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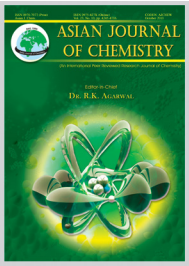

Studies on Enhancement of Solubility Dissolution Rate and Bioavailability of Glipizide by Complexation with β-Cyclodextrin

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The objective of the investigation is to study the complexation of glipizide, biopharmaceutical classification system class II drug with β-cyclodextrin (β-CD) and to evaluate the feasibility of enhancing its solubility, dissolution rate and bioavailability by β-cyclodextrin complexation. Complexation of glipizide with β-cyclodextrin was evaluated by phase solubility, TLC, DSC, XRD and IR spectral studies. Solid inclusion complexes of glipizide and β-cyclodextrin were prepared by kneading method and were evaluated by *in vitro* and *in vivo* methods. The aqueous solubility of glipizide was increased linearly as a function of concentration of the β-cyclodextrin. The phase solubility studies indicated the formation of glipizide-β-cyclodextrin inclusion complex at a 1:1 M ratio in solution. The complexes formed were quite stable. Solid inclusion complexes of glipizide-β-cyclodextrin exhibited higher rates of dissolution and dissolution efficiency values when compared to uncomplexed glipizide. Glipizide-β-cyclodextrin (1:3) complexes exhibited 5.58 fold increase in the dissolution rate and 4.76 fold increase in the dissolution efficiency of glipizide. TLC, DSC and IR spectral studies indicated no chemical interaction between the glipizide and β-cyclodextrin. XRD indicated stronger drug amorphization and entrapment of glipizide in β-cyclodextrin. All pharmacokinetic parameters estimated [C_{max}, T_{max}, K_a and $(AUC)_0^{\infty}$] indicated rapid and higher absorption and bioavailability of glipizide when administered as β-cyclodextrin complex. The absorption rate constant (K_a) was increased from 2.08 h^{-1} for glipizide. $(AUC)_0^{\infty}$ was increased from 86.2 μg h/mL for glipizide to 120.6 μg h/mL for β-cyclodextrin complex. Thus, complexation with β-cyclodextrin has markedly enhanced the absorption rate and bioavailability (both rate and extent of absorption) of glipizide, a biopharmaceutical classification system-class II drug.

Key Words: Glipizide, β-Cyclodextrin, Solubility, Dissolution rate, Bioavailability.

INTRODUCTION

Many of the modern drugs belong to the class II category under biopharmaceutical classification system[1] (BCS), which are characterized by low solubility and high permeability. These drugs are insoluble in water and aqueous fluids in the pH range of 1.0 - 7.5 and exhibit low and variable dissolution and bioavailability. There is a great need to develop technologies for these 'BCS' class II drugs for enhancing their dissolution rate and bioavailability.

Cyclodextrin (CD) complexation is an efficient technique to enhance the solubility and dissolution rate of BCS class II drugs. Cyclodextrins are cyclic (α-1-4) linked oligosaccharides of α-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. Cyclodextrins are reported to form inclusion complexes with lipophilic drugs. As consequence of the inclusion process many physico-chemical properties, such as solubility, dissolution rate, stability and bioavailability can be favourably affected[2,3] without altering their pharmacodynamic properties. Cyclodextrins have been receiving increasing application in pharmaceutical formulations in recent years due to their approval by various regulatory agencies[4,5].

Glipizide a second-generation sulfonylurea belongs to BCS class II. It is insoluble in water and its dissolution is considered to be the rate-determining step in its absorption from the gastrointestinal fluids[6]. The present work has been undertaken with an objective of studying the cyclodextrin complexation of glipizide, BCS class II drug with β-cyclodextrin to evaluate the feasibility of enhancing its solubility, dissolution rate and bioavailability by β-cyclodextrin complexation.

EXPERIMENTAL

Glipizide was a gift sample from Micro Labs, Bangaluru. β-Cyclodextrin was a gift sample from Gangwal Chemicals Pvt. Ltd., Navi Mumbai. Methanol (SD fine chemicals) was known for commercial sources. All other materials used were of pharmacopeial grade.

Phase solubility studies: The phase-solubility technique permits the evaluation of the affinity between β-cyclodextrin

and glipizide in water. Phase solubility studies were performed according to the method reported by Higuchi and Connors[7]. Glipizide, in amounts that exceeded its solubility, was taken in to 25 mL stoppered conical flasks to which were added 15 mL of distilled water (pH 6.8) containing various concentrations of β-cyclodextrin (1-15 mM). These stoppered conical flasks were shaken for 72 h at room temperature on a rotary shaker. Subsequently, the aliquots were withdrawn (2 mL), using a syringe at 1 h intervals and samples were filtered immediately by using 0.45 μ nylon disc filter. The filtered samples were diluted suitably. A portion of a sample was analyzed by UV spectrophotometer (SHIMATZU 1700) at 276 nm, against blanks prepared in the same concentration of β-cyclodextrin in water so as to cancel any absorbance that may be exhibited by the β-cyclodextrin molecules. Shaking was continued until 3 consecutive estimations were equivalent. The solubility experiment was conducted in triplicate.

The apparent stability constant (K_c) was calculated from the slope of the corresponding linear plot of the phase solubility diagram according to the equation, K_c = slope/S_o (1-slope), where So is the solubility of the drug in the absence of solubilizer.

Preparation of solid inclusion complexes: The solid inclusion complexes of glipizide and β-cyclodextrin were prepared in 2:1, 1:1, 1:2 and 1:3 mM ratios by Kneading method as follows. β-Cyclodextrin and a solvent blend containing of (1:1) ratio distilled water: methanol was mixed together in a mortar so as to obtain a homogeneous paste. Drug mM (Glipizide) was then added slowly by triturating. The mixture was then kneaded for 45 min. During this process, an appropriate quantity of water was added to the mixture in order to maintain a suitable consistency. The paste was then dried in oven at 70 ºC for 2 h until dry. The dried complex was powdered and passed through sieve No. 100.

Drug content uniformity: Glipizide content in the β-cyclodextrin inclusion complex was estimated by UV spectrophotometric (SHIMADZU 1700) method. An accurately weighed sample of glipizide and β-cyclodextrin complex prepared by kneading method was dissolved with 25 mL methanol in a 100 mL volumetric flask and the volume was adjusted up to 100 mL by using phosphate buffer of pH 7.4. The solution was filtered. The filtrate was assayed for drug content by measuring the absorbance at 276 nm after suitable dilution, against phosphate buffer of pH 7.4 as blank. Reproducibility of the above method was studied by analyzing six individually weighed samples of glipizide. The mean error (accuracy) and relative standard deviation (precision) were found to be 0.6 and 0.8 % respectively.

Dissolution rate study: The dissolution rate of glipizide from its β-cyclodextrin inclusion complexes was studied by using USP-thermo lab eight station dissolution test apparatus in phosphate buffer solution of pH 7.4. The test sample of drug-β-cyclodextrin complex (100 mg equivalent to pure drug in complex) was tithed in a muslin bag with a paddle stirrer, which rotates at a speed of 50 rpm, the temperature of water bath, was maintained at 37 ± 0.5 ºC throughout the experiment. 5 mL aliquot of dissolution medium was withdrawn at a specific time intervals and replaced with 5 mL fresh medium of phosphate buffer solution of pH 7.4 to maintain sink condition. The withdrawn sample was filtered, after suitable dilutions with phosphate buffer solution of pH 7.4 the absorbance was determined by using UV spectrophotometer (SHIMADZU 1700) at 276 nm against blank (phosphate buffer solution of pH 7.4). The absorbance was recorded. The release of pure drug was also studied to compare with release of drug from complexes. All studies were conducted in triplicate; the average data obtained were computed for further calculations.

Thin layer chromatography: The thin layer chromatography studies were carried out on aluminium preparative sheets using *n*-hexane:butyl acetate:ethyl acetate:water (60:15:15:20) as developing solvent (mobile phase) and dilute sulphuric acid as spraying solution. The R_f value was calculated for both the standard and sample, using the following formula.

R_f value = Distance travelled by solute/Distance travelled by mobile solvent.

Differential scanning calorimetry: Differential scanning calorimetry was used to characterize the glipizide-β-cyclodextrin solid complexes prepared by kneading method were recorded on NETZSCH DSC 204 METTLER STARe Model. Samples (2-5 mg) were sealed in to aluminum pans and scanned at a heating rate of 10 ºC min^{-1} over a temperature range 30-300 ºC under a nitrogen gas stream.

X-ray diffractometry: X-ray powder diffraction patterns were recorded using a Phillips model powder diffractometer with monochromatized CuK_α radiation. Glipizide, β-cyclodextrin and solid inclusion complexes prepared by kneading method were scanned at room temperature in the continuous scan mode over the 5º-50º 2θ range with 0.1 2θ step size and with counting time of 0.6 sec.

Infrared spectroscopy: IR spectra of glipizide, β-cyclodextrin and inclusion complexes of glipizide with β-cyclodextrin prepared by kneading method were recorded on FTIR spectrophotometer (SHIMADZU spectrophotometer) using KBr disc.

Pharmacokinetic evaluation: Pharmacokinetic evaluation was done on β-cyclodextrin complex of glipizide in comparison to glipizide pure drug. Cyclodextrin complex prepared at a drug: cyclodextrin ratio of 1:3 were used in the pharmacokinetic evaluation.

***In vivo* study:** Healthy rabbits of either sex (weighing 1.5-2.5 kg) were fasted overnight. Glipizide and its β-cyclodextrin complex were administered at a dose equivalent to 4 mg of drug/kg. Each product was repeated 4 times (n = 4). The *in vivo* experiments were conducted as per crossover RBD in healthy rabbits of either sex (n = 4) with a washout period of one month.

After collecting the 0 h blood sample (blank), the product in the study was administered orally in a capsule shell with 10 mL of water. Blood samples (3 mL) were collected from marginal ear vein at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 h after administration. The blood samples were collected in heparinized tubes and were centrifuged at 10000 rpm for 10 min. The plasma separated was collected into dry tubes. All the samples were stored under refrigerated conditions prior to assay. Plasma concentrations of the glipizide were determined by HPLC method as follows.

The HPLC system (make; M/s Shimadzu Corporation, Japan) used consisted of UV-visible detector (Shimadzu,

Model; SPD-10 AVP), C_{18} column (Phenomenex, DESC: Gemini 5µ C 18 110 A, Size: 250 X 4.6 mm, S/No; 288063-23) 2 pumps (Model: LC-10 ATVP) and a microsyringe of capacity 25 µL (Model:Microliter R#702, Mfd.,by: M/s Hamilton). The mobile phase is a mixture of water containing 0.1 % w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitrile (34: 66). The mobile phase was filtered through 0.45 µ membrane filter before use and was run at a flow rate of 1 mL/min. The column effluent was monitored at 230 nm.

To 0.5 mL of plasma 1 mL of acetonitrile was added, mixed thoroughly and centrifuged at 5000 rpm for 20 min. The organic layer (0.5 mL) was taken into a dry tube and the acetonitrile was evaporated. To the dried residue 0.5 mL of mobile phase [a mixture of water containing 0.1 % w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitrile (34: 66)] was added and mixed for reconstitution. Subsequently 20 µL were injected into the column for HPLC analysis.

From the time *versus* plasma concentration data various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half-life ($t_{1/2}$), percent absorbed to various times and absorption rate constant (K_a) were calculated in each case as per standard methods[8,9].

RESULTS AND DISCUSSION

The complexation of glipizide with β-cyclodextrin was investigated by phase solubility studies. The phase solubility diagram for the complex formation between glipizide and β-cyclodextrin is shown in Fig. 1. The aqueous solubility of the glipizide was increased linearly as a function of the concentration of β-cyclodextrin. The phase solubility diagram of glipizide β-cyclodextrin complexes can be classified as A_L type according to Higuchi and Connors[7]. Because the straight line had a slope < 1 in each case, the increase in solubility was due to the formation of a 1:1 M complex in solution with β-cyclodextrin. The estimated K_c value of glipizide-β-cyclodextrin complexes is 677.9 M^{-1}. The value of K_c indicated that the complexes formed between glipizide and β-cyclodextrin is quite stable.

Solid inclusion complexes of glipizide-β-cyclodextrin in 2:1, 1:1, 1:2 and 1:3 ratios were prepared by kneading method. All the complexes prepared were found to be fine and free flowing powders. There was no significant loss of drug during the preparation of solid inclusion complexes. Low c.v. values in the per cent drug content ensured uniformity of drug content in all batch. The coefficient of variation (c.v.) in the per cent drug content was found to be less then 1.0 % in all the batches prepared. The dissolution rate of glipizide from β-cyclodextrin complex system was studied using phosphate buffer solution of pH 7.4 as a dissolution fluid. The dissolution of glipizide was higher from all the glipizide-β-cyclodextrin complexes prepared when compared to glipizide pure drug. The dissolution data were fitted into various mathematical models such as zero order, first order and Hixson-Crowell's cube root models to assess the kinetics and mechanism of dissolution. The dissolution data obeyed first order kinetic model as well as Hixson-Crowell's cube root model. Dissolution efficiency (DE_{30}) values were calculated as per Khan[10]. T_{50} (time taken for 50 % dissolution) and PD_{10} (%) values were recorded from the dissolution profiles. The dissolution parameters are summarized in Table-1.

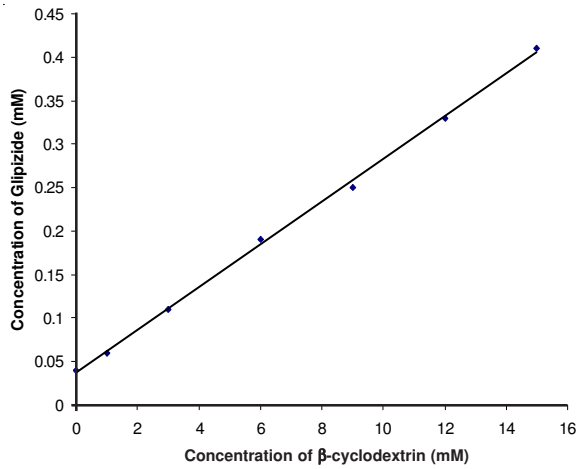

Fig. 1. Phase solubility study-effect of β-cyclodextrin concentration on the solubility of Glipizide

TABLE-1
DISSOLUTION PARAMETERS OF GLIPIZIDE β-CYCLODEXTRIN COMPLEXES

Formulation	Dissolution parameters				
	PD_{10} (%)	T_{50} (min)	DE_{30} (%)	K_1 (min^{-1})	Increase K_1 (folds)
Glipizide	11.86	> 60	12.60	0.004	-
GB 1	40.38	24.06	39.13	0.013	3.34
GB 2	51.68	08.56	49.36	0.018	4.55
GB 3	59.98	06.24	55.72	0.020	5.12
GB 4	64.80	05.16	60.09	0.022	5.58

GB 1-Glipizide: β-cyclodextrin (2:1), GB 2-Glipizide: β-cyclodextrin (1:1), GB 3-Glipizide: β-cyclodextrin (1:2), GB 4-Glipizide: β-cyclodextrin (1: 3)

All β-cyclodextrin complexes exhibited higher rates of dissolution and dissolution efficiency values than glipizide, indicating higher dissolution of glipizide form its β- cyclodextrin complexes. The K_1 and DE_{30} values were increased as the proportion of β-cyclodextrin in the complex system was increased.

Differential scanning calorimetry was used to characterize the glipizide-β-cyclodextrin solid complexes. The differential scanning calorimetry thermograms of glipizide β-cyclodextrin are shown in Fig. 2. The differential scanning calorimetry curve of glipizide showed a single sharp endothermic peak at 214.91 ºC corresponding to its melting point. β-Cyclodextrin showed broad endothermic peaks. In the thermograms of glipizide-β-cyclodextrin, the intensity (or height) of the endothermic peak at 214.91 ºC was reduced indicating interaction of glipizide with β-cyclodextrin and absence of crystalline drug and its complete complexation with β-cyclodextrin.

XRD patterns of glipizide and complexes with β-cyclodextrin are shown in Fig. 3. XRD of glipizide exhibited characteristic diffraction peaks indicating its crystalline nature. The diffraction peaks were much reduced in the case of glipizide-β-cyclodextrin complexes. The much reduced diffraction peaks of glipizide confirmed the stronger drug amorphization and entrapment in β-cyclodextrin.

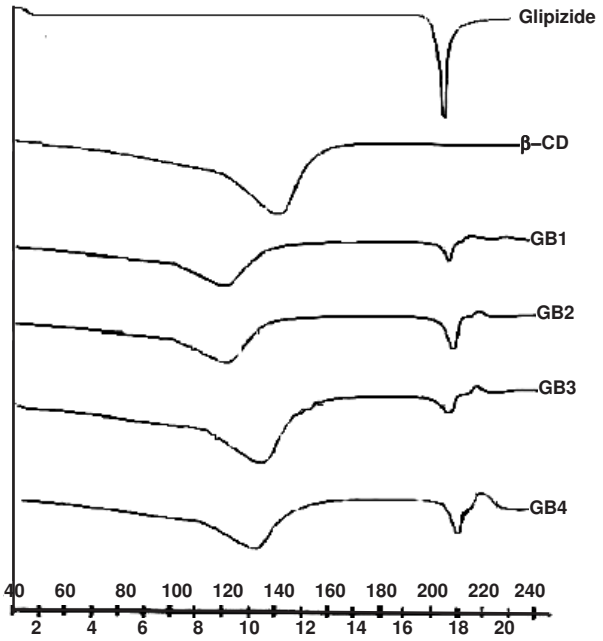

Fig. 2. Differential scanning profiles of Glipizide and its β-cyclodextrin complexes; GB 1- Glipizide: β-cyclodextrin (2:1), GB 2- Glipizide: β-cyclodextrin (1:1), GB 3- Glipizide: β-cyclodextrin (1:2), GB 4- Glipizide: β-cyclodextrin (1:3)

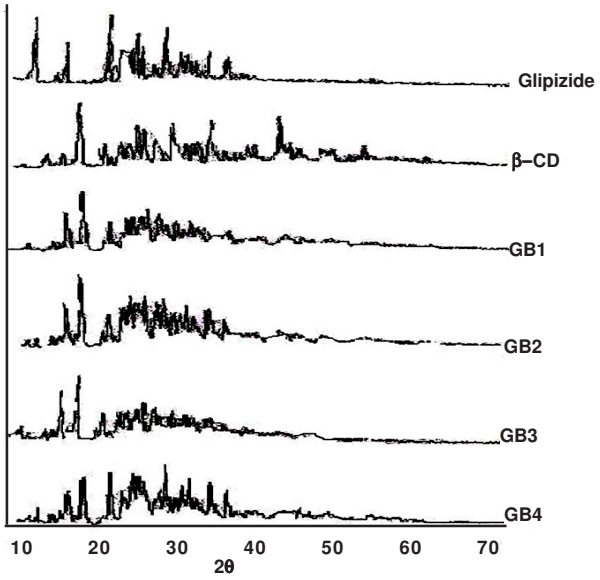

Fig. 3. XRD profiles of Glipizide and its β-cyclodextrin complexes; GB 1-Glipizide: β-cyclodextrin (2:1), GB 2- Glipizide: β-cyclodextrin (1:1), GB 3- Glipizide: β-cyclodextrin (1:2), GB 4- Glipizide: β-cyclodextrin (1:3)

The IR spectra of glipizide and glipizide-β-cyclodextrin are shown in Fig. 4. The IR spectra of glipizide and glipizide-β-cyclodextrin are identical indicating no interaction between glipizide and β-cyclodextrin.

In TLC study the R_f values were found to be 0.795 and 0.804 for pure glipizide and glipizide-β-cyclodextrin complex

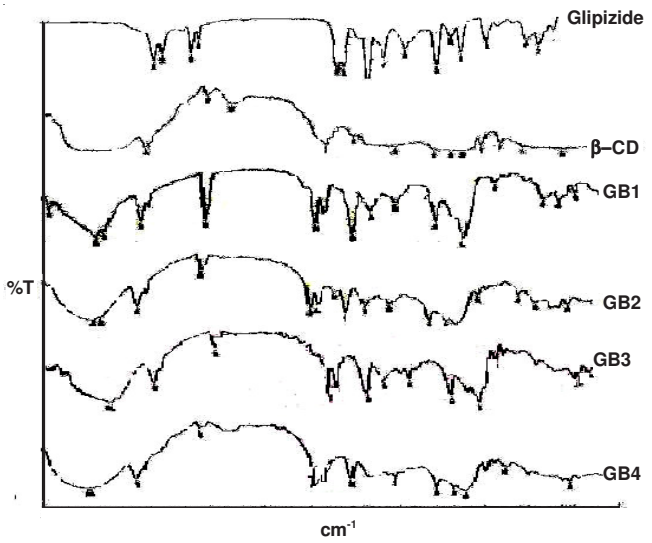

Fig. 4. IR Profiles of Glipizide and its ß-CD complexes; GB 1-Glipizide: β-cyclodextrin (2:1), GB 2- Glipizide: β-cyclodextrin (1:1), GB 3- Glipizide: β-cyclodextrin (1:2), GB 4- Glipizide: β-cyclodextrin (1:3)

respectively. As the R_f values of glipizide and glipizide-β-cyclodextrin complex coinciding and as no other spots were observed it can be concluded that glipizide and β-cyclodextrin are compatible without any chemical change or reaction.

The pharmacokinetic parameters estimated following the oral administration of glipizide and glipizide-β-cyclodextrin complex are summarized in Table-2.

Glipizide was found to be absorbed slowly with a K_a of 2.08 h^{-1}. A peak plasma concentration (Fig. 5) (C_{max}) of 16.25 μg/mL was observed at 3.0 h after administration. All pharmacokinetic parameters (Table-2) estimated [C_{max}, T_{max}, K_a and (AUC_0^{∞})] indicated rapid and higher absorption and bioavailability of glipizide when administered as β-cyclodextrin complex. Higher C_{max} and lower T_{max} values were observed with the β-cyclodextrin complex when compared to those of glipizide pure drug. The absorption rate constant (K_a) was found to be 4.60 h^{-1} with β-cyclodextrin complex, where as in the case of glipizide K_a was only 2.08 h^{-1}. AUC08 was also much higher in the case of β-cyclodextrin complex when compared to glipizide pure drug. (AUC_0^{∞}) was increased from 86.2 μg h/mL for glipizide to 120.6 μg h/mL for β-cyclodextrin complex.

The $t_{½}$ estimated following the administration of glipizide and its β-cyclodextrin complex was 4.25 and 3.65 h respectively. The close agreement of these $t_{½}$ values indicated that the elimination characteristics of glipizide have not altered when it was administered as β-cyclodextrin complex.

Conclusion

The aqueous solubility of glipizide was increased linearly as a function of concentration of the β-cyclodextrin. The phase solubility studies indicated the formation of glipizide-β-cyclodextrin

TABLE-2
PHARMACOKINETIC PARAMETERS OF GLIPIZIDE AND IT β-CYCLODEXTRIN (CD) COMPLEX

Product	$t_{½}$ (h)	C_{max} (μg/mL)	T_{max} (h)	$(AUC)_0^{\infty}$ (μg h/mL)	$(AUC)_0^{\infty}$ (μg h/mL)	BA (%)	K_a (h^{-1})	Absorbed (%)	
								0.5 h	1.0 h
Glipizide	4.25	16.25 ± 1.1	3.0	66.8	86.2	100	2.08	28.5	76.4
Gz-β-CD (1:3)	3.65	30.10 ± 0.7	1.0	110.2	120.6	139.9	4.60	45.2	98.2

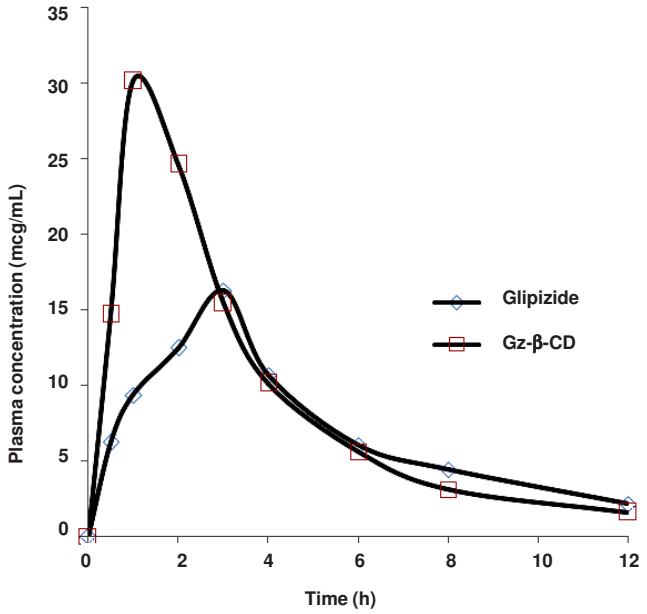

Fig. 5. Plasma concentrations of Glipizide following oral administration of Glipizide and its β-cyclodextrin complex

inclusion complex at a 1:1 M ratio in solution. The complexes formed were quite stable. Solid inclusion complexes of glipizide-β-cyclodextrin exhibited higher rates of dissolution and dissolution efficiency values when compared to uncomplexed glipizide. Glipizide-β-cyclodextrin (1:3) complexes exhibited 5.58 fold increase in the dissolution rate and 4.76 fold increase in the dissolution efficiency of glipizide. TLC, DSC and IR spectral studies indicated no chemical interaction between the glipizide and β-cyclodextrin. XRD indicated stronger drug amorphization and entrapment of glipizide in β-cyclodextrin. All pharmacokinetic parameters estimated [C_{max}, T_{max}, K_a and (AUC_0^∞)] indicated rapid and higher absorption and bioavailability of glipizide when administered as β-cyclodextrin complex. The absorption rate constant (K_a) was increased from 2.08 h^{-1} for glipizide to 4.60 h^{-1} with glipizide-β-cyclodextrin complex. (AUC_0^∞) was increased from 86.2 μg h/mL for glipizide to 120.6 μg h/mL for β-cyclodextrin complex. Thus, complexation with β-cyclodextrin has markedly enhanced the absorption rate and bioavailability (both rate and extent of absorption) of glipizide, a BCS-class II drug.

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