Formulation and Evaluation of Fast Dissolving Gliclazide Tablets by Complexation with Hydroxypropyl-β-Cyclodextrin

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ABSTRACT

Gliclazide (GZ) is practically insoluble in water and its bioavailability is limited by dissolution rate. The aim of the present study was to enhance the dissolution rate and bioavailability of GZ by complexation with hydroxypropyl (HP)-β-cyclodextrin (CD) applying three different methods; physical mixing, kneading technique and spray drying technique. Also, to evaluate the dissolution rate and the hypoglycemic effect of the prepared complexes, in comparison with the GZ market product (Glizide tablets) in Saudi market. The produced complexes were characterized and evaluated using Differential Scanning Calorimetry (DSC), X-ray Diffractometry (XRD), Scanning Electron Microscope (SEM) and the in vitro release studies.

All the methods of preparation of complexes were found to be effective in improving the solubility of gliclazide in comparison with the commercial product (Glizide tablets). The formation of inclusion complexes was evident in these formulations as shown by DSC and XRD studies. The inclusion complexes prepared by spray drying method in 1:1 molar ratios were the most effective method for improving the solubility of GZ. The in-vivo hypoglycemic effect of the complexed GZ-HP-β-CD prepared by spray drying significantly improved the biological performance and therapeutic efficacy of the drug compared to Glizide market product.

KEYWORDS: Gliclazide-Hydroxypropyl-β-Cyclodextrin; Complexation; hypoglycemic effect.

Introduction

Cyclodextrins (CDs) form a group of structurally related oligosaccharides with cylinder-shaped cavities that have the capacity to form inclusion complexes with many drugs by taking a whole drug molecule, or a part of it, into the cavity (Pitha, 1986; Duchene, 1987). CDs have widespread pharmaceutical applications mainly because of their effect on enhancing the solubility and bioavailability of many drug formulations. The interaction of ketoprofen and ibuprofen with β-CD in solution and in a solid state has been studied by Mura et al., 1998. The nuclear magnetic resonance of the inclusion complexation of gliclazide (GZ) with β-CD and the enhancement of its aqueous solubility also has been investigated (Moyano et al., 1986, Ozkan et al., 2000, and Hashem et al., 2013). Many other drugs have been tested for CD inclusion to enhance solubility such as bropirimine, ibuprofen, tolbutamide, and doxorubicin and daunorubicin (Echezarreta-Lopez M. and Torres-Labandeira, 2000; Ghorab MK. and Adeyeye MC, 2001; Veiga FJ, et al., 2001 and Bekers O, et al., 1990). The improved bioavailability of many drugs complexed with CDs has been documented in several articles (Uekama et al., 1983; Seo and Uekama,1995, Sanghavi et. al., 1999; Yuen, and Yuen, 2001; Yap, et al., 2001; Wong and Yuen, 2001).

Gliclazide, [1-(3-azabicyclo(3,3,0)oct-3-yl)-3-p-tolylsulfonylurea] is a second generation hypoglycemic sulfonylurea which is widely used for the treatment of non-insulin dependent diabetes mellitus (NIDDM) (Reynolds, 2012). The drug shows good tolerability, low incidence of hypoglycemia, and a low rate secondary failure (Harrower, 1994). In addition, it has a potential for slowing the progression of diabetic retinopathy. So, GZ appears to be a drug of choice in long term sulfonylurea therapy for the control of NIDDM (Harrower, 1994 and Palmer and Brogden, 1993). GZ is a white crystalline powder, relatively insoluble in water. The pKa of GZ is 5.8. GZ exhibits slow GI absorption rate and inter individual variations of its bioavailability (Palmer and Brogden, 1993). The slow absorption rate of drug usually originates from either poor dissolution of drug from the formulation or poor permeability of drug across GI membrane. The slow dissolution can be attributed, at least in part, to hydrophobicity of gliclazide powder as evidenced by poor wetting of powder surface by water. For porower soluble and highly permeable (class-II) drugs, the rate of oral absorption is often controlled by the dissolution rate in the gastrointestinal tract (Lobenbergand Amidon, 2000). Therefore, together with
permeability, the solubility and or dissolution rate of a drug are key determinants of its oral bioavailability (Desai, 2003).

The main objective of this investigation was the enhancement of dissolution and consequently the hypoglycemic effect of gliclazide by the preparation of GZ-HP-β-CD inclusion complexes using physical mixing, kneading and spray drying techniques.

**Materials and Methods**

**Chemicals and materials**

Gliclazide, and Streptozotocin were from Sigma Chemical Company (St. Louis, USA). Hydroxyl propyl-β-cyclodextrin (HP-β-CD), 97% was from Acrosorganics, "Hungary". GliZide tablets from Tabuk Pharmaceutical Company Batch No. 1 KY336. Hydrochloric acid (HCl) "Merk, Germany". All chemicals were used as received without any further purification.

**Methods**

**Preparation of GZ-HP-β-CD solid complexes**

The preparations of solid GZ-HP-β-CD complexes [GZ-HP-β-CD] were performed with a molar ratio 1:1 using three different techniques:

1. **Physical mixture**

Physical mixture was performed by thoroughly mixing of equimolar (1.54 mmole) of both GZ (0.5 gm) and HP-β-CD (2.008 gm) in a ceramic mortar.

2. **Kneading**

HP-β-CD (2.008 gm (1.54 mmole) was putted a ceramic mortar and wetted by few drops of distilled water and properly kneaded with 0.5 gm of GZ (1.54 mmole) by the addition of few drops of distilled water. The dough mass pressed and stretched with fingers, folded over, and rotated through 90º repeatedly until the dough is elastic and smooth then allowed to dry under fume hood at room temperature for 24 hr.

3. **Spray drying**

Spray-drying was performed by mixing 0.5 gm of GZ (1.54 mmole) dissolved in 10 ml 60% methanol solution with 2.008 g of HP-β-CD (1.54 mmole) dissolved in 50 ml of distilled water in a sonicator (Ultrasonicator, Transsonic Ts-540 "Elma", Germany) at room temperature for 25 min. The obtained solution is dried by spray drying using Mini-Spray drier (Buchi B.290, Buchi labortechnik, Switzerland) under purified nitrogen gas. The inlet was adjusted at a flow rate of 880 ml/min at 120°C. The nitrogen gas adapted at a flow rate of 357 NL/hu and outlet temperature was 85 °C.

**Differential scanning calorimetry (DSC)**

The DSC measurements were performed on a Differential Scanning Calorimetry, (Shimatzu DSC60, Japan) with a thermal analyzer. All accurately weighed samples (1.2 to 1.8 mg). The measurements were carried out at a heating rate of 10 °C/ min. In order to provide the same thermal history, each sample were placed in sealed aluminum pans, before heating under nitrogen flow (20 mL/min) at a scanning rate of 10 °C min⁻¹ from 30 to 200 °C or 10 to 250°C.

**X-ray Diffraction Studies (XRD)**

The X-ray powder diffraction patterns were obtained at room temperature using X-ray diffractometer Philips PW3040, Holland at Ni-filtered CuKα radiation, with voltage 50 kV, current 30 mA, and scanning speed of 1° (2θ)/min, investigating the samples in the 2θ range 0-30°.

**Scanning Electron Microscopy (SEM)**

Electron micrographs of crystals were obtained using a scanning electron microscope (LEO 440i, UK) operating at 15 kV. The specimens were mounted on a metal stub with double-sided adhesive tape and coated under vacuum with gold in an argon atmosphere prior to observation.

**In-vitro dissolution properties**

The dissolution studies were performed using a dissolution test apparatus (Turu Grau mod. D-6, Pharmatest, Germany). The dissolution medium used was 0.1N HCl (pH 1.2) Aliquots were analyzed spectrophoto-metrically (UV spectrophotometer shimadzu UV-2550, Japan) for drug content at λmax = 226 nm. Each determination was performed in triplicate.

**Preparation of GZ-HP-β-CD tablets**

**Tablet preparation**

The ingredients of the formula stated bellow were sufficiently mixed using a cubic mixer (AR 400, Erweka GmbH, Heusenstamm, Germany) for 10 min. The mixture was directly compressed into 200 mg convex tablets (10 mm in diameter) using a constant-rate tableting machine (EKO, Korsch GmbH, Heusenstann, Germany).

**Tablet formula:**

- GZ-HP-β-CD complex (prepared by spray drying)..................................................................................50mg
- Aerosil.................................................................1mg
- Magnesium stearate...........................................1mg
- Talc...........................................................................2mg
- Hydroxypropyleulose.........................................6mg
- Maize starch........................................................6mg
- Avicel PH102.........................................................34mg

**Animals**

16 Adult male Sprague-Dawley rats of 200-250 g were used. The animals were housed in the animal care unit and facility at the King Khalid University, college of pharmacy, observing all national guidelines for care and use of laboratory animals. All animals were housed for at least 24 hr.prior to experimental procedures under 12/12 hr light/dark cycles with free access to food and water. This will allow acclimatization of the animals to the lab environment and thus will obviate or minimize stress prior to experimental procedures.
**Induction of diabetes**

An experimentally induced model of type 2 diabetes (NIDDM) was produced by intraperitoneal injection of freshly prepared 60 mg/kg of streptozotocin dissolved in 0.02M sodium citrate buffer (pH 4.0), after fasting the rats for 24 hours.

The degree of diabetes was assessed 3 days later by measuring the blood glucose levels (from the ear vein) using fast take glucometer (Accu-check Performa®). Rats with blood glucose above 300 mg/dl at fed conditions were selected for the experiment.

**Assessment of therapeutic efficacy**

The in vivo hypoglycemic effect of the tablets prepared by spray drying in comparison with the market product (Glizide® 80 mg), were performed. The diabetic rats were dividing into two groups each of 8 rats one for the prepared tablets containing GZ-HP-β-CD complex and the other for the commercial product Glizide. The rats were then applied 10 mg/kg dispersed in (4.6% glycerin, 87.6% polyethylene glycol 400, 7.8% distilled water) and a third group without treatment (control). Samples were withdrawn at 0, 1, 2, 4, 6, 12, 24 hr and the blood samples were collected, and the blood glucose level (BGL) determination test was performed immediately. All in vivo experiments began at 8:00 a.m. and the rats are kept on their standard fed condition (Hashem FM, 2013). The hypoglycemic response was evaluated as percentage decrease in blood glucose level calculated as follows:

\[
\% \text{ Decrease in BGL} = \frac{\text{BLG at } t = 0 - \text{BLG at } t = 24}{\text{BLG at } t = 0} \times 100
\]

**Data analysis**

The pharmacodynamics parameters taken into consideration were maximum percentage decrease in blood glucose level, time for maximum response (t max), time at which half peak percentage decrease in BGL prevails (t 1/2) and area under percentage decrease in BGL versus time curve (AUC 0–24hr) which was calculated adopting the trapezoidal rule (Wagner SG, 1975).

Statistical analyses of data were performed using Student’s t-test. Data reported as means (±) standard deviation (SD). Statistical differences between the groups were considered significant if the (p) value was < 0.05 unless otherwise reported. This statistical analysis was computed with the instat3 software (Bilim V., et al., 2008).

**Results and Discussion**

The phase-solubility of GZ with HP-β-CD were studied according to the method described by Higuchi and Connors, 1965 (Fig1). Inclusion complex showed a typical AL-type solubility curve. The extent of complexation in aqueous media (i.e., the stability of the formed complex) is characterized by the stability constant \( K_s \). Hence, \( K_s \) values were calculated from the initial straight line portion of the solubility diagrams by assuming that a 1:1 complex was initially formed (the slope was smaller than 1).

\[
K_s = \frac{\text{Slope}}{S_0 (1 - \text{Slope})}
\]

Where \( K_s \) is the stability constant for the complex, and \( S_0 \) is the solubility of gliclazide (intercept of the solubility diagram). The stability constant of the inclusion complex in distilled water was found to be 92 M⁻¹.

**Differential Scanning Calorimetry (DSC)**

The DSC thermograms of all the prepared complexes are represented in Figure (2). GZ exhibit a broad endothermic peak at 169.44 °C. The DSC thermogram of HPβ-CD shows a very broad endothermic peak around 70 °C, corresponding to the release of water molecules, and sharp endothermic peak at 275 °C. The thermogram of the physical mixture and kneaded mixture showed reduction in intensity in the melting endothermic peak characteristic of pure GZ. Also, the broad peak corresponding to the dehydration of HP-β-CD was evident. The disappearance of the endothermic peak at 275 °C can suggest some drug-cyclodextrin interaction (Kim K., et al., 1985). On the other hand, spray-drying complexes revealed complete disappearance of the endothermic peak of GZ that may be attributed to the formation of an amorphous solid product or the inclusion of the drug inside the HP-β-CD cavity, or both.

**X-ray diffraction**

The X-ray diffraction patterns of pure GZ, HP-β-CD, and their physical mixture, kneaded and spray-dried inclusion complexes are represented in Figure (3). The diffract gram of GZ, indicating the presence of GZ in the crystalline state, The differences in the diffraction patterns of these complexes (physical, kneaded, and spray dried complexes) from each isolated constituent seem to indicate the formation of a new solid phase as a result of the formation of inclusion complexes, furthermore a reduction in peak height in diffraction pattern of the binary systems was observed in all complexes indicated also a reduction in crystallinity.
Fig. 2. Differential scanning calorimetric thermogram of pure Gliclazide, HP-β-cyclodextrin, physical, kneaded, and spray-dried mixture of GZ and HP-β-cyclodextrin.

Fig. 3. X-ray diffraction pattern of gliclazide, HP-β-cyclodextrin, physical, kneaded, and spray-dried mixture of gliclazide and HP-β-cyclodextrin.

Scanning Electron Microscopy (SEM)

The untreated GL crystals are observed in Fig. 4 using electron microscopy (600×) and micro crystals of formulation complexed GZ prepared by spray drying technique, kneaded mixture and physical mixture are shown in Fig.4 A, B, C, D and E (600×) respectively. The micrographs show that micro crystals were spherical shaped and about 6.3–8 μm in size while the mean diameter of the untreated drug is about 63 μm and the crystals are rod-shaped.

Fig. 4. Scanning Electron Microscope (SEM) micrographs (600X) for; (A) Untreated GZ crystals, (B) Hydroxypropyl-β cyclodextrin (HP-β-CD), (C) for Spray dried Complexed GZ with (HP-β-CD), (D) for Kneaded mixture of GZ & (HP-β-CD), and (E) Physical mixture of GZ & HP-β-CD).
**Dissolution properties of gliclazide-HP-β-cyclodextrin complex**

As illustrated in Figure (6) and Tables (1,2), it is clear that all inclusion complexes exhibited higher dissolution characteristic than pure gliclazide, in both 0.1N HCl and in distilled water. The dissolution profiles were evaluated by the dissolution efficiency (DE) parameter (Khan KA., 1975) (26), the dissolved percentage (DP) and relative dissolution rate at 5 min. (RDR5 min) as listed in Table (1) and (2). The DE of gliclazide and its solid complexes were calculated from the following equation:

\[
\text{Dissolution efficiency (D.E)} = \frac{\int_{t_1}^{t_2} y \, dt}{y_{100} - (t_2 - t_1)} \times 100
\]

Where \( y \): the percentage of dissolved product
\( t_1 \) and \( t_2 \): two time points
\( y_{100} \): maximum percentage of the dissolved product

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**Fig. 5.** Dissolution profiles of gliclazide and corresponding HP-β-cyclodextrin inclusion complex prepared by different methods, in 0.1N HCl (pH 1.2). Each point represents the mean and error bars represent the standard deviation of the mean of measurements from three samples.

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**Fig. 6.** Mean percentage decrease in blood glucose level of diabetic rats after administration of single dose of innovated drug product (GLIZIDE), and complexed gliclazide tablets with HP-β-cyclodextrin.
TABLE 1
Percentage of gliclazide dissolved from the prepared complexes in comparison with the Glizide (market product) in 0.1N HCl (pH 1.2).

<table>
<thead>
<tr>
<th>Time (minute)</th>
<th>Physical mixturea</th>
<th>Kneaded mixturea</th>
<th>Spray-dried mixturea</th>
<th>Glize (Market product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>18.9</td>
<td>59.4</td>
<td>71.1</td>
<td>81.9</td>
</tr>
<tr>
<td>10</td>
<td>28.9</td>
<td>62.1</td>
<td>69.3</td>
<td>85.5</td>
</tr>
<tr>
<td>30</td>
<td>28.8</td>
<td>81.9</td>
<td>72.6</td>
<td>86</td>
</tr>
<tr>
<td>50</td>
<td>38.7</td>
<td>89.1</td>
<td>72.7</td>
<td>86</td>
</tr>
<tr>
<td>60</td>
<td>40.1</td>
<td>92.7</td>
<td>72.9</td>
<td>86</td>
</tr>
<tr>
<td>120</td>
<td>48.6</td>
<td>100</td>
<td>72.9</td>
<td>86</td>
</tr>
</tbody>
</table>

b All the examined samples are equivalent to 10 mg gliclazide.
a Mixture of equimolar ratio of gliclazide and HP-β-cyclodextrin.

Statistical analysis of all dissolution parameters showed that for all the examined complexes the rank order in terms of % dissolution efficiency at 60 min, in 0.1N HCl was spray dried >physical mixture > kneaded mixture > market product > pure gliclazide.

The mean percentage decrease in blood glucose level of diabetic rats after administration of complexed gliclazide with HP-β-cyclodextrin inclusion complexes by different method, in 0.1 N HCl pH (1.2) at 37°C ± 0.5°C.

TABLE 2
Dissolution efficiency after 5 min (DE5min) and 60 min (DE60min), % drug dissolved after 30 min (DP30min), and relative dissolution rate after 5 min (RDR5min) of gliclazide and corresponding HP-β-cyclodextrin inclusion complexes by different method, in 0.1 N HCl pH (1.2) at 37°C ± 0.5°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DE5min</th>
<th>DE30min</th>
<th>DE60min</th>
<th>RDR5min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliclazide</td>
<td>9.9</td>
<td>28.9</td>
<td>28.8</td>
<td>1</td>
</tr>
<tr>
<td>Physical mixturea</td>
<td>29.7</td>
<td>80.4</td>
<td>81.9</td>
<td>3.14</td>
</tr>
<tr>
<td>Kneaded mixturea</td>
<td>35.5</td>
<td>76</td>
<td>72.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Spray-dried mixturea</td>
<td>41.3</td>
<td>86.8</td>
<td>86</td>
<td>4.3</td>
</tr>
<tr>
<td>Market producta</td>
<td>15.1</td>
<td>41.1</td>
<td>52.3</td>
<td>1.57</td>
</tr>
</tbody>
</table>

‡ All the examined samples are equivalent to 10 mg gliclazide.

Conclusions
All the methods of preparation of complexes were found to be effective in improving the solubility of gliclazide in comparison with the commercial product (Glizide tablets). The formation of inclusion complexes was evident in these formulations as shown by DSC and XRD studies. The inclusion complexes prepared by spray drying method in 1:1 molar ratios were the most effective method for improving the solubility of GZ. The in-vivo hypoglycemic effect of the complexed GZHP-β-CD prepared by spray drying significantly improved the biological performance and therapeutic efficacy of the drug compared to Glizide market product.

References


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