

## Improvement of Glibenclamide Bioavailability Using Cyclodextrin Inclusion Complex Dispersed in Polyethylene Glycol

Aiman A. Obaidat <sup>✉</sup> and Nadia M. Ababneh

<sup>1</sup> Department of Pharmaceutical Technology, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan

### ABSTRACT

The specific aim of this study was to improve the aqueous solubility of the oral hypoglycemic agent, glibenclamide, so that to improve its oral absorption, and hence bioavailability after oral administration. This was accomplished by complex formation between glibenclamide and  $\beta$ -cyclodextrin ( $\beta$ -CD) and dispersion in polyethylene glycol 6000 (PEG 6000). Differential Scanning Calorimetry (DSC) and X-Ray Diffractometry (XRD) results confirmed the complex formation between glibenclamide and  $\beta$ -CD. The solubility of glibenclamide increased as a function of increasing the concentration of  $\beta$ -CD and PEG 6000. The dissolution rate of glibenclamide from the prepared complex was more rapid than that from the commercial brand Glibil 5<sup>®</sup> and the physical mixture of the pure components. The oral bioavailability of the prepared formulation was investigated by administration to 12 rabbits and compared to that of Glibil 5<sup>®</sup>. The proposed formulation produced higher bioavailability compared to Glibil 5<sup>®</sup> in terms of the maximum plasma concentration (C<sub>max</sub>) and the area under the curve (AUC) where they were significantly higher compared to those after administration of Glibil 5<sup>®</sup>. Our results confirmed that the oral bioavailability of glibenclamide was significantly improved by complexation with  $\beta$ -CD and dispersion in PEG 6000. Therefore, it can be concluded that PEG 6000 increases the solubilizing effect of  $\beta$ -CD, and hence, reducing the amount of  $\beta$ -CD needed to prepare such a solid dosage form of glibenclamide.

**Keywords:** Glibenclamide,  $\beta$ -cyclodextrin, Polyethylene glycol 6000, Inclusion complex, Dissolution, Oral bioavailability.

### INTRODUCTION

Glibenclamide is a potent oral hypoglycemic agent from the second generation sulphonylureas used in the treatment of type II diabetes mellitus. It is poorly soluble or practically insoluble in water with very poor in vitro dissolution. This causes low bioavailability of the drug where only about 45% of the oral dose is absorbed through the gastrointestinal tract <sup>(1)</sup>. Therefore, the dissolution of glibenclamide is considered to be the rate limiting step for its absorption. Increasing the solubility of glibenclamide was attempted by several

methods. Such methods include inclusion and complexation with cyclodextrins (CDs) <sup>(1)</sup>, solid dispersion <sup>(2)</sup>, the use of surface active agents <sup>(3)</sup>, glass formation <sup>(4)</sup>, and co-administration of water-soluble polymers such as HPMC <sup>(5)</sup>. Co-administration of water-soluble polymers with the drug-CD complex has increased the efficiency of CD in increasing the aqueous solubility of glibenclamide with using minimal amount of CD <sup>(5)</sup>. CDs are very widely used in the pharmaceutical preparations for their desirable effect on changing the physical characteristics of drugs mainly by increasing solubility, stability, and bioavailability. This is due to their unique structure that enables them to engulf the drug or part of it inside their hydrophilic cavity. Such interaction with the drug by non-covalent bonding, that does

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Received on 22/9/2008 and Accepted for Publication on 28/12/2008.

<sup>✉</sup> E-mail: aobaidat@just.edu.jo

not exert a chemical change in the structure of the drug, leads to easy release of the drug from the cavity of CDs. Although its solubility is low,  $\beta$ -CD and its derivatives are of the most commonly used for the purpose of complexation due to their cavity dimension, relative safety, cost and availability. Derivatives of  $\beta$ -CD are increasingly used recently due to their higher solubility<sup>(6)</sup>.

Water-soluble polymers work as suitable drug carriers in many dosage forms. Polyethylene glycol (PEG) polymers (macrogels) are of the most commonly used polymers. They range between liquid, semisolid and solid according to their molecular weight. Thus, providing wide options to choose from, which makes them suitable for a wide range of applications<sup>(6)</sup>.

In this study, increasing the solubility and dissolution rate of glibenclamide was attempted by combining two methods which are complexation with  $\beta$ -CD and solid dispersion in the water-soluble polymer PEG 6000. Each method alone increases the solubility of glibenclamide and both  $\beta$ -CD and PEG 6000 have a positive interaction, when combined together, in increasing the solubility of glibenclamide. We propose a simple formulation to prepare an oral dosage form of glibenclamide which is expected to give a high bioavailability. This will be accomplished by combining  $\beta$ -CD inclusion complex with the water-soluble polymer PEG 6000. This will minimize the amount of  $\beta$ -CD needed in the formulation as well as the amount of glibenclamide which may lead to minimizing the side effects produced by the drug and lowering the cost of the formulation. Physical characteristics of the solid form of this formulation were examined by Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD). Phase solubility and dissolution studies were conducted to examine the release of the drug. In vitro release of and animal in vivo studies were conducted to evaluate the bioavailability and the absorption profile of glibenclamide from the proposed formulation compared to the commercial product, Glibil 5®.

## EXPERIMENTAL

### Materials

Glibenclamide was a gift sample from Hikma Pharmaceutical Company (Amman-Jordan).  $\beta$ -CD and PEG

6000 were obtained from Scharlau Chemicals (Spain). Glibil5®, tablets containing 5 mg of glibenclamide, manufactured by Hikma Pharmaceutical Company, was obtained from the local market. All other chemicals were of analytical reagent grade and used as supplied.

### Methods

#### *Preparation of the formulation*

The formulation was composed of equimolar amounts of glibenclamide and  $\beta$ -CD added to a sufficient amount of PEG 6000 in order to have 5 mg of glibenclamide in each 500 mg of the formula. Each 50 g of the formula was composed of 0.5 g glibenclamide, 1.15 g  $\beta$ -CD, and 48.35 g of PEG 6000. The mixture was heated in an oven at 150 °C for 45 min during which the mixture was thoroughly mixed with a spatula three times to ensure good dispersion of the components. This temperature was chosen to be below the melting point of glibenclamide which is 169 °C. A heating time of 45 min was found to be enough for melting and mixing of the components and to avoid degradation of PEG 6000 which is expected to happen after more than one hour of heating. Fast cooling of the mixture was performed in an ice bath and left for 24 hr and then pulverized in a mortar and sieved to obtain the portion of particle size in the range of 355-500  $\mu$ m. All further experiments were carried out using this range of particle size. Samples from this formula were compressed as tablets using compression machine (Roell & Korthaus RKM50, Germany) under a force of 25 KN  $\text{cm}^{-2}$  for 10 seconds to have a uniform shape tablets weighing 500 mg and each tablet contains 5 mg of glibenclamide.

#### *Preparation of physical mixtures*

Binary physical mixtures of glibenclamide and either  $\beta$ -CD or PEG 6000 were prepared in 1:1 ratios. Tertiary physical mixtures were also prepared in 1:1:1 ratios as the same ratios as in the formulation. All of the components were accurately weighed into a glass screw capped bottles and shaken for 2 min. Each physical mixture was divided into two portions, one is tested as such and the other is treated by heating similar to treatment performed

on the formulation. Therefore, physical mixtures are referred to as treated or untreated according to whether it was heat processed or not.

#### *Content uniformity*

For the determination of content uniformity of glibenclamide in the formulation, 10 tablets were randomly chosen and analyzed. The tablet to be tested was transferred into 100 ml volumetric flask containing methanol and sonicated for 5 min. Then 5 ml of the filtered solution were transferred to a 50 ml volumetric flask and diluted in phosphate buffer (pH 7.4) and analyzed for the content of glibenclamide using a validated HPLC method against a standard sample prepared by the same method. HPLC analysis was performed on a Merk HPLC system equipped with a photodiode array detector and an autosampler. The mobile phase consisted of 0.02 M ammonium acetate in methanol:acetonitrile:water (30:30:40). The flow rate was 1.0 ml/min with a loop size of 100  $\mu$ l at 240 nm. The column was LichroCART® 125 mm-4mm, Purospher®, STAR, RP-18e (5  $\mu$ m) <sup>(2)</sup>.

#### *Characterization of the complexes*

DSC patterns of the pure, binary and tertiary physical mixtures, and the formulation were obtained by DSC analysis in crimped aluminum pans using Shimadzu DSC-50 (Shimadzu, Japan) equipped with a computerized data station. The temperature range was 25-350 °C at a scanning rate of 10 °C/min under a nitrogen flow of 20 ml/min. Indium was used as a standard to calibrate the machine.

Powder X-ray diffraction patterns of the pure components, physical mixtures, and the formulation were recorded using Philips PW 1729 X-ray diffractometer (Philips, Netherlands) at 30 KV over a 2 $\theta$  range of 5-50 at a scanning speed of 0.054 s/2 $\theta$  ( $\lambda = 1.5148 \text{ \AA}$ )

#### *Phase solubility*

The solubility of the formulation and glibenclamide was tested. An excess amount from the formulation or the drug was transferred to a 20 ml screw capped glass

bottles to which 10 ml of phosphate buffer (pH 7.4) was added. Different molar concentrations of  $\beta$ -CD and PEG 6000 or both of them were added to excess amount of glibenclamide. The mixtures were shaken in a water bath at 37 $\pm$ 0.5 °C for 72 hr. All samples were filtered through 0.45  $\mu$ m membrane filters, diluted with buffer and analyzed for glibenclamide using the HPLC method mentioned above.

#### *Dissolution study*

In vitro release of glibenclamide from the physical mixtures and the prepared formulation compared to the commercial brand Glibil 5® was examined using dissolution apparatus I (Erweka, Type DT-D6, Germany). The dissolution medium was 1000 ml of phosphate buffer (pH 7.4) at a temperature of 37 $\pm$ 0.5 °C and rpm of 100. Samples of 5 ml were withdrawn at specified time intervals, replaced with equal volume of fresh phosphate buffer, filtered through 0.45  $\mu$ m membrane filters and then analyzed using the HPLC method mentioned above.

#### *In vivo study*

This study was conducted by administering a single oral dose of 5 mg of Glibil5® or the prepared formulation under fasting conditions to twelve rabbits with an average weight of 2 kg. A crossover design study was performed with a wash out period of one week between the two phases of the study. The tablet under investigation was given to each rabbit orally through a disposable solution administration set. The tube of which is designed with a wider end of plastic where the tablet can be fixed and the other end of the tube was attached to a 20 ml syringe filled with 5% dextrose solution. The end with the tablet fixed on was pushed into the pharynx of the rabbit and then the syringe is pressed to push the tablet and the solution into the stomach by directly preventing the rabbit from chewing the tablet. Dextrose solution was given instead of water to prevent hypoglycemia due to administration of glibenclamide. Blood samples were collected from the rabbit's ear vein at pre-determined time intervals and were transferred into heparinized tubes and plasma was separated promptly and immediately

frozen at -20 °C until assayed. The plasma samples were analyzed using a sensitive, specific, and validated modified HPLC method. The pharmacokinetic parameters of the drug were calculated as the average of the results obtained from twelve rabbits, using Winonline Software (Pharsight, USA)

HPLC analysis of plasma samples was performed on a Merk HPLC system equipped with a photodiode array detector and an autosampler. The column was LichroCART® 125mm-4mm, Purospher®, STAR, RP-18e (5 µm). The mobile phase consisted of 0.05 M ammonium dihydrogen phosphate:acetonitrile (52:48) adjusted to pH 3.5 using phosphoric acid. The flow rate was 1.0 ml/min at a wavelength of 240 nm with a loop size of 100 µl. Diazepam was used as internal standard.

## RESULTS AND DISCUSSION

The content of glibenclamide in the prepared

formulation was approximately 97% of the actually added amount of glibenclamide during preparation. This test was performed to make sure that our formulation is not overloaded with more than 5 mg of glibenclamide since we compared our results to the commercial brand Glibil 5®. The content uniformity of glibenclamide in Glibil 5® was found to be in the range of 98.4-104%.

DSC thermograms of the pure components used in the study are shown in Figure 1. The thermogram of pure glibenclamide was typical of a crystalline anhydrous substance showing a sharp endothermic peak at 175 °C corresponding to the melting point of glibenclamide. The DSC curve of β-CD showed a broad peak starting at 40 °C indicating the loss of crystal water and an endothermic peak at about 130 °C. The DSC curve of PEG 6000 is also typical of a crystalline anhydrous substance with a sharp endothermic peak at about 62 °C corresponding to the melting point of PEG 6000.

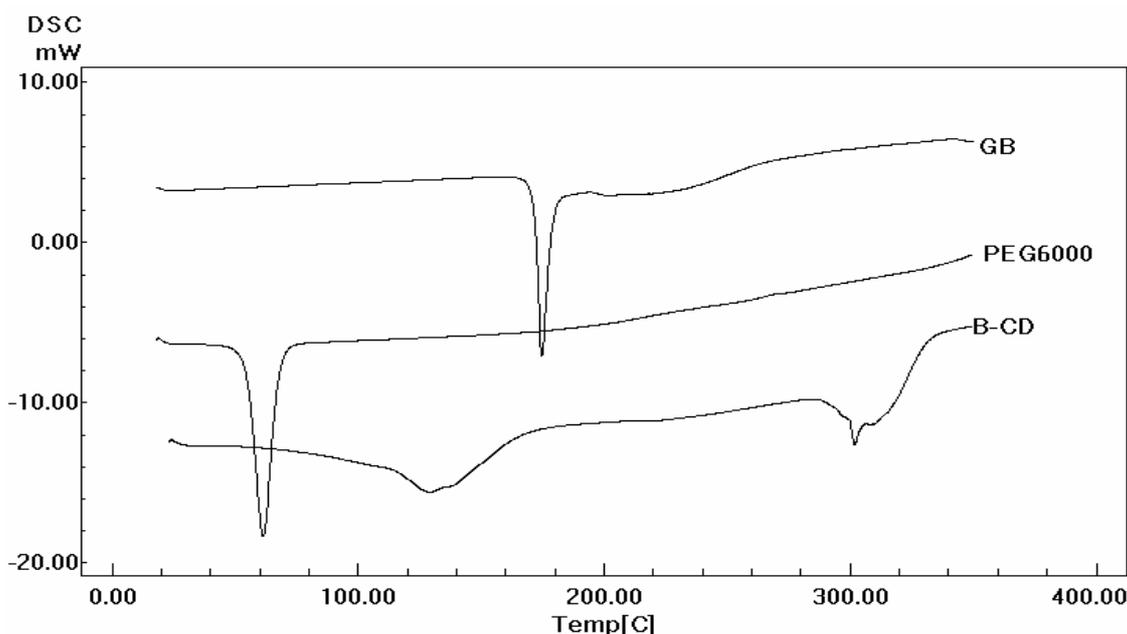


Fig. (1): DSC thermograms of glibenclamide, PEG 6000, and β-CD.

The DSC thermogram of the physical mixture was approximately the superposition of the raw materials where the individual components are clearly distinguishable. However, there was a depression in the melting points of glibenclamide and PEG 6000 by few degrees. This may indicate some kind of weak interaction in the physical mixture by mechanical activation or heating due to the DSC scanning process itself<sup>(7-9)</sup>.

Inclusion of a guest molecule in the cavity of  $\beta$ -CD leads to changes in the thermal behavior of such a molecule. The DSC thermogram of the formulation is shown in Figure 2. There was a complete disappearance of the characteristic endothermic peaks of glibenclamide and  $\beta$ -CD in this thermogram. This is attributed to the

formation of a true inclusion compound, and hence, the disappearance of the endothermic peak of glibenclamide due to its inclusion in the  $\beta$ -CD cavity. However, there was a sharp endothermic peak at about 61 °C due to the melting of PEG 6000. The presence of this peak was expected due to the high ratio of PEG 6000 in this formulation and it is not expected that  $\beta$ -CD would form an inclusion complex with PEG 6000. The cavity of  $\beta$ -CD is too small to fit a large molecular weight substance such as PEG 6000<sup>(10)</sup>. Considering these results, we can conclude the formation of an amorphous complex of glibenclamide and  $\beta$ -CD dispersed in the PEG 6000 matrix.

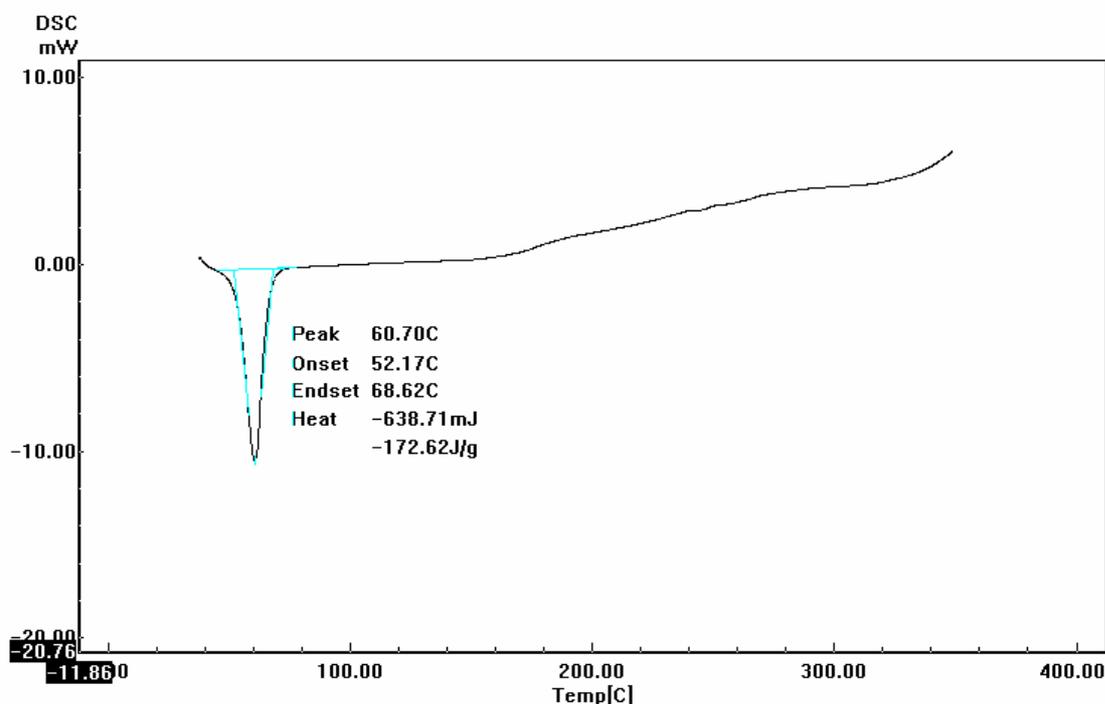


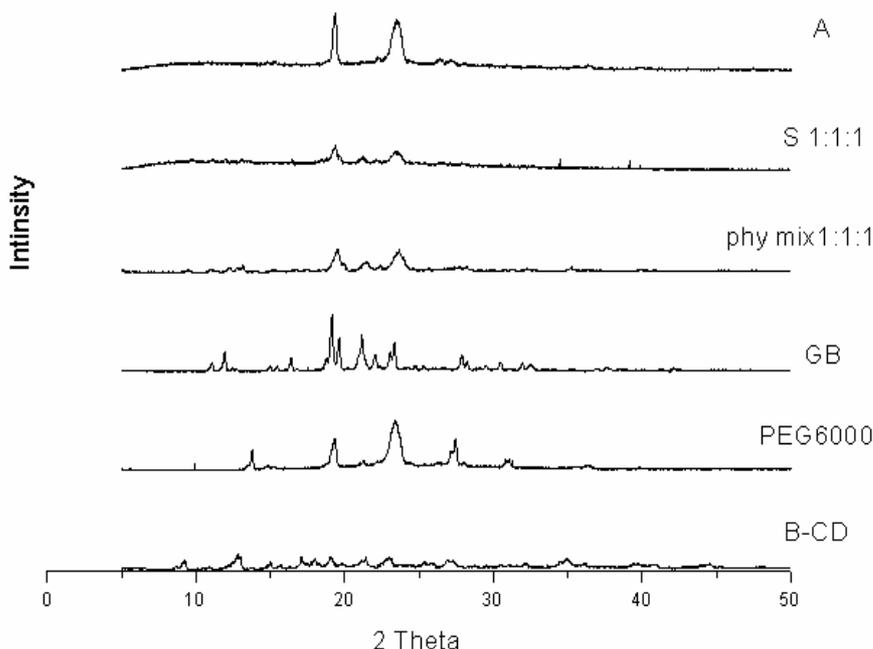
Fig. 2. DSC thermogram of the formulation.

Figure (3) shows the X-ray diffraction patterns of glibenclamide,  $\beta$ -CD, PEG 6000, physical mixture, and the formulation. The X-ray diffraction patterns of glibenclamide,  $\beta$ -CD, and PEG 6000 revealed several high intensity peaks indicative of their crystalline nature.

Comparing the diffraction patterns of the pure components with that of the physical mixture, it can be noticed that there are no marked dissimilarities in their patterns since they were relatively the superposition of the pure components. The characteristic peaks of

glibenclamide nearly completely disappeared from the X-ray diffraction pattern of the formulation. This indicates the formation of an amorphous complex of glibenclamide and  $\beta$ -CD dispersed in PEG 6000. Converting a drug to an amorphous nature can be a consequence of the heating process. Therefore, the X-ray data cannot discriminate whether that the heated product obtained is a true inclusion complex or a homogenous dispersed mixture of

the amorphous components<sup>(11)</sup>. Nevertheless, considering the results of the DSC analysis as well as the lack of some important diffraction peaks in the pattern obtained for the formulation and nearly the complete disappearance of some of glibenclamide characteristic peaks, it can be concluded that there is a formation of an inclusion complex between glibenclamide and  $\beta$ -CD dispersed in PEG 6000<sup>(11)</sup>.



**Fig. (3): X-ray diffraction patterns of  $\beta$ -CD, PEG 6000, Glibenclamide (GB), untreated physical mixture of the components, treated physical mixture of the components (S), and the prepared formulation (A).**

#### Phase Solubility

The equilibrium phase-solubility diagram of glibenclamide with increasing concentration of  $\beta$ -CD is shown in Figure 4. Glibenclamide solubility was observed to increase with increasing the concentration of  $\beta$ -CD which indicates the formation of an inclusion complex. The phase solubility diagram obtained at the concentrations used in this study can be classified as type  $A_L$  diagram. Since the slope of the straight line in the diagram was less than 1, it was assumed that the observed

increase in the solubility is due to the formation of a complex of 1:1 stoichiometry. The apparent stability constant,  $K_c$ , was calculated to be  $2035 \text{ L mol}^{-1}$ , and it was calculated using the slope of the straight line of the phase solubility diagram and the following equation:

$$K_c = \text{slope} / [S_o(1 - \text{slope})]$$

where  $S_o$  is solubility of pure glibenclamide without cyclodextrin<sup>(12)</sup>.

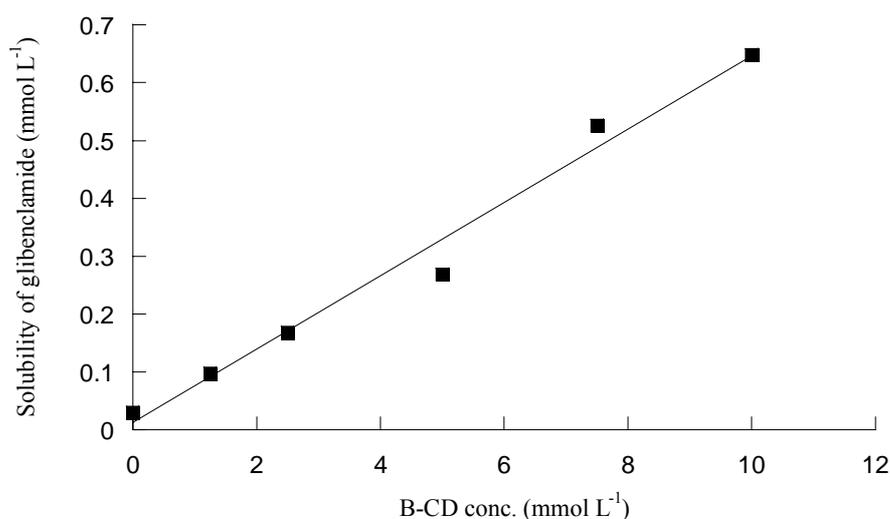


Fig. 4. Phase solubility diagram of glibenclamide as a function of  $\beta$ -CD concentration.

The solubility of glibenclamide increased non-linearly as a function of increasing concentrations of PEG 6000 alone as shown in Figure 5. This system of phase of phase solubility diagram is classified as  $A_p$  type where it exhibits a positive deviation indicating the formation of 1:1 and/or 1:2 complex between glibenclamide and PEG 6000 at the concentrations used in this study. Figure 5 also shows the

effect of increasing concentration of PEG 6000 on the solubility of glibenclamide in the presence of a constant concentration of  $\beta$ -CD of 2.5 mmol L<sup>-1</sup>. The solubilizing effect of PEG 6000 and  $\beta$ -CD when combined was more than additive. The effect was synergistic since a greater extent of solubilization was achieved more than when PEG 6000 and  $\beta$ -CD were used separately.

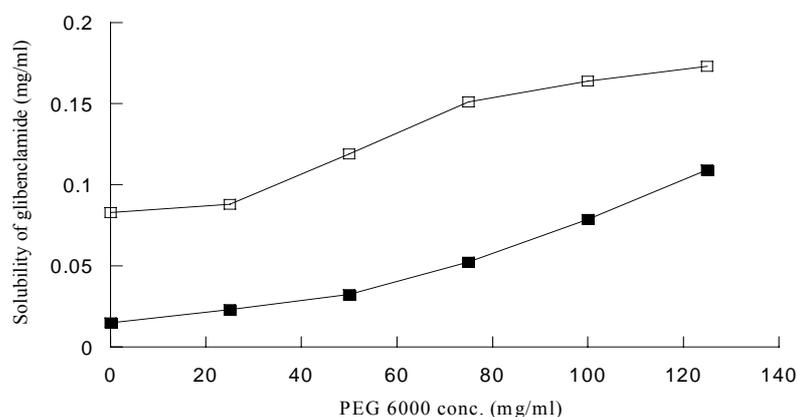
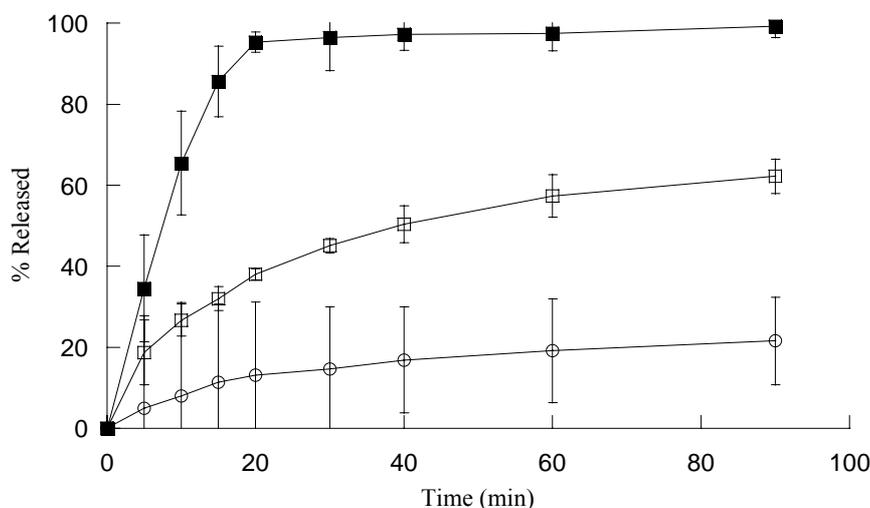


Fig. 5. Phase solubility diagram of glibenclamide as a function of PEG 6000 concentration alone (■), and in the presence of a fixed concentration of  $\beta$ -CD of 2.5 mmol L<sup>-1</sup> (□).

Water-soluble polymers have been reported to increase the apparent binding constants of the drug-CDs complexes which results in enhanced solubility of many drugs<sup>(13)</sup>. Increasing the concentration of PEG 6000 in the presence of fixed concentration of  $\beta$ -CD changed the phase solubility diagram dramatically than from those for both separately. However, previous studies showed that increasing the concentration of  $\beta$ -CD in the presence of a fixed amount of the polymer increased the solubility but did not change the type of the phase solubility diagram<sup>(5)</sup>.

#### Dissolution Study

Dissolution studies were conducted on the physical mixture, Glibil 5®, and the formulation. The physical mixture showed the lowest release were less than 12% of the drug was released in the first 15 min followed by Glibil 5® with only 32% was released within 15 min. Considering the same time of 15 min, the percent released of the drug from the formulation was 85%. The release profiles are shown in Figure 6.



**Fig. 6. Dissolution of glibenclamide in phosphate buffer of pH 7.4 from physical mixture (○), Glibil 5® (□), and the prepared formulation (■).**

The highest rate and extent of dissolution were obtained from the formulation compared to the physical mixture and Glibil 5®. The improved dissolution rate of the prepared inclusion complex may be due to the increase in solubility as well as marked reduction in the crystallinity of glibenclamide.

#### In Vivo Study

The in vivo availability of glibenclamide from the prepared formulation compared to Glibil 5® was studied in rabbits. The time course of the mean plasma concentration of glibenclamide is shown in Figure 7. It

was found out that the formulation produced a higher maximum plasma concentration ( $C_{max}$ ) of glibenclamide compared to that of Glibil 5® with values of 3208.33 ng/ml and 1971.67 ng/ml, respectively. The time to reach  $C_{max}$  ( $T_{max}$ ) for the formulation was also longer where it was about 4.6 hr compared to 2.5 hr for Glibil 5®. The extent of absorption of glibenclamide from the formulation, as represented by the total area under the curve (AUC), was also higher compared to Glibil 5®. AUC produced by the formulation was 19858.95 ng.hr/ml compared to 10879.92 ng.hr/ml for

Glibil 5®. The pharmacokinetic parameters obtained for glibenclamide after the in vivo study for both of the formulation and Glibil 5® are presented in Table 1. The statistical analysis of the pharmacokinetic parameters using one way ANOVA test revealed that the mean values obtained for the formulation were higher and

significantly different from those obtained for Glibil 5®. These findings indicated that this formulation produced higher bioavailability compared to Glibil 5®. There was a correlation between the in vitro dissolution of the drug from the formulation and the results obtained from the in vivo study.

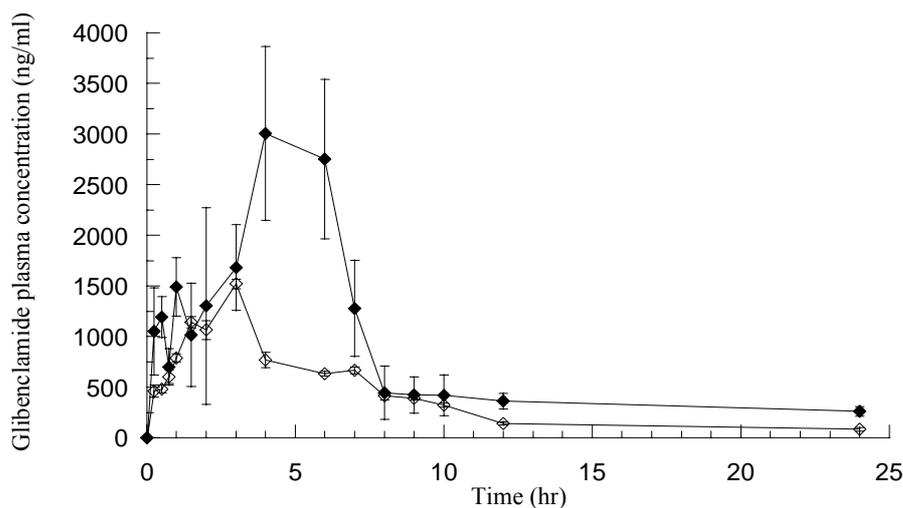


Fig. 7. Glibenclamide plasma concentration versus time in rabbits after administration of Glibil 5® (◇), and the prepared formulation (◆). n = 12.

Table (1). Pharmacokinetic parameters for Glibil 5® and the prepared formulation obtained after administration to rabbits.

Pharmacokinetic Parameter	Glibil 5®	RSD%	Formulation	RSD%
C <sub>max</sub>	1971.67 ng/ml	22.23	3208.33 ng/ml	23.39
T <sub>max</sub>	2.58 hr	35.56	4.66 hr	22.16
AUC <sub>0-12 hr</sub>	6552.71 ng.hr/ml	23.2	14253.20 ng.hr/ml	24.14
AUC <sub>0-∞</sub>	10879 ng.hr/ml	32.73	19858.95 ng.hr/ml	45.6
Ke	0.0897 hr <sup>-1</sup>	13.7	0.0296 hr <sup>-1</sup>	64.5
t ½	7.8 hr	13.7	29.6 hr	64.5

Results are the average obtained from administration to 12 rabbits. C<sub>max</sub>: maximum concentration of the drug in the plasma; T<sub>max</sub>: time to reach the maximum plasma concentration; AUC: area under the curve; Ke: elimination rate constant; t ½: elimination half-life; RSD: relative standard deviation

**CONCLUSION**

In conclusion, the results showed that the solubilizing effect of  $\beta$ -CD increased by the addition of PEG 6000. Therefore, the pharmaceutical usefulness of  $\beta$ -CD can be substantially improved by co-administration of a water-soluble polymer such as PEG 6000.

**ACKNOWLEDGEMENT**

This study was supported by a grant from the Deanship of Scientific Research at Jordan University of Science and Technology, Irbid-Jordan.

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