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Design, Preparation and Characterization of Cyclodextrin Inclusion Complexes of Glimipiride

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ABSTRACT

Over the years, inclusion complexation of drugs with β-cyclodextrin has emerged as a viable attempt to improve the dissolution of water insoluble drugs. The aim of the present work was to improve the dissolution rate of Glimepiride, by inclusion complexation with β-cyclodextrin. The stoichiometric ratio determined by phase solubility analysis for inclusion complexation of glimepiride with β-cyclodextrin was 1:1, 1:2, & 1:5. The solubility of glimepiride increased with increasing amount of β-cyclodextrin in water. Complexes of glimepiride were prepared with β-cyclodextrin by kneading method and physical mixture. The complexes were characterized by Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), and X-ray diffraction (XRD) patterns. These studies indicated the inclusion of glimepiride in the cavity of β-cyclodextrin. The complexation resulted in a marked improvement in the solubility of glimepiride. An optimum increase in the dissolution rate of the drug was observed at a drug-polymer concentration of 1:5 concentrations. Mean dissolution time of glimepiride decreased significantly after preparation of complexes of glimepiride with β-cyclodextrin.

Keywords: Glimepiride, β-cyclodextrin, inclusion complexation, in vitro dissolution studies.

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INTRODUCTION

The rate of absorption and bioavailability of poorly water soluble drugs is often controlled by the rate of dissolution of the drug in the gastrointestinal tract. Many technological methods of enhancing the dissolution characteristics of slightly water-soluble drugs are solid dispersions, micronization, solvent deposition, prodrugs, use of surfactants and inclusion complexation etc. Among the various methods, cyclodextrin complexation is an industrially accepted technique.[1]

The efforts to improve dissolution and solubility of poorly and practically water insoluble drugs remains one of the most challenging tasks in drug development. Several methods have been introduced to increase dissolution rate and thereby oral absorption and bioavailability of such drugs. Among various approaches, cyclodextrin complexation has shown promising results in improving solubility, wettability, dissolution rate of drug and subsequently its bioavailability of products that are orally administered. The hydrophilic cyclodextrins have been extensively used to enhance the oral bioavailability of several drugs and the improvement is mainly ascribable to the increase in drug solubility through the formation of inclusion complexes.

Glimepiride (Figure 1) is one of the third generation sulphonylurea, antidiabetic drug, which stimulates insulin release. It is used for treatment of non-insulin-dependent diabetes mellitus. Glimepiride is classified under class II according to biopharmaceutical classification system. The drug shows low, pH dependent solubility. In acidic and neutral aqueous media, Glimepiride exhibits very poor solubility at 37ºC (<0.004 mg/ml). In media pH>7, solubility of drug is slightly increased to 0.02 mg/ml.[2] From an economic point of view, low oral bioavailability results in wasting of a large portion of an oral dose and adds to the cost of drug therapy, especially, when the drug is an expensive one.

The approach of complexation has been frequently used to increase the aqueous solubility and dissolution rate of water insoluble and slightly soluble drugs in an effort to increase oral bioavailability. However, in certain instances, this approach can be used to increase drug stability, particularly esters, control drug release rate, improve organoleptic properties and maximize the gastrointestinal tolerance by reducing drug irritation after oral administration. Generally speaking, cyclodextrins are potential carriers for achieving such objectives (Higuchi and Kristiansen, 1970).

Cyclodextrins (CDs) (Figure 2) form a group of structurally related oligosaccharides with cylinder- shaped cavities that have the capacity to form inclusion complexes with many drugs by taking a whole drug molecule, or a part of it, into the cavity. Because of the large number of

hydroxyl groups on CDs, they are water-soluble. They are known for their ability to molecularly encapsulate a wide variety of drugs into their hydrophobic cavity without the formation of any covalent bonds.[3] Complexation with cyclodextrins has been reported to enhance the solubility, dissolution rate and bioavailability of poorly water soluble drugs. Many other drugs have been tested for CDs inclusion to enhance solubility.

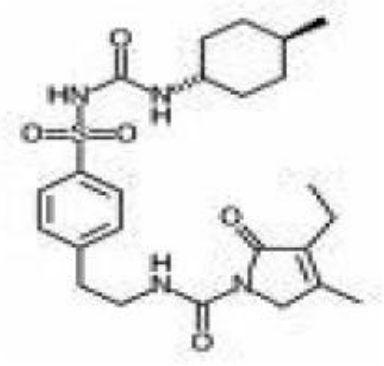

Figure 1: Structure of Glimepiride(GLI)

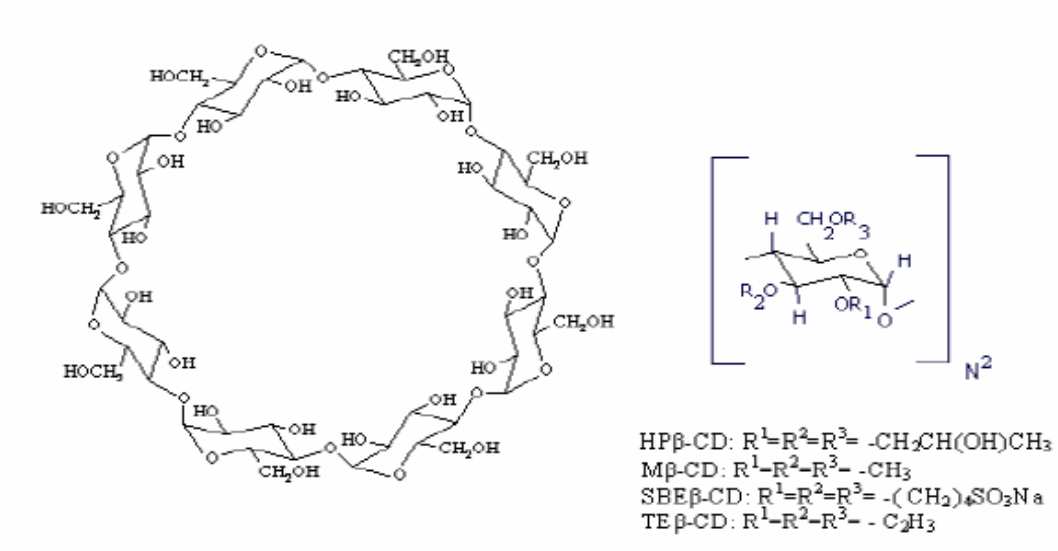

Figure 2: Structure of β- cyclodextrin

In vitro dissolution testing provides an easy and convenient means to evaluate the performance of pharmaceutical preparations. In this study an attempt was made to in vitro dissolution profiles of Glimepiride (GLI) from complexes of 1:1, 1:2, &1:5. Mean dissolution time (MDT) reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process that provides an accurate drug release rate.[4] Also, during storage, the excipients may interact with the drug and affect its dissolution characteristics. There are several reports showing marked changes due to aging, which adversely affect dissolution and, hence, the bioavailability of oral sulphonylurea drugs.

To overcome these difficulties, several approaches have been used, namely, the formation of a complex between glimepiride and β-CD, preparation of solid dispersions using water soluble carriers to improve the dissolution rate of glimepiride and subsequently its bioavailability. The aim of present work was to examine the potential for complex formation between glimepiride and β-CD and its implication on the physicochemical properties of the drug. Improvement of aqueous solubility in such a case is a valuable goal to improve therapeutic efficacy. The study was planned to improve the aqueous solubility and dissolution rate of GLI by preparing complexes with β-cyclodextrin (β-CD) employing methods such as kneading. The study further aimed to characterize the interaction between GLI and β-CD.

MATERIALS AND METHODS:

Materials:

Glimepiride was a gift sample obtained from Dr. Reddy's laboratory; Hydrabad, India, β-cyclodextrin was procured from S.D Fine chemicals, Mumbai. All other materials used are of pharmacopoeial grade.

Preparation of Calibration curve of Glimepiride:

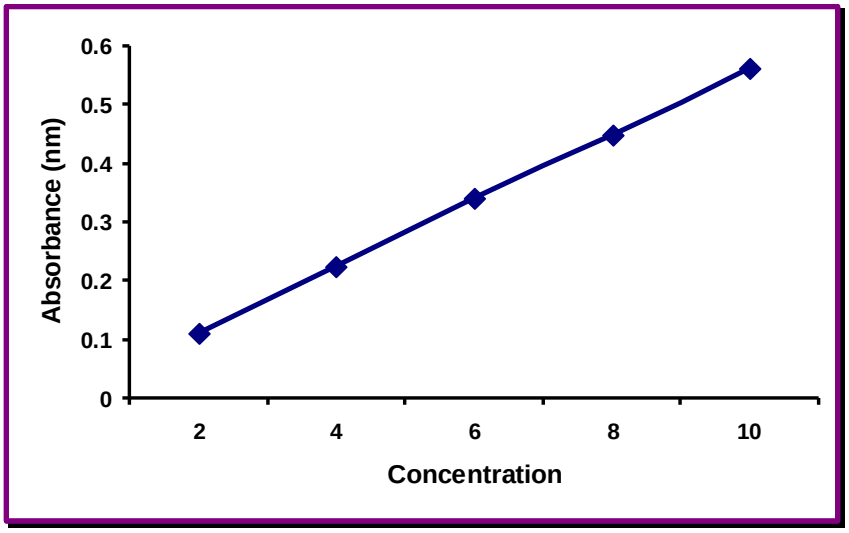

Figure 3: Calibration curve of Glimepiride (GLI) in Phosphate buffer pH 7.

Table 1 : Data for calibration curve of Glimepiride(GLI)

S. No.	Concentration (µg/ml)	Absorbance at 230 nm
1.	2	0.110
2.	4	0.224
3.	6	0.340
4.	8	0.449
5.	10	0.562

The calibration curve for the estimation of the drug (GLI) was constructed employing distilled water. The stock solution of the drug GLI was subsequently diluted with phosphate buffer of pH 7.4 to obtain a series of dilutions containing 2, 4, 6, 8 &10 µg/ml of the drug (GLI). The absorbances of the above dilutions were measured in UV spectrophotometer at 230 nm. The data is given in table 1 and shown in figure 3.

Phase solubility studies of pure drug:

Phase solubility studies of pure drug (GLI) with different concentrations of β- cyclodextrins (3-15 millimoles) were performed by the method described by Higuchi and Connors. An excess of Glimepiride (200 mg) was added to 15ml of triple distilled water containing various concentrations of cyclodextrins such as 0, 1, 3, 6, 9, 12, and 15 x 10^{-3} moles/liter taken in a series of 25ml stopped conical flask and the mixture was shaken for 72 hours at room temperature on a rotary flask shaker. After 72 hrs of shaking to achieve equilibrium, 2ml aliquots are withdrawn at 1 hr interval and filtered through whatman no.1 filter paper. The filtered samples are diluted suitably and assayed for the drug GLI content by specific UV method. Shaking is continued until the consecutive estimations are the same.[5] The solubility experiments are conducted in triplicate.

Physical mixture:

GLI with β -CD in different molar ratios (i.e. 1:1 M, 1:2 M) were mixed in a mortar for about one hour with constant trituration, passed through sieve No. 80 and stored in a desiccators over fused calcium chloride.[6] The formulation codes are A1, A2 & A3 given in table 3.

Kneading method:

GLI with β -CD in different molar ratios (i.e. 1:1 M, 1:2 M & 1:5) were taken. First cyclodextrin is added to the mortar, small quantity of 50% ethanol is added, while triturating to get slurry like consistency. Then slowly drug is incorporated into the slurry and trituration was further continued for one hour. Slurry was then air dried at 25 °C for 24 hours, pulverized and passed through sieve No. 80 and stored in desiccators over fused calcium chloride.[7] The formulation codes are A4, A5 & A6 given in table 3.

Table 2: Phase Solubility Studies of Glimepiride: β- Cyclodextrin Complexes

Sr. No.	Concentration of β-CD (mM)	Concentration of GLI (mM)
1.	0	0.0 40
2.	3	0.0 43
3.	6	0.0 47
4.	9	0.0 51
5.	12	0.0 55
6.	15	0.0 61

Table 3 :Different Formulations of Glimepiride with β-Cyclodextrin and in Molar Ratio

Method	Drug to carrier Complex	Drug to Carrier Ratio	Code for Complex
Physical Mixture	GMP:β-CD	1:1	A1
	GMP:β-CD	1:2	A2
	GMP:β-CD	1:5	A3
Kneading Method	GMP:β-CD	1:1	A4
	GMP:β-CD	1:2	A5
	GMP:β-CD	1:5	A6

Drug Content Estimation:

50 mg of complex was accurately weighed and transferred to 50 ml of volumetric flask and volume was made up to the mark with methanol. From this 1ml was taken in 10 ml volumetric flask and the volume is adjusted up to the mark with same solvent. The absorbance of the solution was measured at 230 nm.[8] The drug content of GLI was calculated using calibration curve data.

***In vitro* dissolution studies for Glimepiride –CD complexes:**

In-vitro dissolution of GLI -inclusion complex was studied in USP XXIV dissolution apparatus (Electrolab) employing a paddle stirrer. 900 ml of phosphate buffer of pH 7.4 was used as dissolution medium at 50 rpm. The temperature of 37 ± 0.5°C was maintained throughout the experiment. Complex equivalent to 50 mg of GLI was used in each test. 5ml of sample of dissolution medium were withdrawn by means of syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 230 nm after suitable dilution with phosphate buffer.[9] The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium. The amount of GLI released was calculated and plotted against time.

FTIR Spectroscopy:

The FTIR spectra of GLI and their complexes were obtained by KBr pellet method by JASCO FT/IR- 5300 spectrophotometer. [10]

Differential Scanning Calorimetry (DSC):

The water content of glimepiride complex with β-CD were determined using differential scanning calorimetry (DSC) over a temperature range from 10°C to 300°C. The DSC scan was carried out using a computerized Instrument: SDT Q600 V20.9 Build 20 instrument under a dynamic N2 purging gas atmosphere at a constant rate of 50 cc/min and a heating rate of 5°C/min.[11]

X-ray diffractometry:

X-ray diffraction patterns of glimepiride and glimepiride-β-CD complex were obtained by using a Diano X-ray diffractometer equipped with Co K. The tube operated at 45 kV, 9 mA.[12]

RESULTS AND DISCUSSION:

Preparation of Calibration curve of Glimepiride:

The calibration curve for the estimation of the drug (GLI) was constructed employing distilled water.

Phase Solubility Studies:

The complexation of GLI with β -CD was investigated by Phase Solubility Studies. The aqueous solubility of GLI was increased linearly as a function of concentration of CD. It is assumed that the increase in solubility observed was due to the formation of a 1:1 M, 1:2 M & 1:6 M proportion of inclusion complexes. The solubility constant (Kc) was calculated from the slope of the linear plot of the phase solubility diagram according to equation.1,

$$K_{a:b} = \frac{\text{slope}}{S_0\,(1\text{-slope})} \qquad \textbf{.1}$$

where So is the solubility of the drug in absence of CD. The calculated Kc value was 32.95 M^{-1} and = 42.57 M^{-1} with β -CD respectively. The highest solubility of inclusion complexes(1:5) proportion with kneading method. The results are given in table 2 and shown in figure. 4.

Table 2: Phase Solubility Studies of Glimepiride: β- Cyclodextrin Complexes

Sr. No.	Concentration of β-CD (mM)	Concentration of GLI (mM)
1.	0	0.0 40
2.	3	0.0 43
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4.	9	0.0 51
5.	12	0.0 55
6.	15	0.0 61

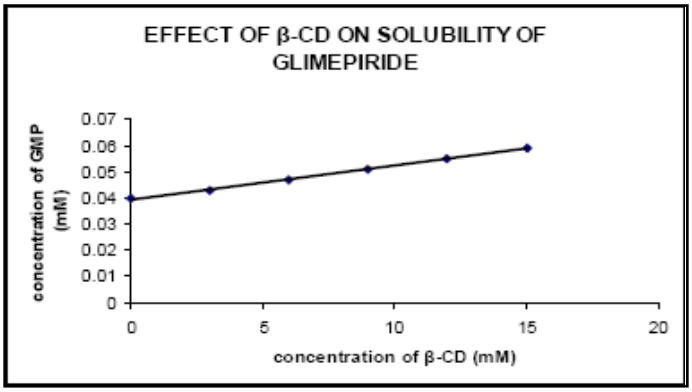

Figure 4: Effect of β-CD on solubility of Glimepiride (GLI)

Drug Content Estimation:

The inclusion complexes prepared by kneading method showed nearly 99.77 ± 0.72 % drug content. But the inclusion complexes prepared by Physical mixture method were found to be slightly less shown in figure.5 and table 4.

Table 4: Drug Content Estimation of Glimepiride in the β-CD complexes

Formulation code	Percent Drug Content ± SD *
A1	95.07 ± 0.39
A2	95.21 ± 0.63
A3	96.19 ± 0.51
A4	97.39 ± 0.19
A5	97.61 ± 0.19
A6	99.77 ± 0.72

* Indicates average of three determination ± SD

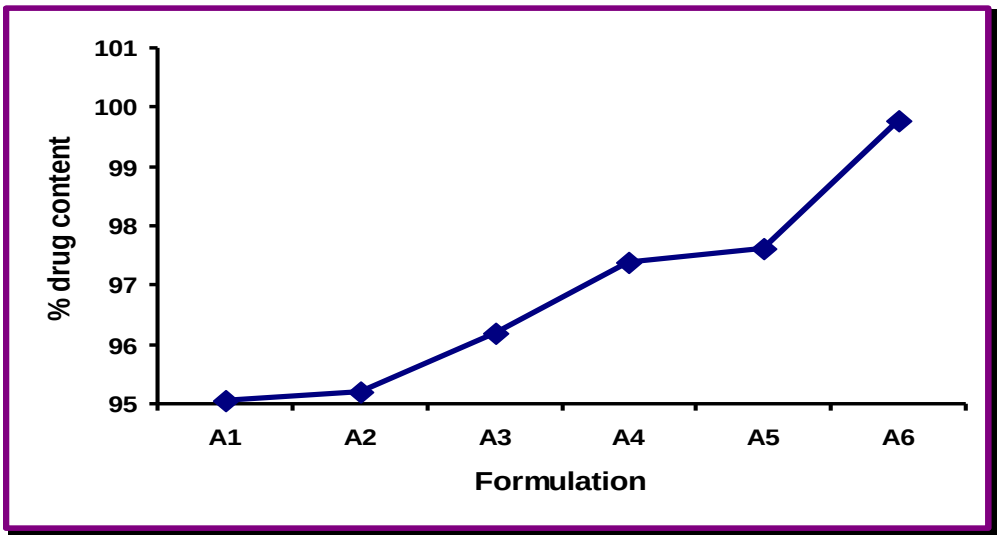

Figure 5: Drug Content Estimation of Glimepiride in the β-CD

Table 5: In- vitro Dissolution Profile of different formulation in Phosphate Buffer pH 7.4

Time in min	Percentage drug release ± SD *					
	A1	A2	A3	A4	A5	A6
0	0	0	0	0	0	0
5	12.91±0.13	17.01±0.16	15.11±0.28	19.11±0.27	21.51 ±0.29	23.6±0.38
10	17.99±0.64	21.37±0.14	19.79±0.25	27.31±0.12	23.72±0.37	23.98±0.42
15	28.11±0.38	29.72±0.27	30.95±0.11	39.15±0.64	41.28±0.29	43.74±0.35
20	35.72±0.26	45.77±0.38	42.15±0.16	51.57±0.43	52.75±0.32	54.38±0.17
25	47.91±0.12	57.41±0.35	50.67±0.41	61.72±0.51	63.52±0.31	65.41±0.15
40	59.25±0.29	65.92±0.46	62.95±0.63	70.97±0.62	71.84±0.25	74.86±0.12
50	70.77±0.26	72.96±0.58	72.11±0.72	83.01±0.39	84.63±0.22	88.72±0.36
60	81.52±0.13	83.79±0.62	84.43±0.68	91.01±0.28	95.27±0.27	98.25±0.49

* Indicates average of three determination ± SD

***In vitro* dissolution study:**

The dissolution characteristics of GLI (pure drug) and complexes are shown in table 5 and fig. 6. The inclusion complexes produces pronounced enhancement in its dissolution rate of drug. The inclusion complexes prepared by kneading method with β-CD of 1:5 proportion shows higher dissolution rate. Among these the complex prepared with β-CD i.e. formulation A6 shows higher dissolution rate (98.25 ± 0.49) at 2 hrs. than the other methods.

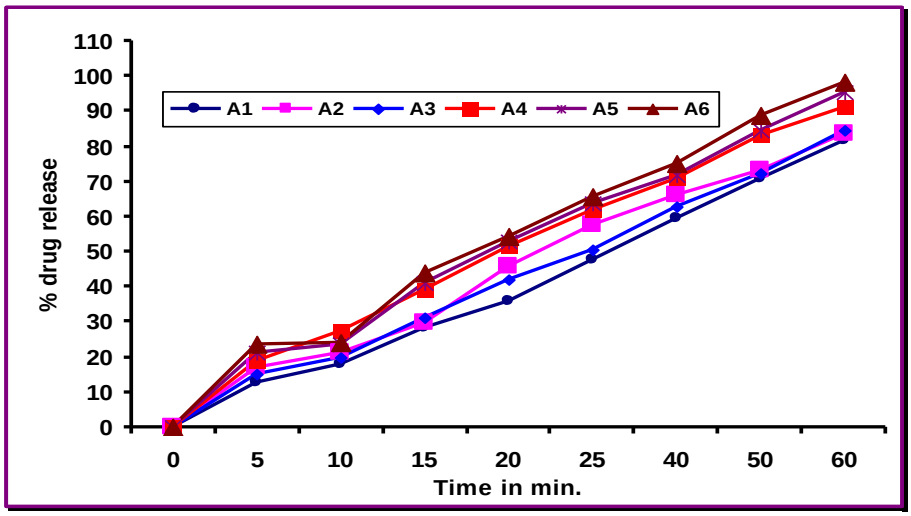

Figure 6: In- vitro Dissolution Profile of different formulation in Phosphate Buffer pH 7.4

FTIR Spectroscopy:

FTIR Spectra of pure drug, β -CD and inclusion complexes of GLI with β -CD prepared by different methods, results shown in fig. 7. As clearly seen from the spectra, the characteristic peaks of GMP at 709, 1082, 1444, 1674, 1705, 2360 and 3369 were modified significantly as a result of complex formation.[13]

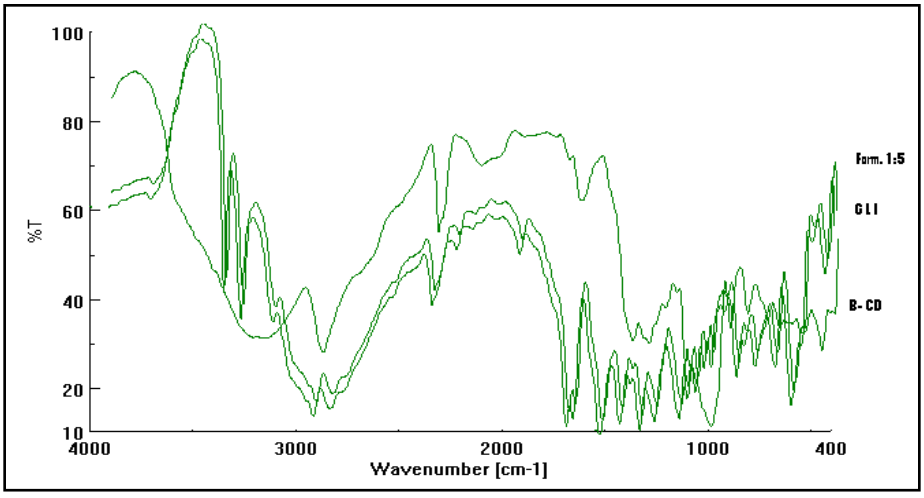

Figure 7: Overlain FTIR spectra of Glimepiride,β- Cyclodextrin, Glimepiride:β-Cyclodextrin (1:5)

Differential Scanning Calorimetry (DSC):

The thermal behavior GMP - β-CD complex was studied using DSC in order to confirm the formation of complex. DSC thermogram of GLI and A6 formulation are shown in fig. 8 and 9 respectively. The DSC thermogram of GMP showed an endothermic peak at 214**°C** corresponding to its melting point. The thermogram of A4 showed endothermic peak at 237°C which is different from the pure drug, which gives clear evidence that there is formation of the complex.[14]

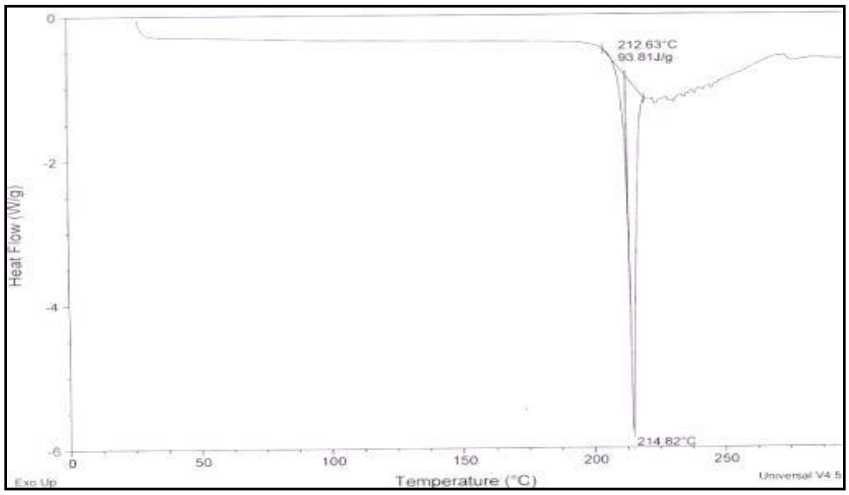

Figure 8: DSC Thermograms of Glimepiride (GLI)

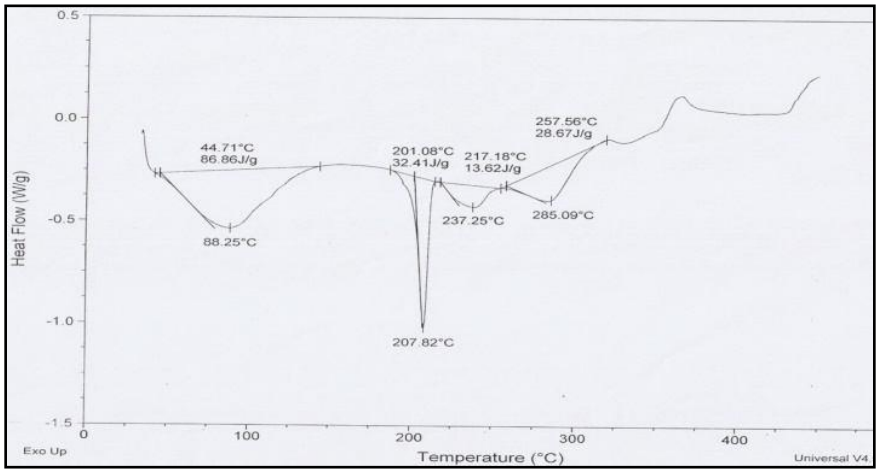

Figure 9: DSC Thermograms of A6 formulation

X-ray diffractometry:

Crystallinity can be determined by comparing some representative peak heights in the diffraction pattern of the binary system with that of a reference.[15] This reduction in crystallinity is attributable to a new solid phase with low crystallinity indicating inclusion complex formation (more water soluble). Furthermore, a reduced number of signals, of markedly lower intensity, are noticeable in the complex, indicating the more amorphous nature of the inclusion compound

compared with the free molecules. X-ray diffraction patterns of glimepiride and its cyclodextrin complex shown in figure.10.

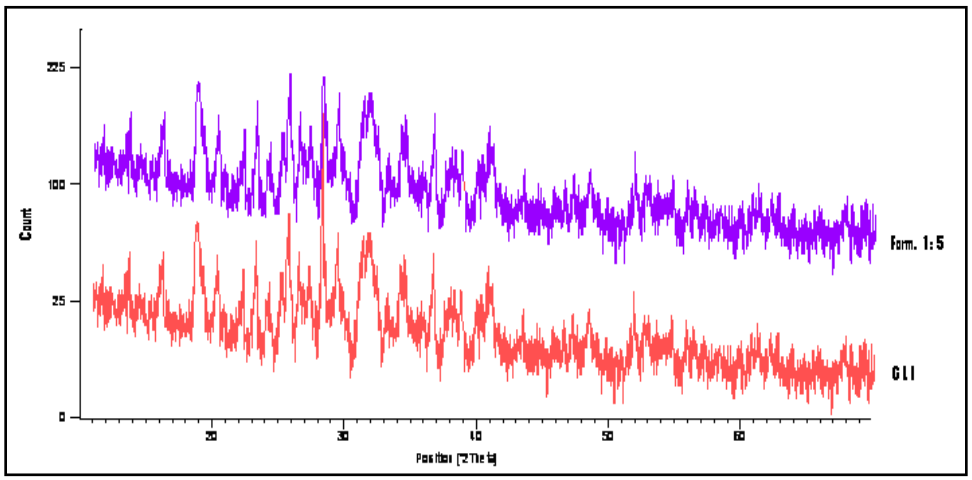

Figure 10: Overlain X-ray diffraction of Glimepiride and A 6 Formulation (1:5)

CONCLUSION:

The Cyclodextrins like β -CD can be used to prepare inclusion complexes of GLI with improved solubility of the drug by kneading method. GLI formed inclusion complexes with β -CD in 1:5 M ratio shows highest solubility and dissolution rate. The inclusion complex prepared with β -CD by kneading method formulation (A6) showed highest solubility and enhancement in dissolution profile.

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