49	C=C Stretch			1616.35	C=C Stretch

When the formation of functional groups was compared between GCM-APM cocrystals, GCM, and APM, there was no new peak. This indicates that there is no new functional group and it can be predicted that no chemical reaction occurred in the GCM cocrystal.

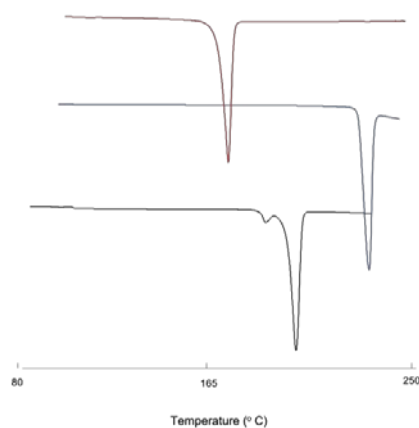The thermogram of GCM aspartame and GCM-APM can be seen in fig. 6.

Fig. 6: The thermogram of GCM, APM, GCM-APM cocrystal

In the thermogram, there was an endothermic peak of pure GCM on 171.96 °C indicating the melting point of GCM. The endothermic peak of APM was on 240.80 °C, indicating that the APM melting point was lower than pure GCM. GCM-APM melting point also was lower than pure GCM; it was 196.65 °C. The changes in the melting point of GCM indicate the crystal changes in GCM and the possible formation of GCM cocrystal [24].

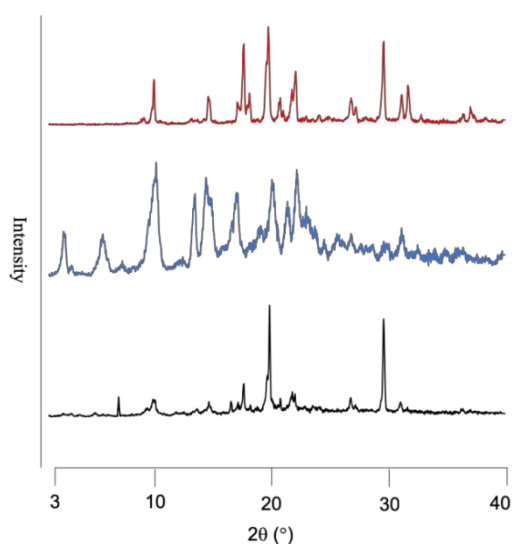The XRD of GCM, APM and GCM-APM can be seen in fig. 7.

Fig. 7: The XRD of GCM (red), APM (blue) and GCM-APM (black)

The intensity diffraction pattern for the 1:2 GCM-APM cocrystal shows different diffraction pattern when compared with the intensities of GCM or APM. X-ray diffraction (XRD) was used to show differences in the shape of the cocrystal compared with pure active GCM. X-ray diffraction (XRD) is used to analyze polymorphisms and to determine changes in the properties of a cocrystal or to determine whether a new phase of the cocrystallization process has occurred. When a new phase of crystals forms or different pattern was found, the diffractogram indicates the formation of a crystal [25].

In addition, different peaks formed for pure GCM and the cocrystal [26, 27]. It can be concluded that new peaks form and the formation of these new peaks or different diffraction pattern can predict the formation of a cocrystal [9, 10].

CONCLUSION

Preparation of the GCM-APM cocrystal (1:2) was conducted using the solvent evaporation method. The solubility and dissolution test of GCM-APM cocrystal (1:2) increased significantly compared to pure GCM and its physical mixture. The FT-IR, DSC, and PXRD confirmations of the cocrystal GCM-APM (1:2) indicated the formation of new solid crystalline phases that differ from pure GCM and its physical mixture.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

All authors have none to declare

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