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Original Article

CHARACTERIZATION AND DISSOLUTION RATE STUDIES OF INCLUSION COMPLEX OF GLIBENCLAMIDE AND HYDROXYPROPYL-B-CYCLODEXTRIN USING CO-GRINDING METHOD

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ABSTRACT

Objective: Glibenclamide belongs to the 2nd generation sulfonylurea group as an oral antidiabetic with low solubility in water and high bioavailability in systemic circulation (Biopharmaceutical Classification System class II). This study aimed to increase the solubility and dissolution rate of glibenclamide by preparing an inclusion complex of Glibenclamide and Hydroxypropyl-β-cyclodextrin.

Methods: Inclusion complexes were prepared by the co-grinding method in two ratios 1:1 and 1:2 mol. Characterizations of inclusion complex were carried out by Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FT-IR) spectroscopy, Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD) analysis. Solubility test was carried out in CO_2-free distilled water and dissolution rate was carried out in phosphate buffer pH 7.4.

Results: The results of the SEM analysis showed changes in particle morphology. FT-IR spectroscopy shows a shift in wavenumber. DSC analysis showed a decrease in the melting point of the inclusion complex. XRD characterization results showed a decrease in the intensity of the inclusion complex. Solubility of inclusion complex of glibenclamide increased nine times 1:1 mol inclusion complex, twelve times 1:2 mol inclusion complex compared to intact glibenclamide. The dissolution of glibenclamide, inclusion complex 1:1, and inclusion complex 1:2 in phosphate buffer pH 7.4 medium at 60 min was 17.19%, 34.15% and 52.83% respectively.

Conclusion: Based on the results of the study, it can be said that the glibenclamide inclusion complex with Hydroxypropyl-β-cyclodextrin successfully increases the solubility and dissolution rate of glibenclamide significantly.

Keywords: Glibenclamide, Hydroxypropyl-β-cyclodextrin, Inclusion complex, Co-grinding, Solubility, Dissolution rate

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INTRODUCTION

Glibenclamide is an oral antidiabetic drug belonging to the sulfonylurea class that works to stimulate insulin secretion in the pancreas. Glibenclamide is practically insoluble in water, so its absorption in the digestive tract is only about 50% [1]. Glibenclamide belongs to the BCS (Biopharmaceutical Classification System) class II with low solubility and high membrane penetration [2].

Many attempts have been made to increase the solubility of drug compounds. One of them is the formation of inclusion complexes [3-5]. The formation of inclusion complexes is mainly influenced by the hydrophobic nature of the drug compound (guest), which interacts with the interior of the cyclodextrin cavity. The guest molecule or the entrained molecule must be of the appropriate size and shape to enter the cavity in the solid structure formed by the host molecule. The hollow space formed by the host molecule can be a channel, a cage, or a layer [6]. The interaction is also influenced by the shape and size of the drug compound; the physicochemical properties of the drug compound can change due to the formation of inclusion complexes [7].

There are many methods that can be used for the formation of inclusion complexes, one of which is co-grinding. Co-grinding is a method that is widely used to reduce the particle size of drugs that are poorly soluble in water with the aim of increasing the dissolution rate and bioavailability of a drug [8]. The co-grinding product was prepared by ball-milling at the desired molar ratio in a high-energy vibration micro-mill at a frequency of 24 Hz for 60 min [9].

Increasing the solubility with the inclusion complex method has been widely carried out and has proven its success. One of the studies that has been carried out is the characterization, formulation, and evaluation of glibenclamide with β-cyclodextrin tablet inclusion complex, which can increase the solubility of glibenclamide [10]. Research on glibenclamide inclusion complex with 2-hydroxypropyl-β-cyclodextrin polymer was found to increase the solubility of glibenclamide in alkaline medium [11].

The formation of inclusion complexes can be carried out with cyclodextrins. The types of cyclodextrins are α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin [12]. This study used hydroxypropyl-β-cyclodextrin, which is a derivative of β-cyclodextrin [13]. Hydroxypropyl-β-cyclodextrin is a chemical modification of cyclodextrin that can be used for parenteral preparations; this is because the solubility of hydroxypropyl-β-cyclodextrin is greater and has a less toxic effect than β-cyclodextrin [7]. Hydroxypropyl-β-cyclodextrin has the chemical formula $C_{42}H_{70}O_{35}$ (C_3H_6) and a molecular weight of 1400 grams/mol [14].

Previous inclusion complex studies have succeeded in increasing the solubility of glibenclamide using β-cyclodextrin polymer [15]. Therefore, this research is a continuation of the modification of the research with hydroxypropyl-β-cyclodextrin polymer using the co-grinding method. The aim is to increase the solubility and dissolution rate of glibenclamide.

The characterization of the inclusion complexes was evaluated by Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FT-IR) Spectroscopy, Differential Scanning Calorimetry (DSC), and X-ray Diffraction (XRD) analysis. Solubility test was carried out in CO_2-free distilled water and dissolution rate was carried out in phosphate buffer pH 7.4.

MATERIALS AND METHODS

Materials

The materials used in this research are glibenclamide (BOC Sciences, USA), hydroxypropyl-β-cyclodextrin (NandR industries, China), methanol pro analysis (Merck, Germany), potassium dihydrogen phosphate (KH2PO4) (Merck, Germany), sodium hydroxide (NaOH) (Merck, Germany), and aqua dest (Novalindo, Indonesia).

Preparation of inclusion complex

Preparation of the inclusion complex with hydroxypropyl-β-cyclodextrin was carried out using the co-grinding method in a ratio

of 1:1 mol (2.60g: 7.40g) and 1:2 mol (1.50:8.50). Glibenclamide and hydroxypropyl-β-cyclodextrin were weighed and mixed, this mixture was then ground using a ball milling device with a milling time of 2 h at a speed of 120 rpm using 42 small balls and 42 large balls [5, 16].

Scanning electron microscopy (SEM) analysis

SEM (Hitachi Type S-3400N®, Japan) analysis was performed on glibenclamide, hydroxypropyl-β-cyclodextrin, and inclusion complex. The samples were coated with a thin layer of palladium-gold prior to analysis. SEM works using a voltage is set at 10 kV and the current is 12 mA [16].

Fourier transform infrared (FT-IR) spectroscopic analysis

FT-IR spectroscopic (Perkin Elmer L1600300 Spectrum Two, USA) analysis was performed on glibenclamide, hydroxypropyl-β-cyclodextrin, and inclusion complex. A small amount of sample (±3 mg) was mixed with 10 mg KBr after which it was placed in the sample holder of the FT-IR spectroscopic instrument and the samples were analysed at room temperature. The spectrum was measured in the range of 450-4000 cm^{-1}wavenumber [5, 16].

Differential scanning calorimetry (DSC) analysis

Thermal analyses on glibenclamide, hydroxypropyl-β-cyclodextrin, and the inclusion complex were carried out using a DSC apparatus (Setaram DSC 131 Evo, France). Samples of 3 mg were placed in a closed aluminium pan. The DSC device is programmed in a temperature range of 50–350 °C, heating speed of 10 °C/min [5, 17].

X-ray diffraction (XRD) analysis

Analyses were carried out on glibenclamide, hydroxypropyl-β-cyclodextrin and inclusion complex. X-ray diffraction analyses of the samples were performed at room temperature using an X-ray diffractometer (Philips X'Pert Pro-PANalytical, The Netherland) with Cu, Kα filter, current 30 mA, voltage 40 kV. Samples were measured in reflection mode at 2 theta with an angle range of 5°–30° [5, 16, 17].

Solubility test

In the solubility test, glibenclamide and inclusion complex were made into a saturated solution using 100 ml of CO_2-free distilled water. A sample equivalent to 25 mg of pure glibenclamide was dissolved in a 100 ml Erlenmeyer, then shaken with an orbital shaker for 24 h at 25 °C. Then the sample was filtered through a 0.45 µm filter (Whatman filter paper) and the concentration of glibenclamide was determined from the absorbance measurement at 229 nm using ultraviolet-visible light (UV-Vis) spectrophotometer (Shimadzu ED23 1800®, Japan) [5, 17, 18].

Dissolution rate profile study

The dissolution rate study of glibenclamide and inclusion complex used the paddle method (Copley Scientific NE4-COPD, UK) at 37±0.5 °C at a speed of 75 rpm for 60 min with mediums phosphate buffer pH 7.4. Five ml of each dissolution medium was pipetted at 5, 10, 15, 30, 45, and 60 min. The absorbance of the solution that had been pipetted from the dissolution medium was measured using a UV-Vis spectrophotometer (at 300 nm) to determine the amount of glibenclamide dissolved [5, 16-18].

RESULTS AND DISCUSSION

The results of the analysis of glibenclamide, hydroxypropyl-β-cyclodextrin, and inclusion complex by SEM can be seen in fig. 1 Glibenclamide appears to be a crystalline solid with an irregular box shape; research that has been done also shows the results of glibenclamide in the form of a collection of crystals with irregular sizes [10]. Hydroxyporpy-β-cyclodextrin appeared to be spherical hollow particles, in accordance with the SEM results of hydroxypropyl-β-cyclodextrin referenced from the literature in the form of spherical crystals [19]. The inclusion complexes of glibenclamide hydroxypropyl-β-cyclodextrin 1:1 mol and 1:2 mol showed that the glibenclamide morphology, which was box-shaped, was no longer visible but instead changed to an irregular shape, it was assumed that glibenclamide and hydroxypropyl-β-cyclodextrin had already been dispersed. The SEM results indicate that the interaction between the two substances affects the crystal morphology of each substance.

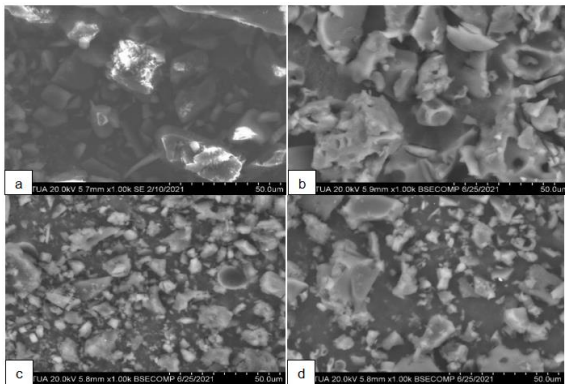

Fig. 1: Scanning electron microscopy analysis of (a) glibenclamide, (b) hydroxypropyl-β-cyclodextrin, (c) 1:1 mol inclusion complex (d) 1:2 mol inclusion complex

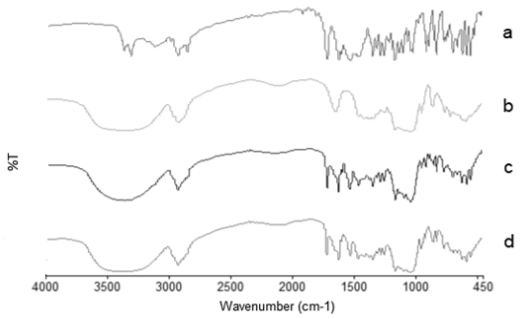

Fig. 2: Fourier transform infrared spectroscopic analysis of (a) glibenclamide, (b) hydroxypropyl-β-cyclodextrin, (c) 1:1 mol inclusion complex (d) 1:2 mol inclusion complex

FT-IR spectroscopy analysis was performed to determine the functional group of an organic compound and to determine the structure of an organic compound by comparing the fingerprint area. The results of the analysis are in the form of a graph showing the percentage of transmittance that varies at each frequency of infrared radiation [20]. The occurrence of a shift in the wave number and chemical interactions between glibenclamide and hydroxypropyl-β-cyclodextrin after the formation of the inclusion complex. The results of the FT-IR analysis can be seen in fig. 2.

In the infrared spectrum of pure glibenclamide, it was seen that the functional groups were N-H 3368.43 cm^{-1}, C=O 1915.00 cm^{-1}, C=C 1673.49 cm^{-1}, C-H 2855.16 cm^{-1}. The results of FT-IR analysis on hydroxypropyl-β-cyclodextrin showed the presence of functional groups N-H 3306.50 cm^{-1}, C=C 1644.39 cm^{-1}, C-H 2929.91 cm^{-1}. The results of the FT-IR analysis on the 1:1 mol inclusion complex showed the presence of groups-H 3368.58 cm^{-1}, C=O 1715.60 cm^{-1}, C=C 1618.21 cm^{-1}, C-H 2930.51 cm^{-1}. The results of the FT-IR analysis of the 1:2 mol inclusion complex were the presence of functional groups N-H 3369.42 cm^{-1}, C=O 1716.24 cm^{-1}, C=C 1618.89 cm^{-1}, C-H 2931.72 cm^{-1}.

The presence of chemical interactions between glibenclamide and hydroxypropyl-β-cyclodextrin in the inclusion complex was indicated by the presence of a new transmittance peak or a shift. The presence of hydrogen bonds formed between two solids is indicated by a shift in wave number [21], namely the interaction between the carbonyl group of glibenclamide and the hydroxyl group of hydroxypropyl-β-cyclodextrin.

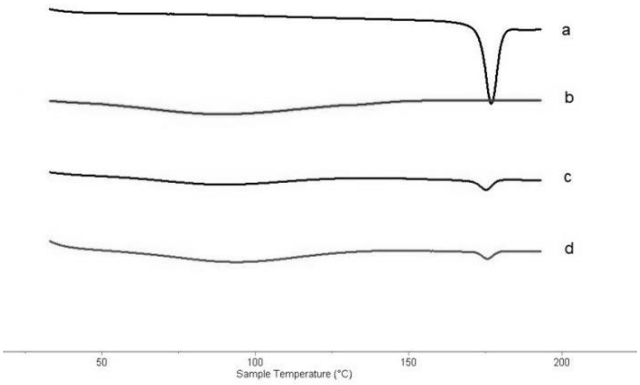

Fig. 3: Differential scanning calorimetry analysis of (a) glibenclamide, (b) hydroxypropyl-β-cyclodextrin, (c) 1:1 mol inclusion complex (d) 1:2 mol inclusion complex

The Differential Scanning Calorimeter (DSC) test is used to determine the heat capacity used to determine the melting point of a substance. DSC is able to measure the amount of heat absorbed or released during the transition from solid to liquid [22]. In this study, the results obtained glibenclamide thermogram showed a sharp endothermic peak at a temperature of 176.926 °C. hydroxypropyl-β-cyclodextrin showed an endothermic peak at 99.332 °C, the inclusion complex thermogram 1:1 mol showed a temperature of 175.18 °C the 1:2 mol inclusion complex also showed a peakendothermic that is at a temperature of 175.618 °C.

From the results of the DSC thermogram, it can be seen that there was a decrease in the melting point of the inclusion complex due to the active substance glibenclamide being mixed with hydroxypropyl-β-cyclodextrin. This indicates that there has been an interaction between glibenclamide and hydroxypropyl-β-cyclodextrin which has formed an inclusion complex [23].

X-ray diffraction analysis was used to evaluate the effect of changes in the degree of crystallinity of the solid drug glibenclamide on the inclusion complex. The results of the glibenclamide diffractogram showed intensive peaks indicating a crystalline form and a very sharp decrease in the intensity of the degree of crystallization occurred from the glibenclamide peak at position 2θ 18,8126 with a peak intensity of glibenclamide 2120.954 units, hydroxypropyl-β-cyclodextrin 409.9468 units, inclusion complex 1:1 mol 821.9536 units, and the inclusion complex 1:2 mol 775.8653 units. The decrease in intensity indicates a change in the degree of crystallinity, this is due to the presence of mechanical energy that occurs during grinding so that glibenclamide and hydroxypropyl-β-cyclodextrin attract each other and form hydrogen bonds with the polymer [24].

A very sharp decrease in intensity from the peak of glibenclamide to approach the profile of the hydroxypropyl-β-cyclodextrin diffractogram also indicates that the glibenclamide molecule has entered the structure of the hydroxypropyl-β-cyclodextrin cavity so that the hydroxypropyl-β-cyclodextrin diffractogram looks more dominant [25]. The decrease in the height of the peak intensity indicates a change in the structure so that the resulting inclusion complex is amorphous [26].

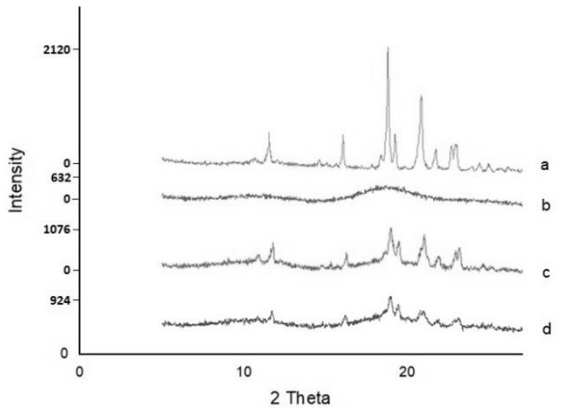

Fig. 4: X-ray diffraction analysis of (a) glibenclamide, (b) hydroxypropyl-β-cyclodextrin, (c) 1:1 mol inclusion complex (d) 1:2 mol inclusion complex

Table 1: Solubility of glibenclamide, 1:1 mol inclusion complex, and 1:2 mol inclusion complex

Compound	Solubility (mg/100 ml)
Glibenclamide	0.44±1.34
1:1 mol inclusion complex	3.95±4.12
1:2 mol inclusion complex	5.34±2.97

[mean±SD, n= 3]

The result of the solubility test of pure glibenclamide in water was 0.44 mg/100 ml. as stated in the Indonesian Pharmacopoeia Ed VI where glibenclamide is practically insoluble in water. The yield for the 1:1 mol inclusion complex was 3.95 mg/100 ml and the 1:2 mol inclusion complex was 5.34 mg/100 ml. The results of the solubility test of the inclusion complex 1:1 mole increased 9 times and the inclusion complex 1:2 mole increased 12 times compared to pure glibenclamide.

Dissolution rate profiles of glibenclamide and inclusion complexes were carried out by paddle method at 75 rpm for 60 min at 37±0.5 °C with mediums phosphate buffer pH 7.4. The dissolution rate profiles of glibenclamide, 1:1 mol inclusion complex, and 1:2 mol inclusion complex in phosphate buffer pH 7.4 can be seen in fig. 5 and table 2, with an increase in dissolution efficiency of 2.3 times and 3 times, respectively. The increase in the dissolution rate was due to being complexed in a matrix that formed a cavity where the inside of the cavity was hydrophobic and the outside of the β-cyclodextrin was hydrophilic. Thus, with the addition of the β-cyclodextrin complexing agent, the substance having solubility and dissolution problems of a single drug can improve the absorption rate and increase the bioavailability of the drug [11]. Previous studies have reported an increase in the dissolution rate and solubility of glibenclamide with different excipients and methods [8, 9, 15].

Table 2: Dissolution efficiency of glibenclamide, 1:1 mol inclusion complex, and 1:2 mol inclusion complex in phosphate buffer medium pH 7.4

Compound	Dissolution efficiency	Increased
Glibenclamide	13.40±0.20	-
1:1 mol inclusion complex	30.72±0.22	2.3 times
1:2 mol inclusion complex	39.94±0.79	3 times

[mean±SD, n= 3]

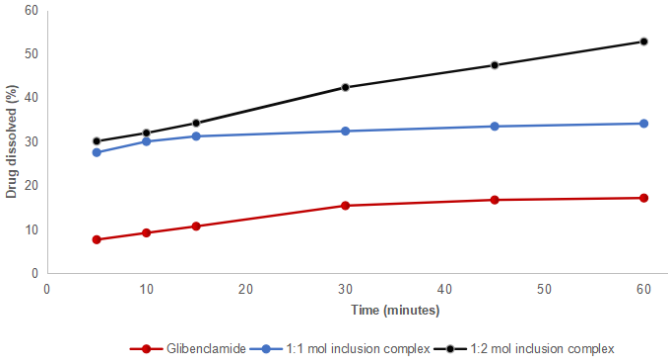

Fig. 5: Dissolution rate profile of glibenclamide, 1:1 mol inclusion complex, and 1:2 mol inclusion complex in phosphate buffer pH 7.4 [mean±SD, n= 3]

CONCLUSION

In this study, the amorphous inclusion complex was successfully formed, which can be seen from the results of the characterization of SEM, FT-IR, DSC, XRD. The glibenclamide inclusion complex, hydroxypropyl-β-cyclodextrin, significantly increased the solubility and dissolution rate of glibenclamide compared to intact glibenclamide.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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