Characterization and Dissolution Rate Studies of Hydrochlorothiazide-β-Cyclodextrin Inclusion Complex Using Co-Grinding Technique

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ABSTRACT

The formation of hydrochlorothiazide inclusion complexes has been carried out to improve the physicochemical properties and increase the dissolution rate of hydrochlorothiazide. Inclusion complexes were made by co-grinding using 8 cyclodextrin at a ratio of 1:1 mol, with variations in milling time, namely 30 minutes (F1), 1 hour (F2), and 2 hours (F3). The inclusion complexes formed were characterized by X-ray diffraction analysis, differential scanning calorimetry (DSC), Fourier transforms infrared (FT-IR), scanning electron microscopy, and dissolution tests. X-ray diffraction results showed a decrease in hydrochlorothiazide intensity, the DSC thermogram showed a decrease in endothermic peaks and enthalpy values, and FT-IR spectrum analysis showed that there was a chemical interaction between hydrochlorothiazide and β-cyclodextrin. All characterization checks carried out met the requirements and confirmed an increase in the dissolution rate. The dissolution yield of hydrochlorothiazide was 41.711%, the physical mixture was 57.135%, the F1 inclusion complex was 59.52%, the F2 inclusion complex was 79.17%, and the F3 inclusion complex was 103.62%. This proves that the formation of inclusion complexes with the addition of β-cyclodextrin can significantly increase the dissolution.

Keywords: Inclusion complex, hydrochlorothiazide, β-cyclodextrin, dissolution studies, co-grinding

Introduction

The bioavailability of an orally administered preparation depends on the rate of dissolution, solubility, and rate of absorption in the gastrointestinal tract. Drugs administered orally are dissolved in an aqueous medium in the gastrointestinal tract for absorption. Improving the solubility and dissolution rate of poorly soluble drugs is the first step toward improving bioavailability (Loftsson & Brewster, 1996). To increase the solubility of a drug that is poorly soluble in water, the inclusion complex method can be used. In inclusion complexes, drug molecules act as guest molecules trapped in hydrophobic cyclodextrin cavities. and the outer part of the cyclodextrin is hydrophilic so it dissolves easily in aqueous media (Bekers, et al., 1991). Cyclodextrins are cyclic oligosaccharide compounds derived from starch. Among the most commonly used forms are α-, β-, γ-cyclodextrins, which have 6, 7, and 8 glucose units, respectively. Cyclodextrins are toroidal or conical in shape and have a rigid structure and a central cavity, the size of which varies according to the type of cyclodextrin. The internal surface of the cavity is hydrophobic and the outer surface of the torus is hydrophilic. This allows the cyclodextrin to accommodate guests in the cavity to form inclusion complexes. Cyclodextrins can be used to form inclusion complexes with various drug molecules, used primarily to improve dissolution and bioavailability to increase solubility and improve chemical and physical stability (Anjana et al., 2013; Rowe, et al., 2009).

Inclusion complexes were prepared by evaporation, coprecipitation, local pressing, freeze drying, and co-grinding methods (Bekers, et al., 1991). The co-milling technique of poorly soluble drug compounds using polymers is very useful in increasing solubility and increasing bioavailability due to modification of the solid properties of drug compounds. At the time of grinding the crystalline solids will transform an amorphous phase in polymer chains (Vadther, et al., 2009; Chono et al., 2008; Bazegar-Jalali et al., 2007).). The energy required for a drug molecule in the crystalline form is greater than in the amorphous form so this change in shape is utilized to increase the dissolution rate and solubility of a drug compound that is poorly soluble in water (Sinko & Sing, 2011).

Hydrochlorothiazide is a thiazide diuretic used as a drug in the treatment of hypertension. Hydrochlorothiazide is a white or almost white crystalline powder, odorless, easily soluble in sodium hydroxide, n-butylamine, and dimethyl formamide, slightly soluble in methanol, poorly soluble in

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water, insoluble in ether, chloroform and dilute mineral acids. In the Biopharmaceutics Classification System, Hydrochlorothiazide is classified as a class IV, which has low solubility and low permeability after oral administration (Masmoudi, et al., 2013).

To increase the solubility of hydrochlorothiazide, it was formulated in the form of an inclusion complex using β-cyclodextrin. The inclusion complexes were made by co-grinding technique with variations of milling time of 30 minutes, 1 hour, and 2 hours respectively called F1, F2, and F3. The formulation was then characterized by its physicochemical properties and its dissolution rate was determined. The formation of the inclusion complex is expected to increase the solubility of hydrochlorothiazide thereby increasing the dissolution rate of hydrochlorothiazide.

**Methods:**

**Materials:**

- Hydrochlorothiazide (IPCA Labotatorium Limited), β Cyclodextrin (PT Signa Husada), 96% ethanol (Bratachelm), methanol (Merck), hydrochloric acid (Merck) and distilled water (Bratachelm).

**Preparation of the physical mixture of hydrochlorothiazide - β-cyclodextrin**

The physical mixture of hydrochlorothiazide-β-cyclodextrin was prepared with the ratio (1:1) mol. All ingredients are mixed and homogenized for 30 minutes. The formed physics mixture is stored in a desiccator before use.

**Preparation of hydrochlorothiazide inclusion complex - β-cyclodextrin**

Hydrochlorothiazide and β-cyclodextrin were weighed according to the formula with a ratio of 1:1 mol. The sample was mixed and then ground using a planetary ball mill PM 100 Retsch grinder using stainless steel balls with a ball diameter of 20 mm at a speed of 200 rpm for 30 minutes, 1 hour, and 2 hours to obtain Hydrochlorothiazide inclusion complex with β-cyclodextrin (Garg & Sing, 2005; Friedrict et al., 2005). The substance adhering to the walls of the grinding balls and grinding balls is cleaned every certain time. The inclusion complex formed was stored in a desiccator before use.

**Determination of Inclusion Complex Characterization**

**X-Ray Diffraction Analysis**

Tests were carried out on Hydrochlorothiazide, β-cyclodextrin, physical mixtures, and inclusion complexes of Hydrochlorothiazide with β-cyclodextrin. X-ray diffraction analysis was determined using the XPert PRO PANalytical at room temperature. The sample is placed in the sample holder and leveled to prevent particle orientation during sample preparation. The measurement conditions were Cu metal target, Kα filter, 40 kV voltage, and 30 mA current, measurement analysis was carried out in the range 2 theta 5 – 60°.

**Differential Scanning Calorimeter**

Tests were carried out on Hydrochlorothiazide, β-cyclodextrin, physical mixture, and hydrochlorothiazide inclusion complex with β-cyclodextrin using a Differential Scanning Calorimeter Setaram, Type Evo-131. Samples were accurately weighed as much as 3 mg in a crucible pan and then closed. The rate of the tool is programmed at a temperature range of 40 °C to 300 °C with a heating speed of 10 °C per minute which is supplied with nitrogen gas at 60 mL per minute.

**Spectroscopy Analysis Fourier Transform – Infra-Red**

Tests were carried out on hydrochlorothiazide, β-cyclodextrin, physical mixtures and hydrochlorothiazide-β-cyclodextrin inclusion complexes using a Perkin Elmer Frontier FT-IR spectrophotometer. The sample was taken a little after the FT-IR program. Then rotate the location of the sample clockwise, put the sample in a clean and dry sample holder then do the sample analysis while rotating it, wait until the sample is analyzed and the spectrum results will come out. The sample spectrum was recorded at wave numbers 400–4000 cm⁻¹.

**Scanning Electron Microscope**

SEM analysis was performed on hydrochlorothiazide, physical mixture, and Hydrochlorothiazide-β-cyclodextrin inclusion complex using a Hitachi S-3400N Scanning Electron Microscopy. Prepare the sample and place the sample on a 1 cm holder that has been coated with carbon tabs and leveled. The powder is coated with a thin layer of gold-palladium under vacuum and observe the morphology of the sample is at various magnifications.

**Dissolution Profiles**

Determination of dissolution profiles of hydrochlorothiazide, physical mixtures, and hydrochlorothiazide –β-cyclodextrin inclusion complexes using a Copley Scientific Type NE4-COPD dissolution apparatus. The dissolution test was carried out using a USP dissolution apparatus type 1. The dissolution flask container was filled with 900 mL of 0.1 N HCl medium at a temperature of 37 ± 0.5°C and a speed of 50 rpm. The test powder was weighed equivalent to 50 mg, then put into the dissolution container. The test sample was taken 5 mL at intervals of 5, 10, 15, 30, 45, and 60 minutes. The percent dissolution was measured using a Shimadzu UVmini-1240 UV-VIS spectrophotometer at a maximum absorption wavelength of 272 nm.
RESULTS AND DISCUSSION

X-Ray Diffraction Analysis

The results of the X-ray diffraction analysis can be seen in Figure 1. The hydrochlorothiazide diffractogram is a crystalline solid with a characteristic and sharp interference peak at angle 2θ: 16.3823°, 21.3636°, and 24.4769°. In the diffractogram of the physical mixture, it can be seen that the hydrochlorothiazide crystalline pattern is visible at angles 2θ: 16.3823°, 21.3636°, and 24.4769°. The diffractogram of the inclusion complexes F1, F2, and F3 shows a decrease in intensity and degree of crystallinity following the diffraction pattern of β-cyclodextrin. The decrease in the intensity of the inclusion complex may be due to the influence of grinding time, and it is possible that the solid obtained leads to an amorphous form. This indicates that an inclusion complex has been formed.

![Figure 1. Diffractogram of hydrochlorothiazide (HCT), β-cyclodextrin, physical mixture (CF), inclusion complex (F1), inclusion complex (F2) and inclusion complex (F3)](image)

Differential analysis of scanning calorimetry

The results DSC analysis can be seen in Figure 2. The hydrochlorothiazide thermogram shows a sharp endothermic peak at 272.656 °C with an enthalpy of 69.877 J/g, and the thermogram of the physical mixture has an endothermic peak at 267.834 °C with an enthalpy of 35.395 J/g, whereas in the inclusion complex F1 the endothermic peak is seen at 266.451 °C with an enthalpy of 5.136 J/g, the F2 inclusion complex thermogram shows an endothermic peak at 265.938 °C with an enthalpy of 4.868 J/g and the F3 inclusion complex thermogram shows an endothermic peak at 264.123 °C with an enthalpy of 1.718 amount of energy required for melting due to a decrease in the degree of crystallinity.

![Figure 2. Diffractogram of hydrochlorothiazide (HCT), physical mixture (CF), inclusion complex (F1), inclusion complex (F2) and inclusion complex (F3)](image)

Fourier spectrophotometer analysis Transform Infra-Red

Fourier Transform Infra-Red spectrophotometer analysis can be seen in Figure 3. The results of the hydrochlorothiazide spectrum showed the presence of the CH functional group at wave number 2945.82 cm⁻¹, the SO₂ at wave number 1154.77 cm⁻¹, and the NH functional group at wave number 3593.29 cm⁻¹. The FT-IR spectrum of β-cyclodextrin shows a wide peak at wave number 3296.54 cm⁻¹ which indicates the presence of the OH functional group, the CH functional group at wave number 2924.11 cm⁻¹, the CO functional group at wavelength 1018.06 cm⁻¹. The FT-IR spectrum of the physical mixture, formula 1, formula 2, and formula 3, showed that the hydrochlorothiazide functional group was missing, namely, the NH group which was seen at wave number 3593.29 cm⁻¹. From this FT-IR analysis, it can be concluded that there was an interaction between hydrochlorothiazide and β-cyclodextrin after the formation of the physical mixture and the formation of the inclusion complex.
Scanning electron microscopy Analysis

The results of scanning electron microscopy analysis can be seen in Figure 4 with a magnification of 1000x. On the results of the Scanning Electron Microscope (SEM), the morphology of hydrochlorothiazide looks like irregular solid lumps, and the morphology of β-cyclodextrin looks like big lumps with a rough surface texture. In the physical mixture, the morphology of hydrochlorothiazide and β-cyclodextrin can still be distinguished, whereas in the inclusion complexes F1, F2, and F3 it can be seen that hydrochlorothiazide is completely wrapped or included in the β-cyclodextrin cavity. This indicates that an inclusion complex has been formed which will affect the increase in the dissolution rate.

Dissolution Profile

Profile results Dissolution can be seen in Figure 5. In determining the dissolution profile of the inclusion complex, the dissolution rate increased compared to hydrochlorothiazide and the physical mixture, while the physical mixture increased the dissolution rate compared to hydrochlorothiazide. This indicates that an inclusion complex has been formed using β-cyclodextrin. The percentage dissolved in the 60th minute of each formula was Hydrochlorothiazide at 41.71%; a physical mixture of 57.13%; F1 inclusion complex at 59.52%; the F2 inclusion complex was 79.17% and the F3 inclusion complex was 103.62%. From this value, it can be concluded that the longer the grinding time, the higher the percentage of hydrochlorothiazide dissolved in the dissolution medium.
Figure 5. Dissolution profile of hydrochlorothiazide (HCT), physical mixture (CF), inclusion complex (F1), inclusion complex (F2) and inclusion complex (F3)

CONCLUSION

Based on research, the formation of hydrochlorothiazide inclusion complexes with β-cyclodextrin can improve the physicochemical properties of hydrochlorothiazide, which can be seen from the characterization of XRD, DSC, FTIR, and SEM. The inclusion complex of hydrochlorothiazide with can increase the dissolution rate of Hydrochlorothiazide.

REFERENCES


