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**INFLUENCE OF PREPARATION METHOD ON INCLUSION COMPLEXES OF  
GLICLAZIDE AND CYCLODEXTRIN**

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**ABSTRACT**

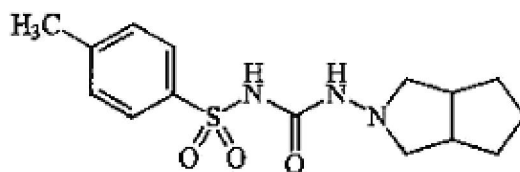
In the present investigation inclusion complexes are formed between Gliclazide (Glz) and  $\beta$ -Cyclodextrin ( $\beta$ -CD) by different methods (physical mixture, kneading method and solvent evaporation method) in different molar ratios (1:1, 1:1.5 and 1:2) The aim of present investigation is to identify the effect of preparation method on the dissolution rate of Glz-  $\beta$ -CD complexes. Solubility studies for Glz and  $\beta$ -CD were performed which reveals that it follows B<sub>S</sub> type profile. FTIR studies were conducted for all the prepared complexes and the results concluded that there were no interactions between Glz and  $\beta$ -CD. Dissolution studies were performed for all the prepared complexes in phosphate buffer of p<sup>H</sup> 7.4. The results conclude that complex prepared by solvent evaporation method at 1:2 molar ratio has faster dissolution rates when compared with all the other complexes.

**Keywords:** Gliclazide,  $\beta$ -Cyclodextrin, Phase solubility studies, FTIR, Dissolution rate.

**INTRODUCTION**

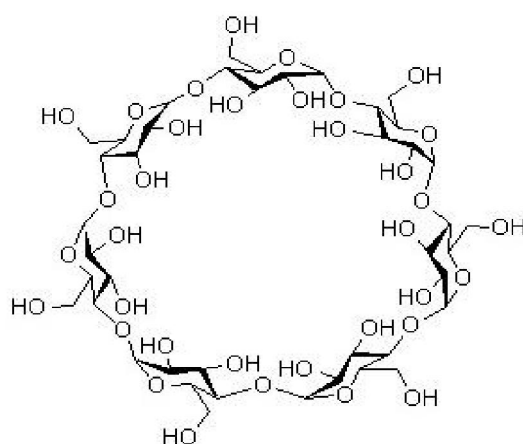
Gliclazide, 1-(3-Azabicyclo (3, 3, 0) oct-3yl)-3-*p*-tolylsulfonylurea] (Fig.i.), is a second generation of hypoglycemic sulfonylureas<sup>1</sup>. It is a white or almost white crystalline powder, odorless, flavorless powder with melting point of 165-170<sup>0</sup> C It is practically insoluble in water, sparingly soluble in acetone, slightly soluble in ethanol and freely soluble in dichloromethane<sup>2</sup>. It is an oral hypoglycemic sulphonylurea used for the treatment of non-insulin dependent diabetes mellitus (NIDDM). The drug is characterized by a low solubility in water, leading to poor oral bioavailability<sup>3, 4</sup>.

**Fig i. Chemical structure of Gliclazide**



- Cyclodextrins are chemically cyclic oligosaccharides containing seven glucopyranose units attached by  $\alpha$ -(1, 4) glycosidic bonds<sup>5, 6</sup> (Fig.ii.). These glucopyranose chains form a cone-like cavity into which drug may enter and form a water-soluble complex and thus change the drugs physicochemical properties of the drug molecule. Cyclodextrins possess a special ability to complex with drugs enabling them to increase solubility, bioavailability and stability, used to reduce bitterness, to convert liquid drugs into microcrystalline powders and to reduce or eliminate unpleasant taste and smell., to reduce tissue irritation upon dosing of drugs<sup>7</sup>. - Cyclodextrin can be found in several marketed oral dosage forms as well as in topical, buccal and rectal drug formulations. - Cyclodextrin is listed in the European pharmacopoeia, USP/NF and JPC. Numbers of studies have been reported by using  $\alpha$ -CD to increase aqueous solubility, dissolution rate and bioavailability of different drugs<sup>8</sup>.

**Figure ii. Chemical structure of  $\beta$ - Cyclodextrin**



The aim of the present study is to prepare, characterize and to assess the invitro dissolution studies of Gliclazide and  $\alpha$ -Cyclodextrin inclusion complexes in phosphate buffer of  $p^H$  7.4, which is the dissolution medium recommended by USP for Gliclazide. To achieve these goals, different preparation techniques, such as solvent evaporation, kneading and physical mixture were evaluated.

## MATERIALS AND METHODS

### Materials:

Gliclazide was supplied by courtesy of Dr.Reddy's Laboratories Ltd, India, and  $\beta$ -CD was provided by Cerestar USA, INC. All other materials were of analytical reagent grade.

#### **Phase solubility studies:**

The stability constant for inclusion complex between Gliclazide and  $\beta$ -CD was determined by using the phase solubility method<sup>10</sup>. 50mg of Gliclazide was added into glass-stopper flasks containing 50ml of  $\beta$ -CD solutions of increasing concentrations (0, 0.02, 0.04, 0.06, 0.08 and 0.1M). The flasks were sealed and shaken at  $25\pm 0.5^\circ\text{C}$ . After equilibration for 72 h, the solutions were filtered through membrane filter (0.22  $\mu\text{m}$  pore size). Then the filtrates were suitably diluted and the concentration of Gliclazide was estimated by UV spectroscopy at 226 nm. The apparent stability constant of the complex with  $\beta$ -CD ( $K_c$ ) was calculated from the phase-solubility diagram using the equation proposed by Higuchi and Connors (1965)<sup>9</sup>.

$$K_c = \frac{\text{slope}}{\text{Intersept}(1 - \text{slope})}$$

Where, the intercept is the apparent solubility of Gliclazide at  $25\pm 0.5^\circ\text{C}$ .

#### **Preparation of the solid complexes:**

Solid complexes of Gliclazide (60mg) with  $\beta$ -CD in 1:1, 1:1.5, and 1:2 molar ratios were prepared by different techniques described below.

#### **Physical mixture:**

The physical mixtures of Gliclazide with  $\beta$ -CD (1:1, 1:1.5, 1:2 molar ratios) were prepared by light mixing the two components in a mortar using the geometric dilution technique<sup>10</sup>. Then it was passed through a 60-mesh sieve and stored in desiccators until used.

#### **Kneading method:**

A solid complex of Gliclazide with  $\beta$ -CD (1:1, 1:1.5, 1:2 molar ratios) was prepared by kneading method<sup>11</sup>. Cyclodextrin was triturated in a mortar with purified water to obtain a paste and then Gliclazide was added. The resulting mixtures were mixed for 30 min and then dried in an oven at  $40^\circ\text{C}$ . The dried mass was pulverized and passed through a 60-mesh sieve and stored in desiccators until used.

#### **Solvent evaporation method:**

The alcoholic solution of Gliclazide is simply added to the aqueous solution of  $\beta$ -CDs (1:1, 1:1.5, 1:2 molar ratios). The resulting mixture is stirred and evaporated under vacuum at 45° C. The dried mass was pulverized and passed through a 60-mesh sieve<sup>12</sup> and stored in desiccators until used.

#### **Infrared Spectroscopic Studies:**

The complexation of Gliclazide with  $\beta$ -CD in solid state was characterized by Infrared Spectroscopy (IR) technique<sup>13</sup>. The Infrared absorption spectra of Gliclazide and its complexes were obtained using an infrared spectrophotometer (Shimadzu IR-470, Japan). Each sample was analyzed using KBr disk method in the range of 500 to 4000  $\text{cm}^{-1}$ .

#### **Gliclazide content in the inclusion complexes:**

Gliclazide content in the various freshly prepared solid complexes was determined before being subjected to any *in vitro* testing. An amount of the prepared system equivalent to 60 mg of the Gliclazide was dissolved in 100 ml of ethanol. Then the solution is diluted suitably and assayed by using UV spectrometer to know the drug content in each of the prepared inclusion complexes. The experiment was carried out in triplicate and the average value was determined.

#### **Dissolution studies:**

The dissolution studies of pure Gliclazide and its various  $\beta$ -CD systems were performed using USP XXIV apparatus (Labindia 2000). In each basket, 900 ml of  $\text{pH}$  7.4 phosphate buffer was used as a dissolution medium<sup>14</sup>. The rotation speed was 100 rpm and the temperature was adjusted at  $37 \pm 0.5^\circ\text{C}$ . An accurately weighed amount of the prepared system equivalent to 60 mg of the drug was added to each flask. Samples were taken at predetermined times and the concentration of the drug was calculated by using UV spectroscopy at 226 nm against blank. For each system, dissolution was run in triplicate and the average percentage of the drug dissolved was determined.

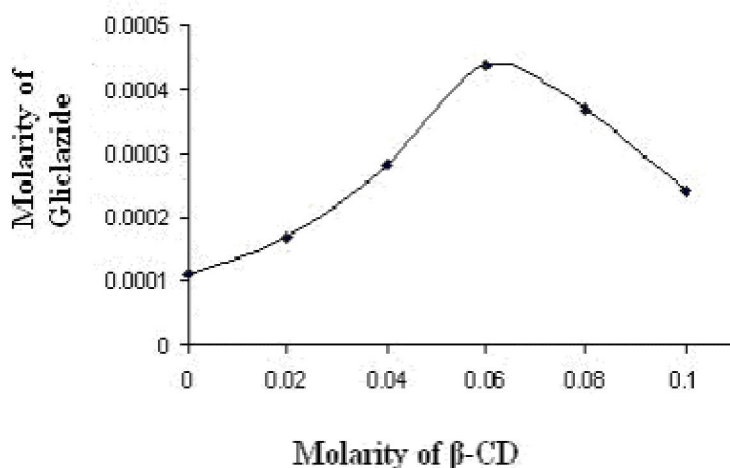
## **RESULTS AND DISCUSSION**

#### **Phase solubility studies:**

Fig.iii. represents the solubility of Gliclazide,  $\beta$ -CD complexes. It shows a B<sub>s</sub> type solubility curve<sup>10</sup>. The first portion of the plot shows that the aqueous solubility of the drug linearly increases as a function of  $\beta$ -CD concentration. Then a short plateau

region is reached which is followed by a decrease in total Gliclazide and precipitation of crystalline complex. Because of the ascending linear portion of the diagram, it was assumed that the solubility increase was due to the formation of 1:1 complex. The apparent solubility constant ( $K_c$ ) was estimated by using equation (1) and was found to be  $61.44\text{M}^{-1}$ .

**Fig.iii. Phase solubility studies of gliclazide and  $\beta$ -CD at room temperature**



### Infrared Spectroscopic Studies:

To confirm the complexation of Gliclazide with  $\beta$ -CD in the solid state, IR spectroscopy was employed (Fig iv.) to compare pure drug,  $\beta$ -CD, physical mixture and inclusion complexes formed by kneading and solvent evaporation methods.

The infrared spectrum of pure Gliclazide (Fig iv.a) showed principal peaks at  $1164.18\text{ cm}^{-1}$  ( $S = 0$  asymmetrical vibration bands),  $1347.5\text{ cm}^{-1}$  ( $S = 0$  symmetrical vibration bands),  $1596.23\text{ cm}^{-1}$  (NH deformation band),  $1710.3\text{ cm}^{-1}$  (C=O stretching band) (C=O deformation),  $3273.6\text{ cm}^{-1}$  (NH stretching band).

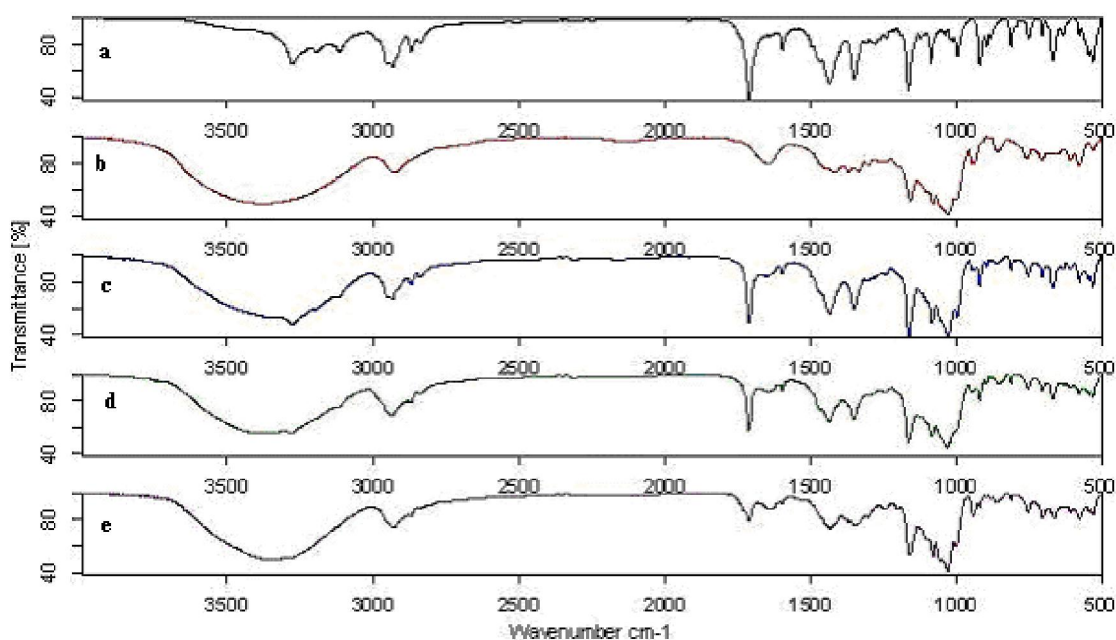
The IR spectrum of  $\beta$ -CD (Fig iv.b) is characterized by vibration of the  $-\text{CH}$  and  $-\text{CH}_2$  groups in the  $2800\text{--}3000\text{ cm}^{-1}$  region. And intense bands at  $3300\text{--}3500\text{ cm}^{-1}$ , associated with the absorption of the hydrogen bonded-OH groups of  $\beta$ -CD

In IR spectrum of physical mixture (Fig iv.c) corresponds simply to the superposition of the IR spectra of the pure Gliclazide and pure  $\beta$ -CD. Absence of additional peaks indicated that there were no interaction between Gliclazide and  $\beta$ -CD.

The IR spectra of the sample prepared by kneading method (**Fig iv.d**) showed small differences when compared with pure drug like Decreased intensity of the NH deformation band at  $1596.3\text{ cm}^{-1}$ , marked reduction in the peak intensity of carbonyl stretching band at  $1710\text{ cm}^{-1}$ , narrowing of the peak at  $3273.61\text{ cm}^{-1}$  (NH stretching band). Absence of additional peaks indicated that there were no interaction between Gliclazide and  $\beta$ -CD.

The IR spectra of the sample prepared by solvent evaporation method (**Fig iv.e**) showed small differences when compared with pure drug like shifting of the S=O asymmetrical vibration bands from  $1164.18$  to  $1159.85\text{ cm}^{-1}$ , decreased intensity of the NH deformation band at  $1596.3\text{ cm}^{-1}$ , marked reduction in the peak intensity of carbonyl stretching band at  $1710\text{ cm}^{-1}$  and narrowing of the peak at  $3273.61\text{ cm}^{-1}$  (NH stretching band). . Absence of additional peaks indicated that there were no interaction between Gliclazide and  $\beta$ -CD.

**Fig.iv.. IR spectra of: a) Gliclazide, b)  $\beta$ -CD, c) Physical mixture of Gliclazide and  $\beta$ -CD, d). Gliclazide- $\beta$ -CD inclusion complexes by kneading method, e) Gliclazide-  $\beta$ -CD inclusion complexes by solvent evaporation method**



**Gliclazide content in the inclusion complexes:**

The drug content of all the systems (physical mixture, kneaded system and the solvent evaporation systems) were indicated in Table.i..The low values of standard deviation in drug content of Gliclazide and  $\beta$ -CD complexes indicated uniform drug distribution in all the complexes

**Table 1: Drug content in gliclazide,  $\beta$ -Cyclodextrin physical mixture, kneaded system and solvent evaporated system**

Molar ratio	gliclazide- $\beta$ CD physical mixture	gliclazide- $\beta$ CD kneaded mixture	gliclazide- $\beta$ CD Solvent evaporated mixture
1:1	59.8	60.13	59.88
1:1.5	60.1	60.7	60.12
1:2	59.96	59.26	60.06

**Dissolution studies:**

Fig-v, vi, vii shows the dissolution behavior of Gliclazide alone, from physical mixture and from inclusion complexes of Gliclazide and  $\beta$ -CD. The release rate profiles were drawn as the percentage Gliclazide dissolved from the pure drug, physical mixture and inclusion complexes versus time. From the dissolution studies it is evident that complex of the drug and  $\beta$ -CD exhibited faster dissolution rates than the pure drug and physical mixture where as the physical mixture exhibited faster dissolution rate than the pure drug. In case of complexes, complex prepared by solvent evaporation method exhibited faster dissolution rates than the complexes prepared by kneading method. The extent of the enhancement of the dissolution rate was found to be dependent on the preparation method. In case of physical mixtures the small increase in dissolution rate is due the surface tension lowering effect of  $\beta$ -CD which results in wetting of the drug surface. In case of complexes prepared by solvent evaporation method the increase in dissolution rate may be due to formation of water soluble complexes of the drug with  $\beta$ -CD.

Fig v. .Dissolution rate profiles of A) Gliclazide B) Gliclazide- $\beta$ -CD physical mixture (1:1 molar ratio),C) Gliclazide- $\beta$ -CD physical mixture (1:1.5 molar ratio), D) Gliclazide- $\beta$ -CD physical mixture (1:2 molar ratio)

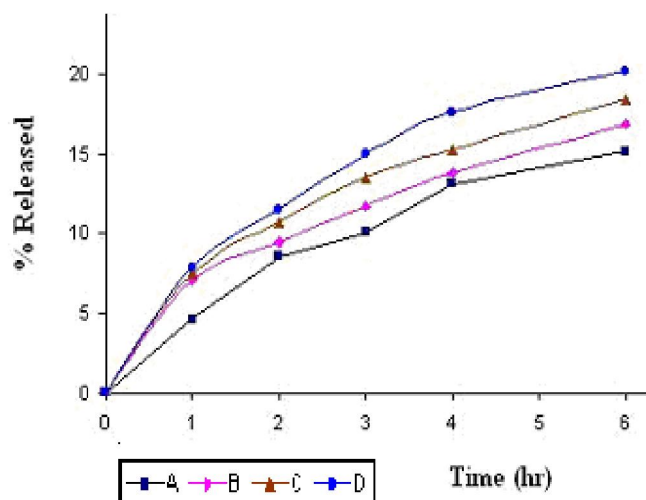


Fig vi. .Dissolution rate profiles E) Gliclazide- $\beta$ -CD complex by kneading method (1:1 molar ratio), F) Gliclazide- $\beta$ -CD complex by kneading method (1:1.5 molar ratios), G) Gliclazide- $\beta$ -CD complex by kneading method (1:2 molar ratio)

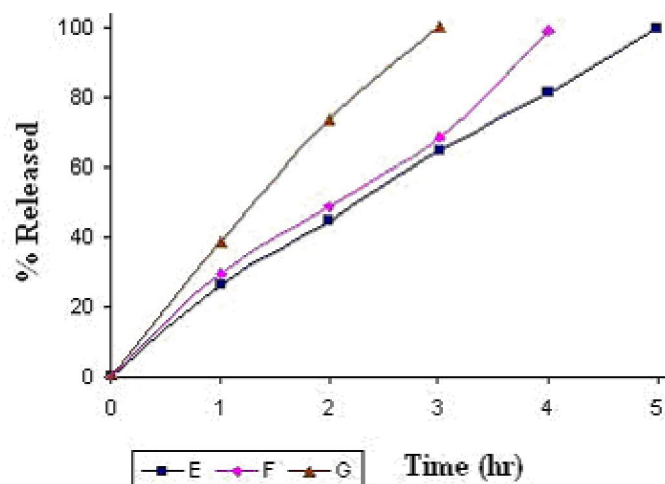
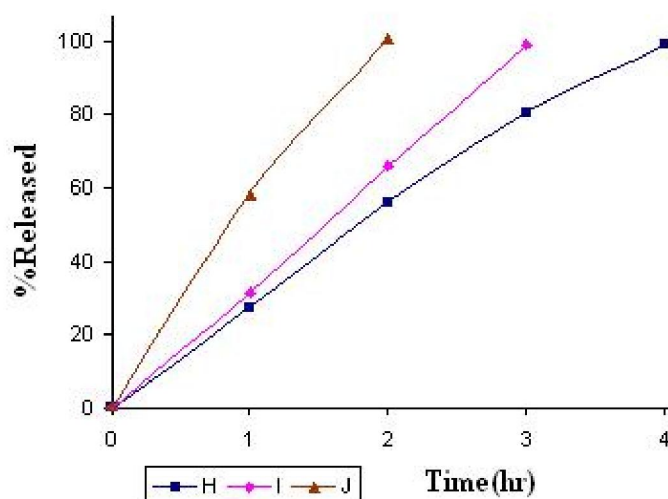




Fig vii. Dissolution rate profiles H) Gliclazide- $\beta$ -CD complex by solvent evaporation method (1:1 molar ratio), I) Gliclazide- $\beta$ -CD complex by solvent evaporation method (1:1.5 molar ratios), J) Gliclazide- $\beta$ -CD complex by solvent evaporation method (1:2 molar ratio)



## CONCLUSION

The results of this study indicate the capacity of  $\beta$ -CD to increase solubility of Gliclazide in pH 7.4 buffer through formation of an inclusion complex. The results showed that the dissolution rate of the drug is high for the complex prepared by solvent evaporation method at 1:2 molar ratios than any other preparations. So these Gliclazide- $\beta$ -CD binary systems are useful in developing formulations of Gliclazide with improved dissolution properties.

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