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# Studies on solubility and hypoglycemic activity of gliclazide β-cyclodextrin-hydroxypropylmethylcellulose complexes

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This study was undertaken with an objective to increase the dissolution rate and bioavailability of a poorly water soluble drug gliclazide (Gz) by complexation with β-cyclodextrin (CD) in the presence of hydroxypropylmethylcellulose (HPMC). Phase solubility studies of Gz were performed in aqueous solutions of different concentrations of CD alone and in the presence of some water soluble polymers. Gz-CD complexes were prepared in 1 : 1 and 1 : 2 drug : CD molar ratios by autoclaving, neutralization and kneading methods. The complexes were also prepared in the presence of 0.05% w/w HPMC. Physical mixtures of Gz-CD in 1 : 1 and 1 : 2 molar ratios were also prepared. Complexes and physical mixtures were characterized and evaluated for *in vitro* dissolution in distilled water and hypoglycemic activity in rats. CD enhanced the dissolution of Gz to 1.5 to 2.0 fold. Presence of water soluble polymer HPMC in Gz-CD complexes further enhanced the rate and extent of drug dissolution to 2.5 fold. Gz-CD-HPMC complexes were found to be more promising as they produced not only an early onset but also more intense hypoglycemic effect as compared to pure drug powder and commercial tablets.

## 1. Introduction

Gliclazide (Gz) is an oral hypoglycemic agent, belonging to the second generation of sulfonylureas and is widely used in the treatment of non-insulin dependent diabetes mellitus (NIDDM). The oral dosage form of Gz suffers from major drawbacks in therapeutic application because of its very low aqueous solubility (≈65 mg/l), low dissolution rate in water and large interindividual bioavailability variation [1, 2].

β-Cyclodextrins (CD) have widely been employed in pharmaceutical formulations to enhance solubility, dissolution rate, stability and bioavailability of poorly water soluble drugs [3]. Enhanced solubility of Gz after complexation with CD is reported [4]. It has also been reported [5–7] that water-soluble polymers have a synergistic effect on the solubility enhancing effect of β-cyclodextrins.

Based on the above facts, the present study was undertaken with an objective to increase the dissolution rate and bioavailability of Gz by complexation with CD in the presence of HPMC.

## 2. Investigations, results and discussion

### *2.1. Phase solubility studies*

Phase solubility studies of Gz in aqueous solutions of different concentrations of CD alone and in the presence of water soluble polymers viz., HPMC, Na-CMC and PEG-4000 were performed. The stoichiometry of the complex (Gz-CD) determined from the initial ascending portion of the $B_s$ type phase diagram (Fig. 1) indicated 1 : 2 stoichiometric molar ratio of Gz and CD in the complex. The apparent stability constant ($K_c$) of the complex in distilled water was found to be 1617.7 $M^{-1}$. The solubility of Gz increased approximately 7-fold in the presence of $4.0 \times 10^{-3}$ M CD containing 0.1% w/v HPMC and, therefore, the complexes prepared for further studies contained HPMC only.

### *2.2. Characterization of complexes*

All the Gz-CD complexes in presence and absence of HPMC were characterized by UV, IR, NMR and XRD analysis which confirmed the formation of Gz-CD complexes.

### *2.3. In vitro dissolution of complexes*

The dissolution profiles of pure Gz and Gz-CD physical mixture and complexes are shown in Figs. 2 and 3. The rate and extent of drug dissolution from pure drug (PD) was significantly ($P < 0.01$) lower than from all the complexes. Evidently, the method of complex preparation, the molar ratio of complex and the presence of HPMC influenced the dissolution rate significantly ($p < 0.05$). The complexes prepared by the autoclaving method (Batch All, A12) exhibited the highest dissolution rate, followed

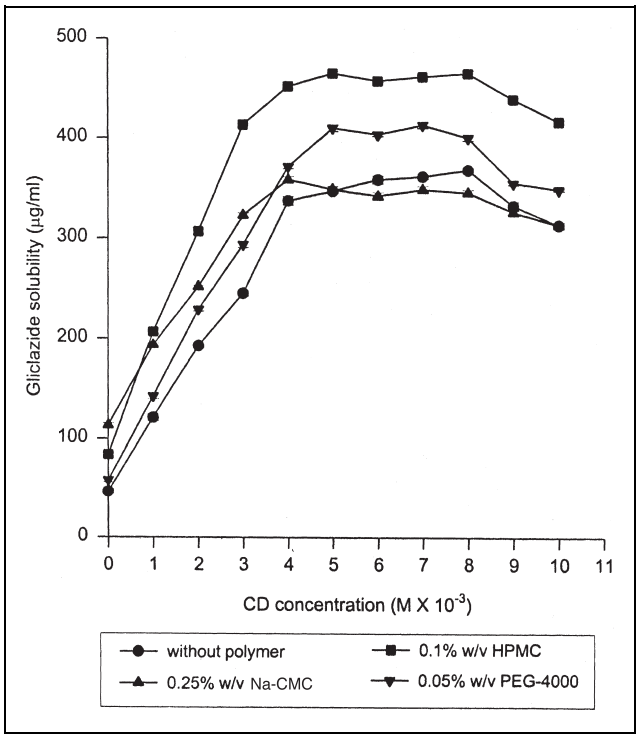


Fig. 1: Phase solubility diagram of gliclazide in CD solutions. Bars represent ±S.D. (n = 3)

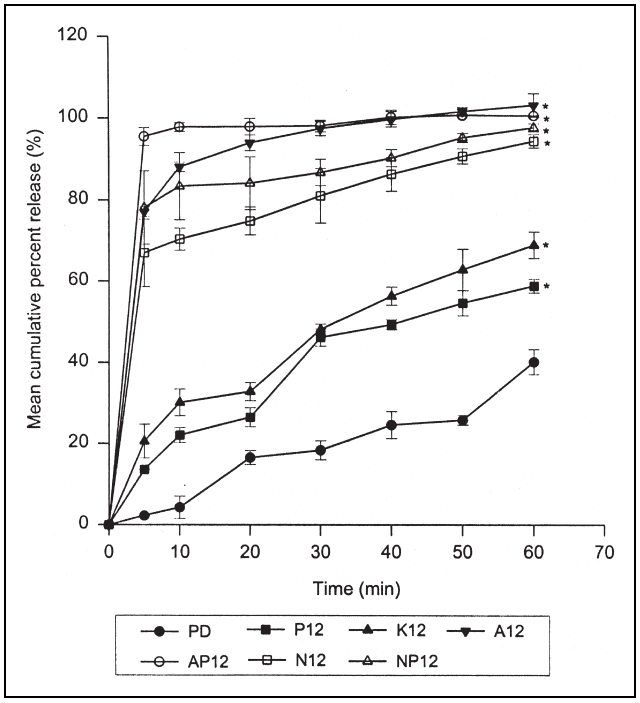


Fig. 2: Dissolution profiles of Gz from pure drug powder (PD), physical mixture (P12) and complexes prepared by: autoclaving – without (A12) and with HPMC (AP12), neutralization – without (N12) and with HPMC (NP12) and kneading (K12) methods. Molar ratio of drug and CD in physical mixture and complexes is 1 : 2. Bars represent ±S.D. (n = 3). * $p < 0.01$ (in reference to PD)

by complexes prepared by neutralization (Batch N11, N12) and kneading (Batch K11, K12) methods. However, the complexes containing HPMC (AP11, NP11, AP12, NP12) exhibited even a much higher rate and extent of drug dissolution than the corresponding complexes without HPMC (A11, N11, A12, N12). Physical mixtures of Gz with CD in 1 : 1 (P11) and 1 : 2 (P12) molar ratios though exhibited lower rate and extent of drug dissolution in comparison to all the complexes but showed higher rate and extent of drug dissolution than the pure drug. Although, the rate and extent of drug dissolution for the complexes, prepared by autoclaving, neutralization and kneading methods in 1 : 1 molar ratio (Fig. 3) of drug and CD, were lower than that with 1 : 2 molar ratio (Fig. 2) of drug and CD, but in the presence of HPMC, 1 : 1 Gz-CD complexes exhibited either similar or higher rate and extent of drug dissolution to that of the corresponding 1 : 2 complexes. The results thus clearly reveal the synergistic solubilization potential of HPMC when mixed with CD.

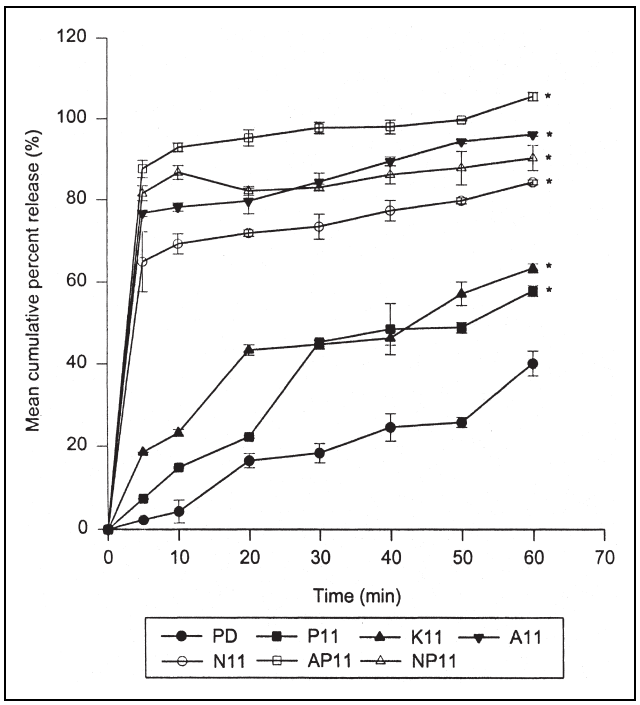


Fig. 3: Dissolution profiles of Gz from pure drug powder (PD), physical mixture (P11) and complexes prepared by: autoclaving – without (A11) and with HPMC (AP11), neutralization – without (N11) and with HPMC (NP11) and kneading (K11) methods. Molar ratio of drug and CD in physical mixture and complexes is 1 : 1. Bars represent ±S.D. (n = 3). * $p < 0.01$ (in reference to PD)

### *2.4. In vivo studies*

The results of hypoglycemic activity following p.o. administration of various formulations in rats are shown in the Table and Fig. 4. It was observed that the Gz-CD-HPMC complexes (AP11 and AP12) not only resulted in early onset of hypoglycemic activity but also produced more intense hypoglycemic effect than all the other formulations. The complexes without HPMC (A12) also produced early and more intense hypoglycemic effect in comparison to pure Gz (PD) and commercial tablet (MKT).

The bioavailability parameters shown in the Table indicate that the relative bioavailability ($F_{rel}$) of complexes and MKT was approximately 6 and 4.5 times higher than that of pure drug.

Thus, it was concluded that the solubility, dissolution characteristics and therapeutic efficacy of the poorly water-soluble drug, Gz can significantly be improved if

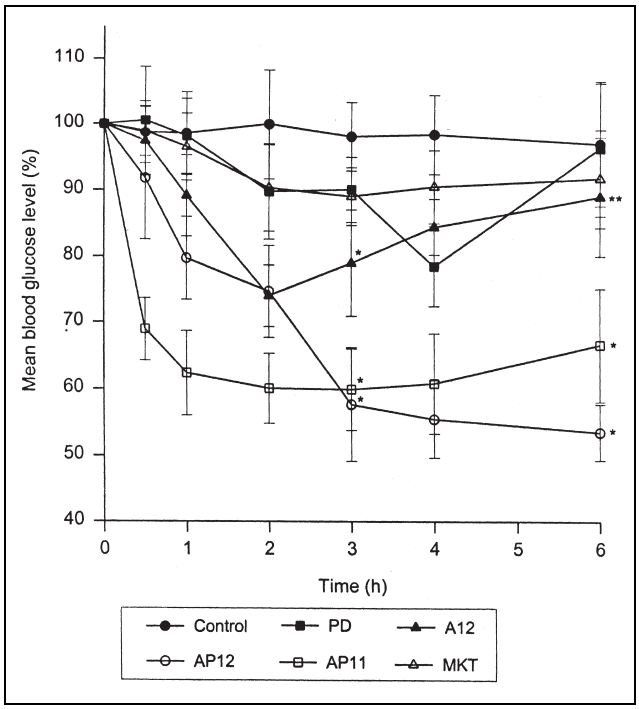


Fig. 4: Profiles of mean blood glucose level (%) after p.o. administration of Gz to rats as 1% w/v carboxymethylcellulose suspension of pure drug powder (PD), marketed tablet (MKT) and complexes prepared by: autoclaving – without (A12) and with HPMC (AP12) (drug : CD molar ratio 1 : 2) and with HPMC having drug : CD molar ratio 1 : 1 (AP11). Suspension without drug is control. Bars represent ±S.D. (n = 5). * $p < 0.01$, ** $< 0.05$ (both in reference to PD)

**Table: Mean (n = 5) bioavailability parameters obtained after single p.o. administration of gliclazide, its complexes and a commercial tablet (MKT) in rats**

| Batch | Bioavailability parameters | | | | |
|---|---|---|---|---|---|
| | $AUC_{(0-6\,h)}$ | PLE[a] (% ± SD) | TPE[b] (h ± SD) | $F^c_{rel}$ (%) | $F^d_{rel}$ |
| PD | 6.13 | 21.48 ± 0.74 | 4.33 ± 0.55 | 100.0 | 1.00 |
| A12 | 36.98 | 25.97 ± 0.28 | 3.0 ± 0.01 | 602.9 | 6.03 |
| AP11 | 32.24 | 48.78 ± 0.71 | 5.0 ± 0.07 | 525.7 | 5.26 |
| AP12 | 36.65 | 43.20 ± 0.29 | 3.5 ± 0.21 | 597.6 | 5.98 |
| MKT | 27.74 | 13.97 ± 0.32 | 4.73 ± 0.36 | 452.3 | 4.52 |

[a] Peak lowering
[b] Time of peak effect
[c] Relative bioavailability in reference to pure drug expressed in percentage
[d] Relative bioavailability in reference to pure drug expressed as ratio

Gz is complexed suitably with CD. Further, incorporation of a small amount of HPMC could have a synergistic effect on aforesaid characteristics of the Gz complexes.

## 3. Experimental

### *3.1. Materials*

Gz was gifted by Recon Ltd, Banglore (India). CD, HPMC and PEG-4000 were purchased from S.D. Fine Chem, Mumbai (India) and Na-CMC from CDH Ltd. Delhi (India). Marketed gliclazide tablet, Glizid®-40 (Panacea Biotech, Delhi, India) was used. All other materials used were of analytical reagent grade.

### *3.2. Phase solubility studies*

Phase solubility studies were preformed according to a reported method [8]. 20 mg of Gz were added to each 20 ml of 0.001 to 0.010 M CD solutions in distilled water (pH 7.0). The solutions in sealed flasks were shaken for 48 h at room temperature. After equilibration for 3 days, filtered aliquots were analysed on a UV spectrophotometer (Jasco 7800 model, Tokyo, Japan) at 225 nm. The apparent stoichiometry and stability constant ($K_c$) of the complexes were determined from the slope of the ascending portion of straight line of the phase solubility diagram.
The phase solubility studies were also performed similarly after incorporation of three water soluble polymers, viz. 0.10% w/v hydroxypropylmethylcellulose (HPMC), 0.25% w/v sodium carboxymethylcellulose (Na-CMC) and 0.05%w/v polyethyleneglycol (PEG-4000) separately to each of the above solutions.

### *3.3. Preparation and characterization of inclusion complexes and physical mixture*

The solid GZ-CD complexes and physical mixture were prepared using the following methods [9] with little modification.

#### *3.3.1. Autoclaving*

Slurries of Gz and CD in 1 : 1 (Batch A11) and 1 : 2 (Batch A12) molar ratios without HPMC and in the presence of 0.05% w/w HPMC (Batches AP11 and AP12, respectively) were prepared separately using minimum volume of water and subjected to autoclaving at 120 °C for 40 min. This causes complete solubilization of both CD and Gz. The hot solutions were then allowed to cool slowly at room temperature with continuous shaking until solid complexes are completely precipitated. The precipitates were then filtered off through vacuum filteration, washed repeatedly with distilled water, dried in hot air (110 °C), sieved through mesh no. 80 and placed in dessicator.

#### *3.3.2. Neutralization*

Gz and CD in 1 : 1 (Batch N11) and 1 : 2 (Batch N12) molar ratio without HPMC, and in same ratios in the presence of 0.05% w/w HPMC (Batches NP11 and NP12, respectively) were dissolved in 100 ml of 0.5 N NaOH solutions separately and stirred on a magnetic stirrer. 50 ml of 0.5 N HCl was then added to each, drop by drop and stirring was continued for 2 h. The precipitated complexes were filtered, dried and stored similarly as above.

#### *3.3.3. Kneading*

Gz and CD in 1 : 1 (Batch K11) and 1 : 2 (Batch K12) molar ratios were placed in a mortar separately, wetted with a few drops of water and kneaded for about 1 h. The pastes obtained were dried, sieved and stored similarly as above.

#### *3.3.4. Physical mixing*

Physical mixtures of Gz and CD in 1 : 1 (Batch P11) and 1 : 2 (Batch P12) molar ratios were also prepared by mixing manually.
The complexes were characterized in solution form through UV spectroscopy and in solid state through X-Ray Diffractometry (XRD), IR and NMR.

### *3.4. Drug content determination*

Accurately weighed 10 mg of each complex and the physical mixture was transfered to a 50 ml volumetric flask, dissolved completely in methanol and made up to the mark. The filtered aliquot was diluted suitably and analyzed spectrophotometrically at 226 nm. The determination was performed in triplicate. Percent efficiency (%) = (theoretical drug content/practical drug content) × 100 was computed.

### *3.5. In vitro dissolution studies*

Dissolution studies of all the batches of complexes, physical mixture and pure drug powder were done for 60 min in triplicate, using the USPXXI dissolution apparatus-II. Powder equivalent to 10 mg Gz was dispersed in 900 ml of distilled water used as dissolution medium (pH 7.0). The stirring rate was 100 rpm and temperature was maintained at 37 ± 0.1 °C. At predetermined time intervals, 5 ml aliquots were withdrawn through a pipette attached with a SG-2 filter and analyzed spectrophotometrically at 225 nm after suitable dilution. The withdrawn volumes were replaced in dissolution medium with fresh distilled water (37 ± 0.1 °C).

### *3.6. In vivo studies in rats*

In vivo evaluation of three Gz complexes (Batches A12, AP12, AP11) in comparison to pure Gz powder (PD) and commercial tablet, Glizid®-40 (MKT) was performed to investigate the hypoglycemic activity for 6 h following single dose peroral administration in healthy non-diabetic albino rats, weighing between 150 and 200 g. The rats were procured from National Fauna Stores (Varanasi, India), housed in standard animal cages in the departmental animal house for 3 days and fed with standard Hind Lever diet. The 5 rats per batch of either sex, fasted overnight with water ad libitum, were dosed with 0.1% w/v carboxymethylcellulose (CMC) aqueous suspension of 3 mg Gz equivalent/kg rat weight. CMC suspension without drug (control) was also dosed to rats. Blood glucose was monitored for 6 h after each administration with a ONE TOUCH™ BASIC™ Glucose Monitoring System (Johnson & Johnson, California, USA). At predetermined time intervals, the distal end (1 mm) of the tail was cut and a drop of blood was taken. Considering the zero time (basal) blood glucose level as 100%, percent blood glucose levels were calculated for each time of estimation.

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