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Short Communication

Effect of pH and Hydroxypropyl- β -Cyclodextrin on Solubility and Stability of Gliclazide

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Abstract

Purpose: Hydroxy propylated β cyclodextrin (HP- β -CD) has been used to improve the aqueous solubility of a variety of pharmacological compounds. Through the complexation of HP- β -CD, it is possible to enhance the water solubility and hence the drug bioavailability after oral administration. The present study was undertaken to examine the effect of pH and concentration of HP- β -CD on the solubility of gliclazide as it shows pH dependent solubility and stability.

Method: The equilibrium solubility of gliclazide in a series of solutions of varying pH (from 1.2 to 11) was determined and compared with the equilibrium solubility of gliclazide in the presence of 20% HP- β -CD at same pH values.

Result: As the pH increases from 9.2 to 11 its solubility relatively constant ($3.5 \mu\text{g/ml} \pm 0.5$). Addition of 20% HP- β -CD increased the solubility to about 9 times at pH 1.2 and 4; 15 times at pH 6.8; 40 times at pH 7.4; 58 times at pH 9.2 and 67 times at pH 11. It was observed that solubility of protonated form is more than neutral molecule. HP- β -CD resulted in increased solubility at all pH values. But inclusion of drug in the cavity of HP- β -CD might depend upon charged state of the molecule.

Conclusion: The solubility of gliclazide can be increased either by the addition of HP- β -CD or by adding pH lowering agents.

Keywords: pH, hydroxypropylated- β -cyclodextrin (HP- β -CD), gliclazide.

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Introduction

Gliclazide (Glz) is a second-generation sulphonylurea oral hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus. It stimulates insulin secretion by pancreatic beta cells. In the long-term, it reduces hepatic gluconeogenesis, and increases insulin effects by acting at receptor or post-receptor sites. It also inhibits platelet aggregation and increases fibrinolysis¹. Because of these therapeutic effects it has emerged as one of the important and promising drug substances for diabetes mellitus, especially due to the noticeable improvement of survival rates in patients with chronic increase in glucose level, but the problem with this potentially useful hypoglycemic agent is that it is practically insoluble in water. This limits its bioavailability and may be the reason for its delayed absorption² and difficulty in its formulation into desired dosage forms.

Cyclodextrins (CDs) are cyclic oligosaccharides, containing a minimum of six D-(+)-glycopyranose units attached by α -1, 4-linkages produced by the action of the cyclodextrin-trans-glycosidase enzyme on a medium containing starch. They have hydrophobic central cavity and a hydrophilic outer surface³. CDs have been found to be very useful in enhancing the solubility of poorly water-soluble drugs owing to the formation of inclusion complex of the drug in its hydrophobic cavity⁴⁻⁸. The most common natural CDs are α -cyclodextrin, β -cyclodextrin and γ -cyclodextrin, which are formed by six, seven, and eight glucose units, respectively. Apart from these naturally occurring CDs, various derivatives are also available^{7,8} which may produce better solubility when complexed^{11,12} but cost and toxicity factors poses limitation in their use. Amongst the various available CDs, β -cyclodextrins (β -CD) are the cheapest and are nontoxic when administered orally. Hydroxypropyl- β -cyclodextrin (HP- β -CD) is hydroxypropylated derivative of β -cyclodextrins and has been used in improving the aqueous solubility of a variety

of pharmacological compounds. It is a cyclic oligosaccharide containing seven D-(+)-glucopyranose units, with an average of one hydroxypropyl group per unit to give a molecular weight of approximately 1540 g/mol. The circular arrangement of the glucose units produces a torus-shaped molecule. The CH₂ groups and ether linkages of the molecule face the hollow interior of the configuration results in a nonpolar cavity and a polar exterior. The interior cavity of the β -cyclodextrin is appropriate in size to accommodate a benzene ring. Because both the interior cavity and benzene are nonpolar HP- β -CD can be used to increase the solubility of compounds containing an isolated benzene ring. When a compound with appropriate geometry and HP- β -CD are in the same solution, the nonpolar aromatic portions of the compound tend to enter the nonpolar interior of the HP- β -CD molecule. This complexation isolates the aromatic portion of the molecule from the water, thereby increasing its aqueous solubility.

We have undertaken this study because gliclazide molecule contains two well-separated benzene and cyclopentenol pyrrol groups that may individually complex with HP- β -CD. The compound is a weak base and the charge state of the drug molecule may influence complex formation, and vice versa. Since gliclazide exhibits pH dependent solubility and stability, the effect of pH and concentration of HP- β -CD on the solubility of gliclazide was examined in this study.

Materials and Methods

HP- β -CD and gliclazide were gifts from Zim Laboratories Ltd, India. All other chemicals were of analytical grade and used without further purifications. Measurements of pH were performed using a calibrated Elico pH meter (Model-140). Gliclazide concentrations were determined at 226 nm using Shimadzu UVPC-2401 spectrophotometer.

As a starting point for this study, the solubility of gliclazide as a function of pH was studied. A series of buffer solutions from pH range 1.2 to 11 were prepared and gliclazide was added in sufficient quantity to saturate each solution. To avoid change in concentration due to evaporation, the solutions were kept in vials sealed with teflon lined screw caps and wrapped with paraffin. All solutions were then placed on a test tube rotator for mixing. They were checked daily for the saturation and pH was adjusted as necessary. To ensure the attainment of equilibrium, all solutions were shaken for one week. The solutions were then diluted as appropriate and absorbance determined spectrophotometrically at 226 nm. Similar studies were repeated after the addition of 20% HP- β -CD to the series of buffer solutions.

To explore the effect of cyclodextrin concentrations on the solubility of gliclazide, phase solubility studies were performed. A series of solutions containing varying concentrations of HP- β -CD (1% to 40%) in pH 7.4 buffer were prepared. Gliclazide was added to each solution in sufficient quantity to ensure saturation, and effect of HP- β -CD concentration on the solubility of gliclazide was determined as previously described¹².

Results and Discussion

Gliclazide contains an α -hydroxyl secondary amine, with a pKa of 7.8. It exhibits pH dependent solubility. The pH dependence of the complexation of gliclazide with HP- β -CD was investigated on the basis of solubility/pH profiles. Figure 1 shows the solubility profile of gliclazide in the presence and absence of HP- β -CD as a function of pH. In the presence and absence of HP- β -CD, gliclazide exhibited pH dependent solubility. Its solubility increases with decreasing pH and then starts decreasing after pH 4. At lower pH values protonated form of the gliclazide and its salt generated in-situ will determine its solubility. The hydrochloride salt generated in-situ in an acidic medium might be less soluble in this medium than the protonated gliclazide itself.

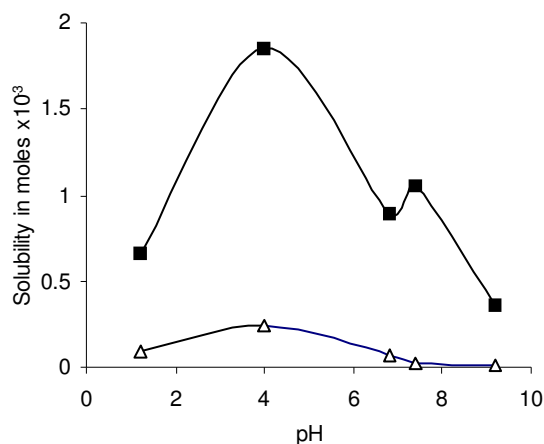


Figure 1: Solubility profile of gliclazide as a function of pH without HP- β -CD (Δ) and in the presence of 20% HP- β -CD (\blacksquare)

At basic pH (as the pH increases from 9.2 to 11) its solubility of the compound was found to be relatively constant (3.5 ± 0.5 $\mu\text{g/ml}$) in the absence of HP- β -CD. The addition of HP- β -CD results in a solubility profile as a function of pH similar in shape to that obtained in the absence of complexing agent. However it shows a significant rise in the solubility of gliclazide at all pH values tested. Addition of 20% HP- β -CD increased the solubility about 9 times at pH 1.2 and 4; 15 times at pH 6.8; 40 times at pH 7.4; 58 times at pH 9.2 and 67 times at pH 11. The percentage rise in the solubility of gliclazide in the presence of HP- β -CD is presented in figure 2. This pattern indicates that the degree of ionization has a decisive influence on the complexibility, and hence on the solubility of gliclazide at different pH. Both protonated and neutral molecules are not included in the HP- β -CD cavity with same ease. At acidic pH, the molecule exists in protonated form and this justifies the limited rise in solubility at acidic pH by HP- β -CD. But at basic pH, the major fraction of the molecules exists in unionized form which is hydrophobic. The interior environment of a cyclodextrin cavity is hydrophobic; hence it can entrap unionized form of the molecule which too is hydrophobic¹³. This can be well

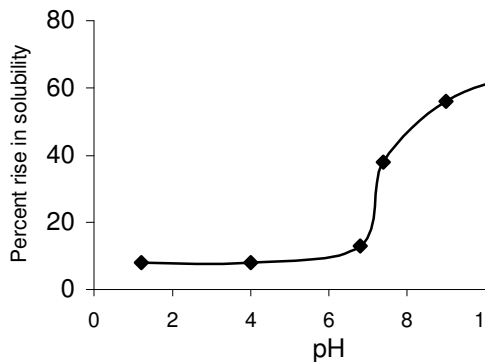


Figure 2: Effect of HP-β-CD on the percent rise solubility of gliclazide

explained by Henderson Hesselbach equation¹⁴

For weak acid,

$$pH = pK_a + \log \left(\frac{\text{ionised} \cdot \text{drug} \cdot \text{concentration}}{\text{unionised} \cdot \text{drug} \cdot \text{concentration}} \right)$$

For weak bases,

$$pH = pK_a + \log \left(\frac{\text{unionised} \cdot \text{drug} \cdot \text{concentration}}{\text{ionised} \cdot \text{drug} \cdot \text{concentration}} \right)$$

Based on the above relationship, gliclazide does not form a complex with β-CD since it is ionization at acidic pH but at the basic pH the compound is in its unionized form and get enclosed in the hydrophobic cavity of β-CD, forming the complex and enhance the solubility is enhanced at basic pH.

The effect of increasing concentration of gliclazide was performed at pH 7.4. Figure 3 shows a linear increase in the solubility of gliclazide with the increasing concentration of HP-β-CD. The apparent stability constant was calculated with the assumption of 1:1 stoichiometry and was found to be 4.25×10^4 at pH 7.4. Adequately stability of the complex was indicated by the stability constant implying that HP-β-CD can be used to improve the aqueous solubility of gliclazide.

Conclusion

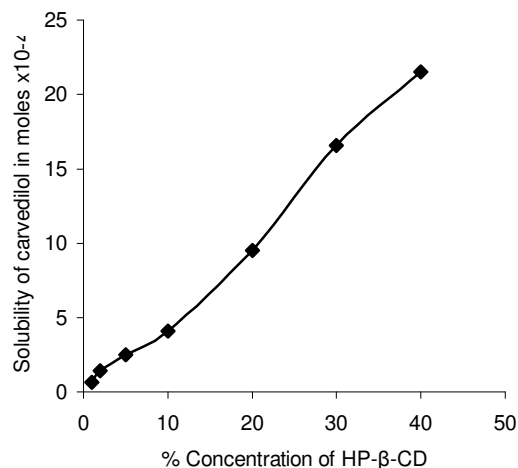


Figure 3: At pH 7.4 solubility of gliclazide versus concentration of HP-β-CD

HP-β-CD can be used to improve the solubility. But since improvement is not constant at all the pH values, due attention should be paid to this fact, while preparing formulation, and carrying out its bioavailability studies. A suitable acidifying agent must be incorporated to get a steady enhancement in solubility.

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