



## ENHANCEMENT OF REPAGLINIDE AQUEOUS SOLUBILITY BY INCLUSION COMPLEX FORMATION WITH CYCLODEXTRINS AND SYNERGISTIC EFFECT OF BASIC pH AND CO-SOLVENT

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### ABSTRACT

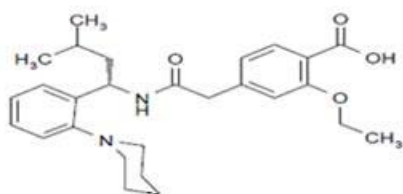
Repaglinide is a hypoglycemic drug and is poorly soluble in water, which affects bioavailability. As per BCS classification, bioavailability depends on both solubility and permeability of drug. In the present work, the possibility of improving solubility of repaglinide by complexation with cyclodextrins and then complex formation of drug with Hydroxypropyl  $\beta$ -cyclodextrin (HP  $\beta$ -CD) in solid form were investigated. The effect of basic pH and co-solvent, ethanol on solubilization of repaglinide by HP  $\beta$ -CD were also studied. The phase solubility studies indicated formation of repaglinide- $\beta$ -CD and repaglinide-HP  $\beta$ -CD inclusion complexes at 1:1 M ratio in solutions with stability constant of  $182.18 \text{ M}^{-1}$  and  $374.54 \text{ M}^{-1}$  respectively. The solubility was markedly enhanced in basic pH and by addition of co-solvent ethanol.

**Keywords:** Repaglinide, solubility, inclusion complexes, cyclodextrins, phase solubility diagram, co-solvent.

### INTRODUCTION

The Biopharmaceutics classification system (BCS) is defined by four major classes and drugs are classified based on three major factors governing bioavailability, namely, dissolution, solubility and permeability<sup>1-3</sup>.

Repaglinide, chemically S (+) 2-ethoxy-4-[2-[3-methyl-1-[2-(1-piperidinyl) phenyl] butyl] amino]-2-oxoethyl] benzoic acid, is an oral hypoglycemic drug administered in patients with type II diabetes mellitus in a dose of 0.5 to 4 mg, 3 to 4 times a day. Repaglinide comes under BCS class II having very low water solubility and high lipophilicity ( $\log P = 5.9$ ). The chemical structure of repaglinide is presented in figure 1<sup>4-7</sup>.



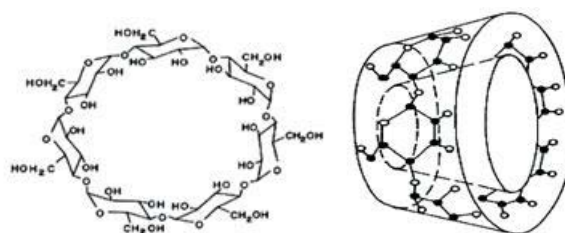
**Figure 1** Chemical structure of repaglinide

Cyclodextrins (CDs) are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity and in general they do not permeate lipophilic membranes. Cyclodextrins are widely used as "molecular cages" in pharmaceutical, agrochemical, food and cosmetic industries. In pharmaceutical industry they are used as complexing agents to increase aqueous solubility of poorly aqueous soluble drugs and to increase their bioavailability and stability.

Cyclodextrin consists of ( $\alpha$ -1,4) linked  $\alpha$ -D-glucopyranose unit as shown in figure 2. Due to chair formation of glucopyranose units, cyclodextrin molecules are shaped

like cones with secondary hydroxyl groups extending from wider edge and primary groups from narrow edge. This gives cyclodextrin molecules a hydrophilic outer surface, whereas lipophilicity of their central cavity is comparable to an aqueous ethanolic solution. The naturally occurring cyclodextrins are  $\alpha$ ,  $\beta$  and  $\gamma$  types containing 6, 7 and 8 glucopyranose units respectively<sup>8</sup>.

$\beta$ -cyclodextrin, most common natural CD, has 21 hydroxyl groups, that is, seven primary and 14 secondary hydroxyls (Figure 2)<sup>9</sup>.



**Figure 2** Chemical structure of beta cyclodextrin

$\beta$ -cyclodextrin has limited aqueous solubility due to strong intermolecular hydrogen bonding in its crystal state. Substitution of H-bond forming -OH group has improved their solubility. Various derivatives that have gained pharmaceutical interest include hydroxypropyl derivatives of  $\beta$ ,  $\gamma$  and methylated  $\beta$ -cyclodextrins, sulfo butyl ether  $\beta$ -cyclodextrin etc<sup>8</sup>.

### Phase-solubility diagram

Higuchi and Connors have classified complexes and it is indicated by phase-solubility profiles as shown in figure 3. A-type phase-solubility profiles are obtained when solubility of substrate (i.e. drug) increases with increasing ligand (cyclodextrin) concentration. When complex is first order with respect to ligand and first or higher order with



respect to substrate then  $A_L$ -type and if complex is first order with respect to substrate, but second or higher order with respect to ligand then  $A_p$ -type phase solubility profile is obtained. It is difficult to interpret  $A_N$ -type phase-solubility profile. B-type phase-solubility profiles indicate formation of complexes with limited solubility in aqueous complexation medium<sup>10</sup>.

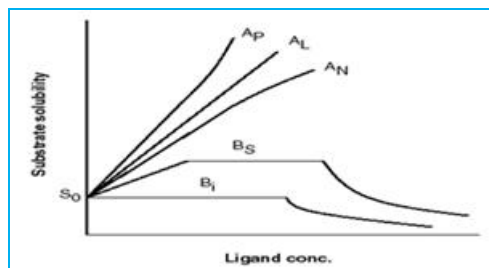


Figure 3 Phase solubility diagram

The most common type of cyclodextrin complexes is 1:1 drug/cyclodextrin (D/CD) complex where one drug molecule (D) forms a complex with one cyclodextrin molecule (CD) and is given in equation 1.



The value of  $K_{1:1}$  is most often between 50 and 2000  $M^{-1}$ . Under such conditions, for an  $A_L$ -type phase-solubility diagram with slope less than unity, stability constant ( $K_{1:1}$ ) of complex can be calculated from slope and intrinsic solubility ( $S_0$ ) of drug in aqueous complexation media (i.e. drug solubility when no CD is present) and is given in equation 2<sup>8</sup>.

$$K_{1:1} = \frac{\text{Slope}}{S_0(1-\text{Slope})} \quad \text{----- (2)}$$

## MATERIALS AND METHODS

Repaglinide was obtained as a gift sample from Torrent Pharma, Ahmadabad (INDIA),  $\beta$ -cyclodextrin from Sun Pharma, Ahmadnagar (INDIA) and HP  $\beta$ -cyclodextrin was obtained from Gangwal Chemicals, Mumbai (INDIA). All other materials used are of analytical grade.

### Construction of calibration curve of repaglinide

Repaglinide (20 mg) was accurately weighed and dissolved in 20 ml methanol. From this solution 10 ml was taken and diluted up to 100 ml with phosphate buffer pH 6.8<sup>11</sup> to make stock solution.

A series of dilutions were made from stock solution (100  $\mu\text{g/ml}$ ) to get concentrations in the range of 0-20  $\mu\text{g/ml}$ . The absorbance was measured spectrophotometrically at  $\lambda_{\text{max}}$  of repaglinide.

### Phase solubility studies

Phase solubility studies on repaglinide with cyclodextrins were performed by method described by Higuchi and Connors<sup>10</sup>.

Excess amount of repaglinide was added to 10 ml double distilled water containing various concentrations of cyclodextrins ( $\beta$ -CD, HP  $\beta$ -CD) (0-25 mM) taken in a series

of 25 ml screw capped vials and mixture was shaken for 24 hrs at room temperature on a water bath shaker. After 24 hr of shaking, to achieve equilibrium, solutions were filtered through whatman no.1 filter paper.

The filtered samples are diluted suitably and assayed for repaglinide content by UV spectrophotometer against blank having same concentration of CDs ( $\beta$ -CD/HP  $\beta$ -CD), so as to cancel any absorbance that may be exhibited by cyclodextrin molecules. The solubility experiments were conducted in triplicate.

Same procedure was followed once again using phosphate buffer pH 6.8 and 7.4.

Similarly, effect of co-solvent ethanol on saturation solubility was done by using increasing concentration of co-solvent.

### Preparation and characterization of inclusion complex in solid form

Inclusion complex of repaglinide with hydroxypropyl  $\beta$ -cyclodextrin (in 1:1 molar ratio) was prepared by kneading method. To a slurry of hydroxypropyl  $\beta$ -cyclodextrin in water (HP  $\beta$ -CD:water 1:3), calculated quantity of repaglinide was added step wise with continuous triturating in same direction. The slurry was then dried at 40°C for 24 hr in hot air oven and resulted complex was ground and passed through sieve no. 150.

In order to characterize complex formation between repaglinide and HP  $\beta$ -CD in solid state, FT-IR spectrum and DSC thermogram were recorded.

### Fourier transforms infrared spectroscopy (FT-IR)

The FT-IR spectra of repaglinide and inclusion complex of repaglinide with HP  $\beta$ -CD were obtained using FT-IR spectrophotometer (SHIMADZU).

### Differential scanning calorimetry

The DSC measurements of repaglinide and inclusion complex were performed on a differential scanning calorimeter (SHIMADZU DSC-60).

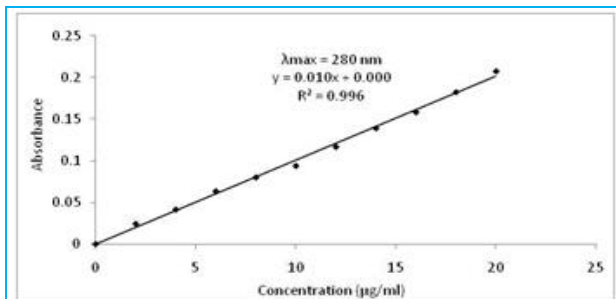
## RESULTS AND DISCUSSION

The calibration curve of repaglinide is as shown in figure 4. The aqueous solubility of repaglinide increases with increase in concentration of cyclodextrins (Figure 5 and 6). According to Higuchi and Connors classification, phase solubility diagrams of inclusion complexes are type A and shows formation of inclusion complexes at 1:1 molar ratio in solution with stability constants ( $K_{1:1}$ ) 182.18  $M^{-1}$  and 374.54  $M^{-1}$  for repaglinide- $\beta$ -CD and repaglinide-HP  $\beta$ -CD inclusion complexes respectively, indicating formation of water soluble complex in presence of HP  $\beta$ -CD. This might be due to much more solubility of HP  $\beta$ -CD in water as compared to  $\beta$ -CD.

Due to low pKa values of repaglinide, effect of basic pH (pH 6.8 and 7.4) on solubilization of repaglinide by HP  $\beta$ -CD was studied (Figure 7). The results indicate increase in solubility of repaglinide in basic pH. It means basic pH

help in formation of inclusion complex between repaglinide and HP  $\beta$ -CD.

The solubility of repaglinide was increased up to  $2.3 \pm 0.084$  mM in presence of ethanol but more solubility was found in presence of HP  $\beta$ -CD, up to  $3.725 \pm 0.051$  mM which indicate synergistic effect of ethanol with HP  $\beta$ -CD on solubilization of repaglinide (Figure 8).



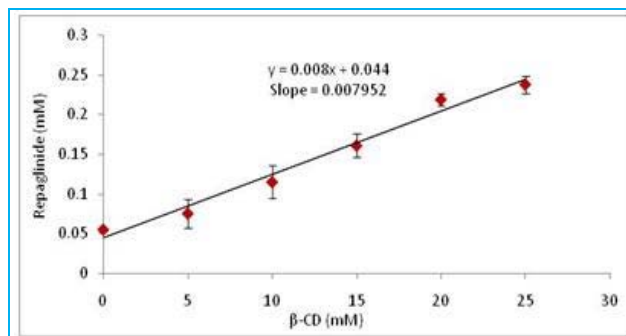
**Figure 4** Calibration curve of repaglinide in pH 6.8 phosphate buffer (0-20 µg/ml)

The results of saturation solubility of repaglinide in presence of  $\beta$ -CD and HP  $\beta$ -CD are as shown in table 1.

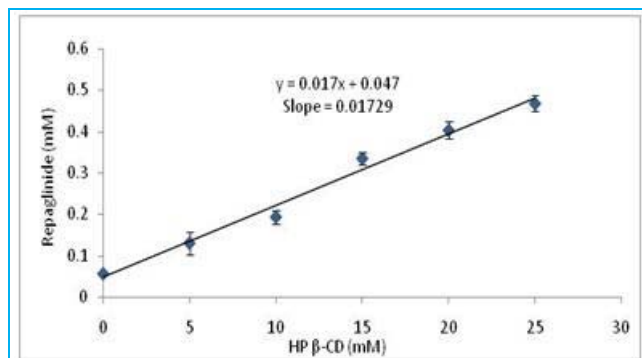
**Table 1** Effect of CDs on saturation solubility of repaglinide in water

| Concentration of $\beta$ -CD/ HP $\beta$ -CD (mM) | Repaglinide (mM) (Mean $\pm$ S.D) <sup>a</sup> |                               |
|---|--|-------------------------------|
|   | In presence of $\beta$ -CD                     | In presence of HP $\beta$ -CD |
| 0   | 0.0551 $\pm$ 0.0023                            | 0.0551 $\pm$ 0.0023           |
| 5   | 0.0753 $\pm$ 0.018                             | 0.129 $\pm$ 0.027             |
| 10  | 0.115 $\pm$ 0.021                              | 0.193 $\pm$ 0.017             |
| 15  | 0.161 $\pm$ 0.015                              | 0.335 $\pm$ 0.014             |
| 20  | 0.219 $\pm$ 0.008                              | 0.403 $\pm$ 0.021             |
| 25  | 0.238 $\pm$ 0.011                              | 0.468 $\pm$ 0.019             |

<sup>a</sup> n=3 standard deviation for three determinations



**Figure 5** Phase solubility diagram of repaglinide with  $\beta$ -CD



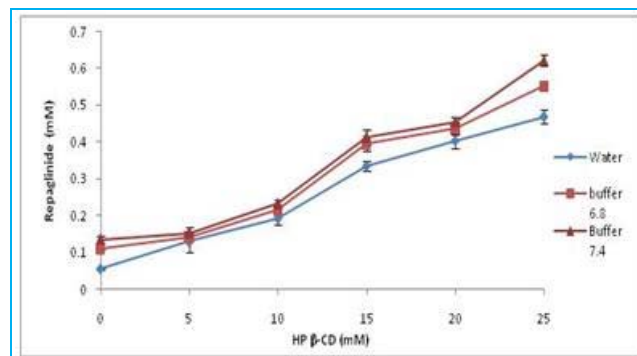
**Figure 6** Phase solubility diagram of repaglinide with HP  $\beta$ -CD

Table 2 and 3 indicates effect of pH and co-solvent on solubilization of repaglinide by HP  $\beta$ -CD, respectively.

**Table 2** Effect of pH on solubilization of repaglinide by HP  $\beta$ -CD

| Concentration of HP $\beta$ -CD (mM) | Repaglinide (mM)                    |   |   |
|--------------------------------------|-------------------------------------|---|---|
|                                      | Water (Mean $\pm$ S.D) <sup>a</sup> | Phosphate buffer pH 6.8 (Mean $\pm$ S.D) <sup>a</sup> | Phosphate buffer pH 7.4 (Mean $\pm$ S.D) <sup>a</sup> |
| 0                                    | 0.0551 $\pm$ 0.0023                 | 0.1105 $\pm$ 0.0031                                   | 0.135 $\pm$ 0.0092                                    |
| 5                                    | 0.129 $\pm$ 0.027                   | 0.142 $\pm$ 0.0073                                    | 0.153 $\pm$ 0.015                                     |
| 10                                   | 0.192 $\pm$ 0.017                   | 0.216 $\pm$ 0.011                                     | 0.232 $\pm$ 0.011                                     |
| 15                                   | 0.334 $\pm$ 0.014                   | 0.396 $\pm$ 0.021                                     | 0.412 $\pm$ 0.021                                     |
| 20                                   | 0.403 $\pm$ 0.021                   | 0.437 $\pm$ 0.018                                     | 0.454 $\pm$ 0.012                                     |
| 25                                   | 0.468 $\pm$ 0.019                   | 0.551 $\pm$ 0.013                                     | 0.621 $\pm$ 0.016                                     |

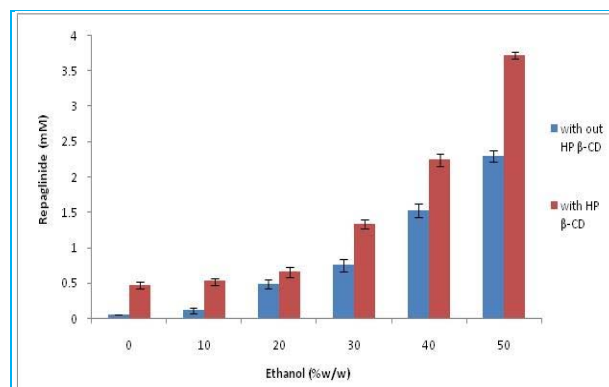
<sup>a</sup> n=3 Standard deviation for three determinations



**Figure 7** Effect of pH on solubility of repaglinide

**Table 3** Effect of co-solvent ethanol on solubilization of repaglinide by HP  $\beta$ -CD

| Concentration of ethanol (%v/v) | Repaglinide (mM)       |                     |
|---------------------------------|------------------------|---------------------|
|                                 | Without HP $\beta$ -CD | With HP $\beta$ -CD |
| 0                               | 0.0551 $\pm$ 0.0023    | 0.468 $\pm$ 0.051   |
| 10                              | 0.113 $\pm$ 0.037      | 0.523 $\pm$ 0.049   |
| 20                              | 0.483 $\pm$ 0.063      | 0.652 $\pm$ 0.072   |
| 30                              | 0.751 $\pm$ 0.085      | 1.338 $\pm$ 0.061   |
| 40                              | 1.532 $\pm$ 0.093      | 2.243 $\pm$ 0.084   |
| 50                              | 2.3 $\pm$ 0.084        | 3.725 $\pm$ 0.051   |



**Figure 8** Effect of co-solvent on solubility of repaglinide

### Characterization of inclusion complex

The FT-IR spectroscopy of repaglinide, HP  $\beta$ -CD and inclusion complex of repaglinide with HP  $\beta$ -CD is as shown in figure 9, 10 and 11, respectively.

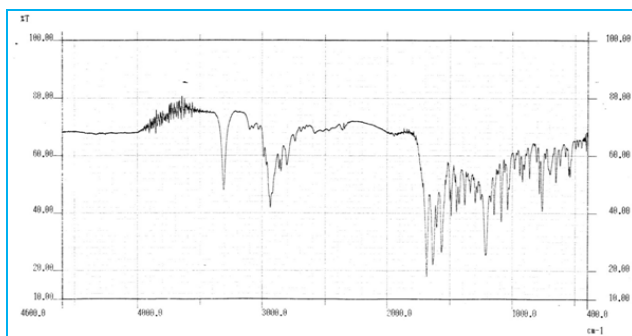


Figure 9 FT-IR spectrum of repaglinide

| Wavelength (cm <sup>-1</sup> ) | Functional group identified |
|--------------------------------|-----------------------------|
| 3308.3                         | N-H stretching              |
| 2936.0                         | Aliphatic C-H               |
| 1686.0                         | C=O                         |
| 1217.2                         | C-N stretching              |

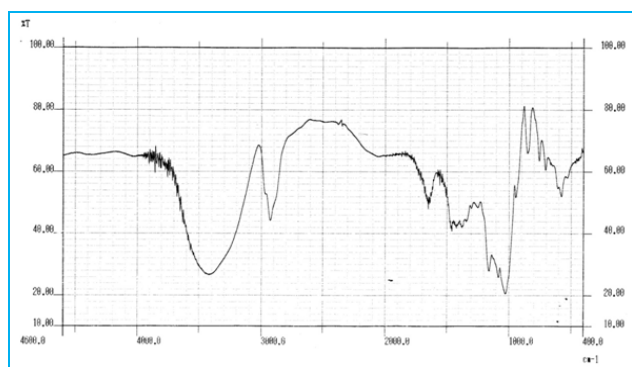


Figure 10 FT-IR spectrum of HP β-CD

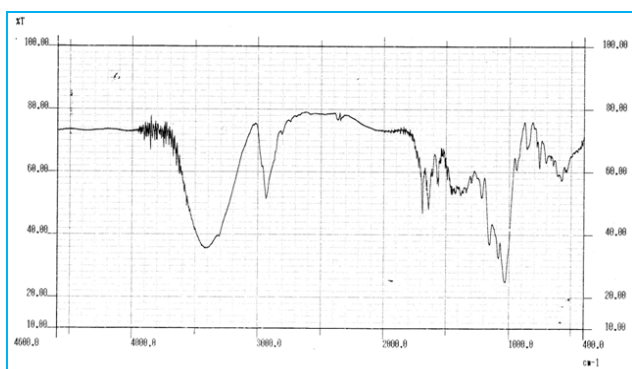


Figure 11 FT-IR spectrum of inclusion complex of repaglinide with HP β-CD

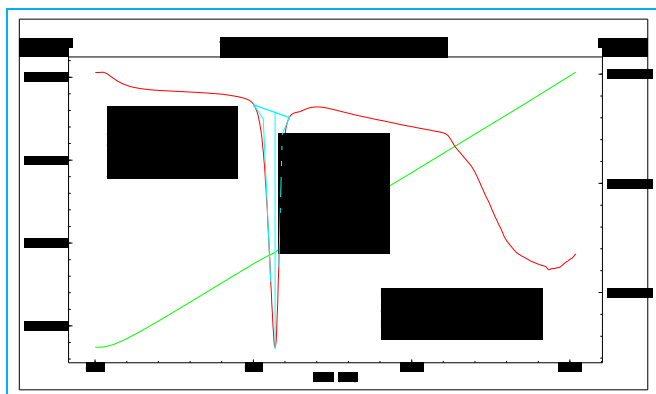


Figure 11 DSC thermogram of repaglinide

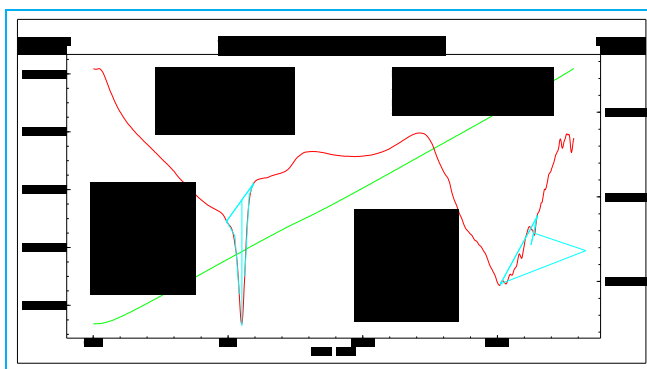


Figure 12 DSC thermogram of inclusion complex of repaglinide with HP β-CD

FT-IR spectrum of inclusion complex shows that characteristic peaks of repaglinide at 3308.3, 2936 cm<sup>-1</sup> etc were disappeared except peaks for C=O and C-N stretching but they are very small. It shows complex has been formed between repaglinide and HP β-CD.

DSC thermogram of Inclusion complex showed that melting point of repaglinide has decreased from 136.71°C to 134.98°C in inclusion complex and height of peak decreased from -14.18 mW to -4.28 mW. It shows that stable complex has been formed between repaglinide and HP β-CD and drug is stable in inclusion complex.

### CONCLUSION

The solubility of repaglinide was increased by complexation with cyclodextrins. It was concluded that repaglinide forms water soluble complex with HP β-CD with stability constant (K<sub>1:1</sub>) 374.54 M<sup>-1</sup> and solubility of repaglinide increases with increase in pH and by addition of co-solvent ethanol. Hence basic pH and co-solvent ethanol has synergistic effect on solubilization of repaglinide by inclusion complex. Repaglinide forms stable inclusion complex with HP β-CD in solid form by kneading method. So inclusion complexation with HP β-CD combined with basic pH and co-solvent, ethanol can be used to enhance solubility of repaglinide in water.

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