

## FORMULATION DEVELOPMENT AND *IN VIVO* EVALUATION OF PIOGLITAZONE INCLUSION COMPLEXES: A FACTORIAL STUDY

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### ABSTRACT

**Objective:** The objective of the study was to evaluate the individual main effects and combined (or interaction) effects of cyclodextrin ( $\beta$  cyclodextrin), surfactant (Poloxamer 407) and polyvinylpyrrolidone K30 (PVP K30) on the solubility and dissolution rate of pioglitazone in a series of  $2^3$  factorial experiments. The inclusion complexes were evaluated for pharmacokinetics and *in vivo* performance in comparison to pioglitazone pure drug.

**Methods:** Among the various approaches complexation with cyclodextrins has gained good acceptance in recent years in the industry for enhancing the solubility and dissolution rate of poorly soluble drugs. As per the phase solubility studies, a  $2^3$  factorial study was used to prepare the solid inclusion complexes and evaluated for the interactions and *in vitro* drug release. The best combinations were selected for *in vivo* performance in healthy albino rabbits. 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.

**Results:** The solubility of pioglitazone in eight selected fluids containing  $\beta$  cyclodextrin ( $\beta$ CD), Poloxamer 407 and PVP K30 as per  $2^3$  factorial studies was determined. Combination of  $\beta$ CD with Poloxamer 407 and PVP K30 resulted in a much higher enhancement (13.85-7.06 folds) in the solubility of pioglitazone than the  $\beta$ CD alone. Solid inclusion complexes of pioglitazone- $\beta$ CD were prepared with and without Poloxamer 407 and PVP K30 by kneading method as per  $2^3$ - factorial design. Analysis of variance (ANOVA) indicated that the individual main effects of  $\beta$ CD, Poloxamer 407 and PVP K30 and their combined effects in enhancing the solubility and dissolution rate ( $K_1$ ) were highly significant ( $P < 0.01$ ). The  $t_{1/2}$  value of pioglitazone estimated (6.92-7.46 h) in the present study was in good agreement with the literature reported value of 6-10 h. Pioglitazone was absorbed slowly when given orally with an absorption rate constant ( $K_a$ ) of  $0.629 \text{ h}^{-1}$  and a peak plasma concentration ( $C_{max}$ ) of  $11.40 \pm 0.7 \mu\text{g/ml}$  was observed at 4.0 h after administration. All the pharmacokinetic parameters namely  $C_{max}$ ,  $T_{max}$ ,  $K_a$  and  $(AUC)_{0-\infty}$  indicated rapid and higher absorption and bioavailability of pioglitazone when administered as  $\beta$ CD complexes. A 3.43 and 4.67 fold increase in the absorption rate ( $K_a$ ) and a 1.49 and 1.67 fold increase in  $(AUC)_{0-\infty}$  was observed respectively with pioglitazone- $\beta$ CD (1:2) and pioglitazone- $\beta$ CD (1:2)-Poloxamer 407 (2%) complexes when compared to pioglitazone pure drug.

**Conclusion:** Combination of  $\beta$ CD with Poloxamer 407 gave higher rates of absorption and bioavailability of pioglitazone than is possible with  $\beta$ CD alone. Hence the combination of  $\beta$ CD with Poloxamer 407 is recommended to enhance the absorption and bioavailability of pioglitazone, a BCS class II drug.

**Keywords:** Pioglitazone,  $\beta$  Cyclodextrin, Poloxamer 407, PVP K30, Solubility, Dissolution rate, Factorial study, Pharmacokinetics

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### INTRODUCTION

Pioglitazone is used to treat type 2 diabetes; condition in which the body does not use insulin normally and therefore cannot control the amount of sugar in the blood. Pioglitazone belongs to thiazolidinedione class. Pioglitazone, a water-insoluble antidiabetic drug belongs to class II under biopharmaceutics classification system (BCS) and exhibit low and variable oral bioavailability due to its poor aqueous solubility. As such its oral absorption is dissolution rate limited and it requires enhancement in solubility and dissolution rate for increasing its oral bioavailability. Among the various approaches complexation with cyclodextrins were used for enhancing the solubility and dissolution rate of poorly soluble drugs. The inclusion complex hides most of the hydrophobic functionality in the interior cavity of the cyclodextrin (CD) while the hydrophilic hydroxyl groups on the external surface remain exposed to the environment and result in the formation of water-soluble CD drug complex [1].

In addition to improving solubility, CDs also impede crystallization of active ingredients by complexing individual drug molecules so that they can no longer self-assemble into a crystal lattice [2]. Cyclodextrins have been receiving an increasing application in pharmaceutical formulation due to their approval by several regulatory agencies [3, 4]. Poloxamer 407 is a polyethylene oxide-polypropylene oxide-polyethylene oxide triblock copolymer of non-ionic nature and is used as a solubilizing agent [5-8].

Polyvinylpyrrolidone (PVP K30) are also reported [9-12] earlier to enhance the solubility of poorly soluble drugs.

Though cyclodextrin complexation and use of surfactants for enhancing the solubility and dissolution rate of poorly soluble drugs have been investigated individually, only a few reports are available on their combined use in enhancing the solubility and dissolution rate. We reported [13] earlier that combination of cyclodextrins ( $\beta$ CD and HP $\beta$ CD) with Poloxamer 407 has markedly enhanced the solubility and dissolution rate of pioglitazone, a BCS class II drug than is possible with them individually. The objective of the present study was to evaluate the pharmacokinetics and *in vivo* performance of the Pioglitazone- $\beta$ CD-Poloxamer 407 inclusion complexes in comparison to pioglitazone pure drug in healthy albino rabbits.

### MATERIALS AND METHODS

#### Materials

Pioglitazone was a gift sample from M/s. Hetero Drugs Ltd., Hyderabad.  $\beta$  cyclodextrin was gift sample from M/s. Cerestar Inc., USA. Methanol (Qualigens), polyvinylpyrrolidone (PVP K30) and Poloxamer 407 were procured from commercial sources. Normal albino rabbits were procured from National Institute of Nutrition, Hyderabad, India. Pellet diet was procured from Rayan's Biotechnologies Pvt. Ltd., Hyderabad, India. All other materials used were of the Pharmacopoeial grade.

### Estimation of pioglitazone

A UV spectrophotometric method based on the measurement of absorbance at 269 nm in 0.1 N hydrochloric acid of pH 1.2 was used for the estimation of pioglitazone. The method was validated for linearity, accuracy, precision and interference. The method obeyed Beer's law in the concentration range of 1-10 µg/ml. When a standard drug solution was repeatedly assayed (n= 6), the relative error and coefficient of variance were found to be 0.68% and 1.02% respectively. No interference by the excipients used in the study was observed.

### Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors [14]. Excess drug (25 mg) in each case was added to 15 ml of double distilled water (pH 6.8) containing various concentrations of βCD (1-15 mmol) or Poloxamer 407 or PVP K30 (0-10% w/v) taken in a series of 50 ml stoppered conical flasks and the mixtures were shaken for 72 h at room temperature (28 °C) on a rotary flask shaker. After 72 h of shaking to achieve equilibrium, 2 ml aliquots were withdrawn at 1 h interval and filtered immediately using 0.45 µ nylon disc filters. The filtered samples were diluted suitably and assayed at 269 nm for pioglitazone against blanks prepared in the same concentration of CDs/Poloxamer 407/PVP K30 in water so as to cancel any absorbance that may be exhibited by the cyclodextrin/Poloxamer 407/PVP K30 molecules. Shaking was continued until three consecutive estimations were the same. The solubility experiments were conducted in triplicate.

### Solubility determination

From the phase solubility data concentration of the βCD, Poloxamer 407 and PVP K30 were confirmed. Each fluid was prepared by known concentration of βCD/Poloxamer 407/PVP K30 as per 2<sup>3</sup> factorial design. Excess drug (50 mg) was added to 15 ml of each fluid, taken in a 25 ml stoppered conical flask and the mixtures were shaken for 24 h at room temperature (28±1 °C) on rotary flask shaker. After 24 h of shaking, 2 ml aliquots were withdrawn at 2 h interval and filtered immediately using a 0.45 µ disk filter. The filtered samples were diluted suitably and assayed for pioglitazone by measuring absorbance at 269 nm. Shaking was continued until two consecutive estimations are the same. The solubility experiments were replicated for four times each (n= 4).

### Preparation of pioglitazone-CD complexes

Solid inclusion complexes of pioglitazone-βCD were prepared in 1:2 ratio with and without Poloxamer 407 (2%) and PVP K30 (2%) by kneading method. Pioglitazone, βCD, Poloxamer 407 and PVP K30 were triturated in a mortar with a small volume of a solvent consisting of a blend of water: methanol (1:1 v/v). The thick slurry formed was kneaded for 45 min and then dried at 55 °C until dry. The dried mass was powdered and sieved to mesh No. 120.

### Characterization

#### Fourier-transform infrared (FTIR) analysis

FTIR studies were performed using a Perkin Elmer FTIR spectrophotometer to investigate the interaction of pioglitazone with the polymers. The samples were pelletized with KBr and the pellet was analyzed at a scanning range of 450-4000 cm<sup>-1</sup>. Characteristic peaks of the respective samples were recorded.

#### Differential scanning calorimetry (DSC) analysis

DSC analysis was carried out using Perkin Elmer, the system in the temperature range 30-250 °C at a heating rate of 10 °C/min in a dynamic nitrogen atmosphere. Approximately 5 mg of powder sample was accurately weighed and crimped in the aluminium lid, and an empty sealed aluminium pan was used as the reference.

#### Dissolution rate study

The dissolution rate of pioglitazone as such and from βCD complex prepared was studied in 900 ml 0.1 N hydrochloric acid of pH 1.2 using Disso 2000 (Labindia) 8-station dissolution test apparatus

with a paddle stirrer at 50 rpm. A temperature 37±1 °C was maintained throughout the study. Pioglitazone or pioglitazone factorial combinations equivalent to 30 mg of pioglitazone was used in each test. Samples of dissolution media (5 ml) were withdrawn through a filter (0.45 µ) at different intervals of time, suitably diluted and assayed for pioglitazone at 269 nm. The sample of dissolution fluid withdrawn at each time was replaced with fresh fluid. The dissolution experiments were replicated three times each (n=3).

### Preparation of pioglitazone-βCD-poloxamer 407/PVP K30 tablets

Compressed tablets each containing 30 mg of pioglitazone were prepared as per 2<sup>3</sup>-factorial study by (i) wet granulation and (ii) direct compression methods employing pioglitazone-βCD-Poloxamer 407/PVP K30 inclusion complexes. The composition, processing information and the results were already reported [15].

### Pharmacokinetic study

Pharmacokinetic evaluation of (i) pioglitazone-βCD (1:2) and (ii) pioglitazone-βCD (1:2)-Poloxamer 407 (2%) inclusion complexes in comparison to pioglitazone pure drug was done in healthy albino rabbits weighing 1.5-2.5 kg (n= 6) of either sex in a crossover randomized block design [16] at a dose equivalent to 10 mg/kg of pioglitazone. The animals were procured from National Institute of Nutrition, Hyderabad, India and kept in individual cages 30 d prior to the study. The animals were maintained under standard laboratory conditions and were fed with a commercial pellet diet and water ad libitum. A constant day-night cycle was maintained and the temperature of the animal room was kept constant throughout the study period. *In vivo* study protocols were approved by the Institutional Animal Ethics Committee (Regd. No 516/01/a/CPCSEA). A washout period of one month was given between testing of two products.

Overnight fasted healthy animals were used for the dosing. After collecting the zero hour blood sample (blank), the product in the study was administered orally in a capsule shell with 10 ml of water. No food or liquid other than water was permitted until 4 h following the administration of the product. Blood samples (2 ml) were collected from marginal ear vein at 0.5, 1, 2, 3, 4, 6, 8 and 12 h after administration. The blood samples were collected in heparinized tubes and were centrifuged at 10000 rpm for 10 min and the plasma separated was collected into dry tubes. All the samples were stored under refrigerated conditions prior to assay on the same day. Plasma concentrations of pioglitazone were determined by a known [17] high-performance liquid chromatography (HPLC) method after revalidation as follows.

### Instrumentation and chromatographic conditions

The HPLC system (Make: M/s Shimadzu Corporation, Japan.) consisted of UV-Visible detector (Shimadzu, Model: SPD-10AVP), C-18 column (Phenomenex, DESC: Gemini 5µ C18 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® # 702, Mfd. by: M/s Hamilton).

The mobile phase consists of a mixture of acetonitrile-water (60: 40 v/v) adjusted to pH 6.0 with 0.1% v/v glacial acetic acid. The mobile phase was filtered through a 0.45 µm membrane filter before use and was run at a flow rate of 1 ml/min. Amikacin (4 µg/ml) was used as internal standard. The column effluent was monitored at 269 nm. The retention time of pioglitazone is 5.310 min. The retention time of internal standard is 3.342 min.

### Estimation of pioglitazone in plasma

HPLC chromatogram of pioglitazone at a concentration of 1 µg/0.5 ml of plasma is shown in fig. 6. For the estimation of pioglitazone in plasma samples, a calibration curve was constructed initially by analyzing plasma samples containing different amounts of pioglitazone as follows:

To a series of tubes containing 0.5 ml of drug-free plasma in each, 0.1 ml of an internal standard solution containing 4 µg of amikacin and 0.1 ml drug solution containing 1, 2, 3, 4, 5 and 6 µg of pioglitazone were added and mixed. To each tube, 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 was added and mixed for reconstitution. Subsequently, 20  $\mu$ l were injected into the column for HPLC analysis. The interday and intraday precision of the method was evaluated by analyzing plasma sample containing 1.0  $\mu$ g/0.5 ml plasma of pioglitazone repeatedly (n=6).

#### Data 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 known standard methods [18, 19].

### RESULTS AND DISCUSSION

#### Phase solubility studies

The phase solubility studies of pioglitazone in presence of  $\beta$ CDs, Poloxamer 407 and PVP K30 were studied. In each case, the aqueous solubility of the drug was increased linearly as a function of the concentration of  $\beta$ CD as well as Poloxamer 407 and PVP K30. The phase solubility diagrams of the drug- $\beta$ CD complex (fig. 1a) can be classified as type AL according to Higuchi and Connors [14]. Because the straight line had a slope < 1, the increase in solubility was due to the formation of a 1:1 M complex in solution with  $\beta$ CD. The apparent stability constant (Kc) in each case was calculated from the slope of the corresponding linear plot of the phase solubility diagram according to the equation,  $K_c = \text{Slope}/S_0$  (1-Slope), where  $S_0$  is the solubility of the drug in the absence of  $\beta$ CD. The estimated apparent stability Constant (Kc) value of Pio- $\beta$ CD complex is 1658.54  $M^{-1}$ . The values of Kc indicated that the complexes formed between drug and  $\beta$ CD were quite stable.

Poloxamer 407 and PVP K30 also increased the solubility of pioglitazone. When the concentration of Poloxamer 407 and PVP K30 has increased the solubility was also increased linearly. Thus the phase solubility studies indicated that the solubility of the pioglitazone could be increased by the use of  $\beta$ CD, Poloxamer 407 and PVP K30.

#### Solubility studies

The individual main effects and combined (interaction) effect of  $\beta$ CD (factor A), Poloxamer 407 (factor B) and PVP K30 (factor C) on the aqueous solubility of pioglitazone were evaluated in a series of 2<sup>3</sup>-factorial experiments. For this purpose, two levels of  $\beta$ CDs (0, 5 mmol), two levels of Poloxamer 407 (0, 2%) and two levels of PVP K30 (0, 2%) were selected in each case and the corresponding eight treatments involved in the 2<sup>3</sup>-factorial study were purified water (1); water containing 5 mmol  $\beta$ CD (a); water containing 2% Poloxamer 407 (b); water containing 5 mmol  $\beta$ CD and 2% Poloxamer 407 (ab); water containing 2% PVP K30 (c); water containing 5 mmol  $\beta$ CD and 2% PVP K30 (ac); water containing 2% Poloxamer 407 and 2% PVP (bc) and water containing 5 mmol  $\beta$ CD and 2% of each of Poloxamer 407 and PVP K30 (abc).

The solubility of pioglitazone in the above-mentioned fluids was determined (n= 4) and the results are given in table 1. The solubility data were subjected to analysis of variance (ANOVA) to find out the significance of main and combined effects of  $\beta$ CD, Poloxamer 407 and PVP K30 on the solubility of pioglitazone. The results of ANOVA indicated that the individual and combined effects of  $\beta$ CD, Poloxamer 407 and PVP K30 in enhancing the solubility of pioglitazone were highly significant (P<0.01). The solubility of pioglitazone was marginally enhanced by  $\beta$ CD (3.42 fold). Whereas Poloxamer 407 and PVP K30 gave a moderate enhancement (4.32 and 9.54 fold) in the solubility of pioglitazone. The order of increasing solubility observed with  $\beta$ CD and surfactants was PVP K30>Poloxamer 407> $\beta$ CD>. Combination of  $\beta$ CD with Poloxamer 407 and PVP K30 resulted in a much higher enhancement (13.85 and 7.06 fold) in the solubility of pioglitazone than the  $\beta$ CD alone.

#### Characterization

##### Fourier-transform infrared (FTIR) analysis

The compatibility of drug and excipients in the drug- $\beta$ CD-Poloxamer 407-PVP K30 complexes prepared was evaluated by FTIR spectral

studies. An FTIR spectrum of pioglitazone is shown in the fig. 1a. The characteristic IR absorption peaks of pioglitazone at 3364  $cm^{-1}$  (N-H stretching vibration, presence of amide), 3084  $cm^{-1}$  (C-H stretching vibration, presence of aromatic), 2928  $cm^{-1}$  (C-H stretching, presence of aliphatic asymmetric), 1743  $cm^{-1}$  (C=O stretching, presence of amide), 1616  $cm^{-1}$  (C=C stretching), 1460  $cm^{-1}$  (C-N stretching), 1242  $cm^{-1}$  (C-S stretching), 1038 and 1084  $cm^{-1}$  (C-O-C stretching, presence of di substituted aromatic ring) were observed in the FTIR spectra of pioglitazone. The FTIR spectra of drug- $\beta$ CD-Poloxamer 407-PVP K30 complexes (fig. 1b) also exhibited all the above mentioned characteristic IR absorption peaks of pioglitazone. The FTIR spectra thus indicated no interaction between pioglitazone and the excipients in the complexes prepared.

##### Differential scanning calorimetry (DSC) analysis

The compatibility of drug and excipients in the drug- $\beta$ CD-Poloxamer 407-PVP K30 complexes prepared was also evaluated by DSC studies. The DSC thermogram of pioglitazone (fig. 2a) exhibited a broad endothermic peak with two sharp divided peaks at 177.5  $^{\circ}C$  and 187.9  $^{\circ}C$  corresponding to its melting point of 188  $^{\circ}C$ . The DSC thermogram of pioglitazone- $\beta$ CD-Poloxamer 407-PVP K30 (fig. 2b) showed a sharp endothermic peak at 178.3  $^{\circ}C$  corresponding to  $\beta$ CD. The DSC thermograms thus indicate no interaction between pioglitazone and  $\beta$ CD, Poloxamer 407 and PVP K30 in the complexes.

##### Dissolution rate study of solid inclusion complexes

To evaluate the individual and combined effects of  $\beta$ CD, Poloxamer 407 and PVP K30 on the dissolution rate of pioglitazone, solid inclusion complexes of pioglitazone- $\beta$ CD were prepared with and without Poloxamer 407 and PVP K30 as per 2<sup>3</sup>-factorial design. For this purpose two levels of  $\beta$ CD (0 and 1:2 ratio of drug:  $\beta$ CD) and two levels of each of Poloxamer 407 and PVP K30 (0 and 2%) were selected and the corresponding eight treatments involved in the 2<sup>3</sup>-factorial study were pioglitazone pure drug (1); pioglitazone  $\beta$ CD (1:2) inclusion binary complex (a); pioglitazone-Poloxamer 407 (2%) binary mixture (b); pioglitazone- $\beta$ CD (1:2)-Poloxamer 407 (2%) ternary complex (ab); pioglitazone-PVP K30 (2%) binary mixture (c); pioglitazone- $\beta$ CD (1:2)-PVP K30 (2%) ternary complex (ac); pioglitazone-Poloxamer 407 (2%)-PVP K30 (2%) ternary complex (bc) and pioglitazone- $\beta$ CD (1:2)-Poloxamer 407 (2%)-PVP K30 (2%) complex (abc).

The  $\beta$ CD complexes were prepared by kneading method. All the solid inclusion complexes of pioglitazone- $\beta$ CD-Poloxamer 407-PVP K30 prepared were found to be fine and free-flowing powders. Low coefficient of variation (c. v.) values (<1%) in the percent drug content indicated uniformity of drug content in each batch of solid inclusion complexes prepared. The dissolution rate of pioglitazone alone and from  $\beta$ CD complexes was studied in 0.1 N hydrochloric acid of pH 1.2. The dissolution of pioglitazone followed first-order kinetics with 'r' (correlation coefficient) above 0.92. Dissolution efficiency (DE<sub>30</sub>) values were calculated as suggested by Khan [20]. The dissolution parameters are given in table 2 and the graphs were given in fig. 3. The dissolution of pioglitazone was rapid and higher in the case of pioglitazone- $\beta$ CD binary and ternary complex systems prepared when compared to pioglitazone pure drug as such.

The dissolution rate ( $K_1$ ) values were subjected to ANOVA to find out the significance of the main and combined effects of  $\beta$ CD, Poloxamer 407 and PVP K30 on the dissolution rate of pioglitazone. ANOVA indicated that the individual main effects of  $\beta$ CD, Poloxamer 407 and PVP K30 and their combined effects in enhancing the dissolution rate ( $K_1$ ) were highly significant (P<0.01) except in the case of 'ac'.  $\beta$ CD alone gave an enhancement of 1.29 fold. Whereas in combination with Poloxamer 407 and PVP K30 gave a marginal enhancement (1.46 and 1.86 fold) in the dissolution rate of pioglitazone. Poloxamer 407 and PVP K30 have given highest enhancement (2.42 and 2.17 fold) in the dissolution rate of pioglitazone.

##### Dissolution rate study of pioglitazone- $\beta$ CD-poloxamer 407/PVP K30 tablets

Pioglitazone tablets formulated employing drug- $\beta$ CD-Poloxamer 407/PVP K30 inclusion complexes and prepared by direct compression method disintegrated rapidly when compared to those

made by wet granulation method. Pioglitazone dissolution was rapid and higher from the tablets formulated employing drug-βCD-Poloxamer 407/PVP K30 inclusion complexes when compared to the tablets containing pioglitazone alone in both wet granulation and direct compression methods. The individual as well as combined effects of the three factors involved i.e., βCD (factor A), Poloxamer 407 (factor B) and PVP K30 (factor C) were highly significant ( $P < 0.01$ ) in enhancing the dissolution rate ( $K_1$ ) and dissolution efficiency ( $DE_{30}$ ) of pioglitazone in both wet granulation and direct compression methods. Among the three factors, Poloxamer 407 (factor B) gave the highest enhancement in the dissolution rate ( $K_1$ ) and dissolution efficiency ( $DE_{30}$ ) of pioglitazone tablets in both wet granulation and direct compression methods. Out of all the βCD combinations, βCD-Poloxamer 407 combination has shown highest dissolution rate ( $K_1$ ) and selected for the pharmacokinetic studies. The alone βCD inclusion complex was also taken to evaluate the Poloxamer 407 effect in the pharmacokinetic study. All the studies were reported and given in brief in another paper [15].

### Pharmacokinetic study

The HPLC method validated was found suitable for the estimation of pioglitazone in plasma samples. The mobile phase consists of a mixture of acetonitrile-water (60: 40 v/v) adjusted to pH 6.0 with 0.1% v/v glacial acetic acid. The retention time for pioglitazone was 5.310 min and for internal standard (amikacin) it was 3.342 min. The linearity of the method was in the concentration range 1-5 μg/ml. The intra and interday coefficient of variation for drug and internal standard was less than 0.698 % showing the high precision of the method. The HPLC chromatograms of drug and internal standard were given in fig. 4.

The pharmacokinetic evaluation was done on pioglitazone-βCD (1:2) and pioglitazone-βCD (1:2)-Poloxamer 407 (2%) inclusion complexes in comparison to pioglitazone pure drug with a view to evaluating the *in vivo* performance of pioglitazone-βCD complexes. Plasma concentrations of pioglitazone following the oral administration of pioglitazone and its βCD complexes are shown in fig. 5. The pharmacokinetic parameters estimated are summarized in table 3.

The biological half-life ( $t_{1/2}$ ) estimated from the elimination phase of the plasma level curves was found to be 7.46, 7.28 and 7.18 h

respectively following the oral administration of pioglitazone, and its CD complexes, pioglitazone-βCD (1:2) and pioglitazone-βCD (1:2)-Poloxamer 407 (2%) complexes. The  $t_{1/2}$  value of pioglitazone obtained in the present study is in good agreement with the literature reported [21] value of 6-10 h. The close agreement of the  $t_{1/2}$  values obtained with the three products indicated that the elimination characteristics of pioglitazone have not changed when it was administered as βCD complexes.

Pioglitazone was found to be absorbed slowly when given orally and a peak plasma concentration ( $C_{max}$ ) of  $11.40 \pm 0.7$  μg/ml was observed at 4.0 h after administration. The absorption rate constant ( $K_a$ ) was found to be  $0.629$  h<sup>-1</sup>.

All the pharmacokinetic parameters (table 3) namely  $C_{max}$ ,  $T_{max}$ ,  $K_a$  and  $(AUC)_{0-\infty}$  indicated rapid and higher absorption and bioavailability of pioglitazone when administered as βCD complexes. Higher  $C_{max}$  values and lower  $T_{max}$  values were observed with the βCD complexes when compared to those of pioglitazone as such. The absorption rate constant ( $K_a$ ) was found to be  $2.16$  h<sup>-1</sup> and  $2.94$  h<sup>-1</sup> respectively with pioglitazone-βCD (1:2) complexes and pioglitazone-βCD (1:2)-Poloxamer 407 (2%) complexes. Whereas in the case of pioglitazone,  $K_a$  was only  $0.629$  h<sup>-1</sup>. A 3.43 and 4.67 fold increase in the absorption rate ( $K_a$ ) was observed respectively with pioglitazone-βCD (1:2) and pioglitazone-βCD (1:2)-Poloxamer 407 (2%) complexes when compared to pioglitazone pure drug.  $(AUC)_{0-\infty}$  (extent of absorption) was also much higher in the case of βCD complexes when compared to pioglitazone pure drug.  $(AUC)_{0-\infty}$  was increased from  $152.27$  μg. h/ml for pioglitazone pure drug to  $226.81$  and  $254.01$  μg. h/ml respectively for pioglitazone-βCD (1:2) and pioglitazone-βCD (1:2)-Poloxamer 407 (2%) complexes. A 1.49 and 1.67 fold increase in  $(AUC)_{0-\infty}$  was observed respectively with pioglitazone-βCD (1:2) and pioglitazone-βCD (1:2)-Poloxamer 407 (2%) complexes when compared to pioglitazone pure drug.

Thus, pioglitazone-βCD (1:2)-Poloxamer 407 solid inclusion complexes exhibited markedly higher rates and extent of absorption of pioglitazone when compared to pioglitazone alone and pioglitazone-βCD (1:2) complexes. Combination of βCD with Poloxamer 407 gave higher rates of absorption and bioavailability of pioglitazone than is possible with βCD alone.

Table 1: Solubility of pioglitazone in various fluids as per 2<sup>3</sup> factorial study

Fluids (code as per 2 <sup>3</sup> -factorial experiment)	Solubility (mg/ml) (n=4) (x±SD)	Increase in solubility (number of folds)	Significance
Distilled water (1)	0.122±0.08	-	-
Water containing 5 mmol βCD (a)	0.418±0.01	3.42	P<0.05
Water containing 2% Poloxamer (b)	0.527±0.043	4.32	P<0.01
Water containing 5 mmol βCD and 2% Poloxamer (ab)	1.691±0.052	13.85	P<0.01
Water containing 2% PVP (c)	1.165±0.054	9.54	P<0.01
Water containing 5 mmol βCD and 2% PVP (ac)	0.863±0.062	7.06	P<0.01
Water containing 2% Poloxamer and 2% PVP (bc)	1.667±0.085	13.65	P>0.05
Water containing 5 mmol βCD, 2% Poloxamer and 2% PVP (abc)	1.779±0.303	14.57	P<0.01

βCD: β Cyclodextrin, Poloxamer: Poloxamer 407 and PVP: Polyvinylpyrrolidone K30, (n= 4, Data presented as mean±SD)

Table 2: Dissolution parameters of pioglitazone-βCD complex systems prepared as per 2<sup>3</sup> factorial study

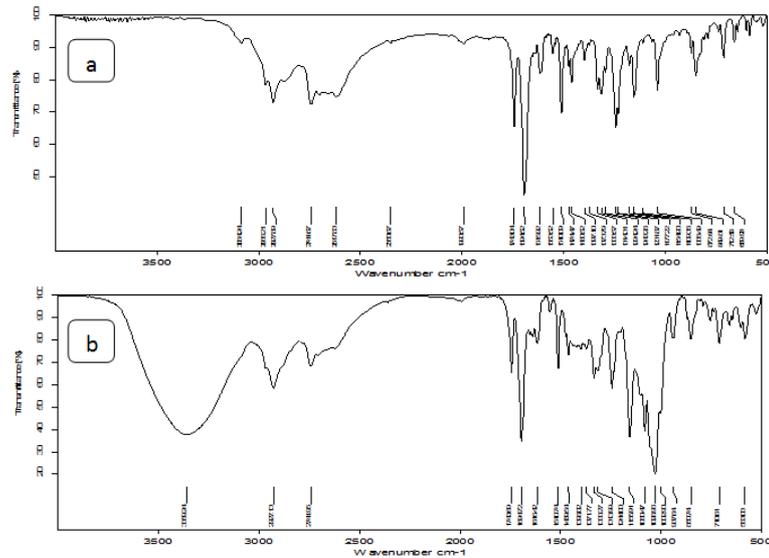
PIO-βCD complexes	Composition	PD <sub>10</sub> (%)	K <sub>1</sub> ×10 <sup>2</sup> (min <sup>-1</sup> )	Increase in K <sub>1</sub> (no. of folds)	DE <sub>30</sub> (%)	Increase in DE <sub>30</sub> (no. of folds)
F <sub>1</sub>	PIO	51.19	4.62	-	16.36	-
F <sub>a</sub>	PIO-βCD (1:2)	59.92	5.98	1.29	19.46	1.19
F <sub>b</sub>	PIO-P 407 (2%)	80.89	11.16	2.42	24.08	1.47
F <sub>ab</sub>	PIO-βCD (1:2)-P 407 (2%)	70.71	6.73	1.46	20.98	1.28
F <sub>c</sub>	PIO-PVP (2%)	81.86	10.01	2.17	21.22	1.30
F <sub>ac</sub>	PIO-βCD (1:2)-PVP (2%)	78.30	8.59	1.86	23.03	1.41
F <sub>bc</sub>	PIO-P 407 (2%)-PVP (2%)	89.82	10.28	2.23	25.36	1.55
F <sub>abc</sub>	PIO-βCD (1:2)-P 407(2%)-PVP (2%)	66.22	7.86	1.70	21.89	1.34

PIO: Pioglitazone; βCD: β Cyclodextrin; P 407: Poloxamer 407; PVP: Polyvinylpyrrolidone K30, (n=3, Data presented as mean)

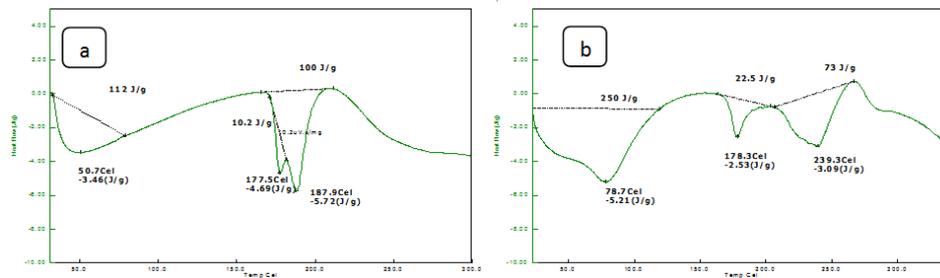
**Table 3: Summary of pharmacokinetic parameters estimated following the oral administration of pioglitazone and its βCD complexes**

Parameter	Pioglitazone	Pioglitazone-βCD (1:2) complex	Pioglitazone-βCD (1:2)-poloxamer 407 (2%) complex
C <sub>max</sub> (μg/ml) (±SD)	11.40±0.7	22.81±0.8	24.48±1.4
T <sub>max</sub> (h)	4.0	2.0	1.0
K <sub>el</sub> (h <sup>-1</sup> )	0.093	0.095	0.097
t <sub>1/2</sub> (h)	7.46	7.28	7.18
(AUC) <sub>0-12h</sub> (μg. h/ml)	92.14	155.03	172.67
(AUC) <sub>0-∞</sub> (μg. h/ml)	152.27	226.81	254.01
BA (%)	100	148.95	166.81
K <sub>a</sub> (h <sup>-1</sup> )	0.629	2.16	2.94
Percent Absorbed			
0.5 h	24.4	71.2	86.9
1.0 h	39.8	88.5	94.7
2.0 h	60.6	100.0	100.0

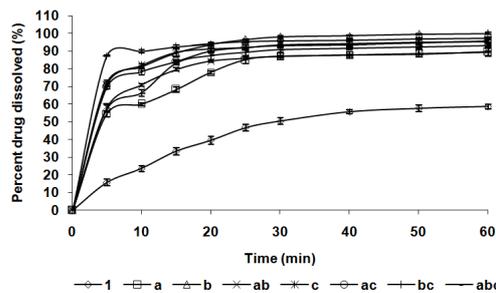
βCD: β Cyclodextrin; n= 6, Data presented as mean±SD.



**Fig. 1: FTIR spectra of a) pioglitazone and b) pioglitazone-βCD-Poloxamer 407-PVP K30 complex**



**Fig. 2: DSC thermogram of a) pioglitazone and b) pioglitazone-βCD-Poloxamer 407-PVP K30 Complexes**



**Fig. 3: Dissolution profiles of pioglitazone-βCD-Poloxamer 407-PVP K30 solid inclusion complexes prepared as per 2<sup>3</sup> factorial study (n=3, Data presented as mean±SD) 1: Pure Drug; a: Pio-βCD; b: Pio-Poloxamer; ab: Pio-βCD-Poloxamer; c: Pio-PVP K30; ac: Pio-βCD-PVP K30; bc: Pio-Poloxamer-PVP K30; abc: Pio-βCD-Poloxamer-PVP K30**

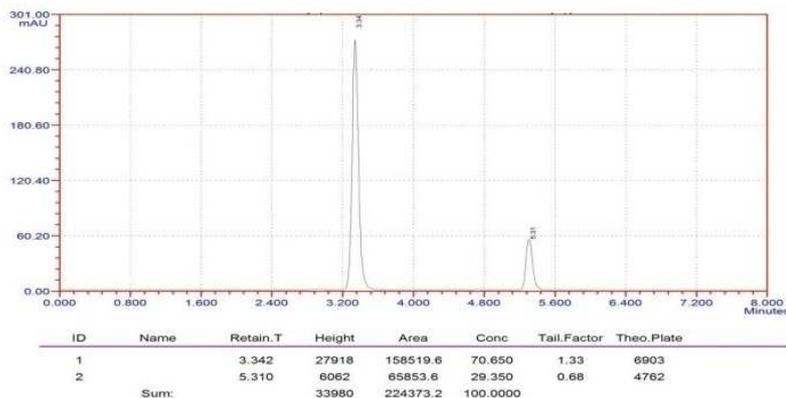


Fig. 4: HPLC chromatogram of pioglitazone (1 µg/0.5 ml of plasma)

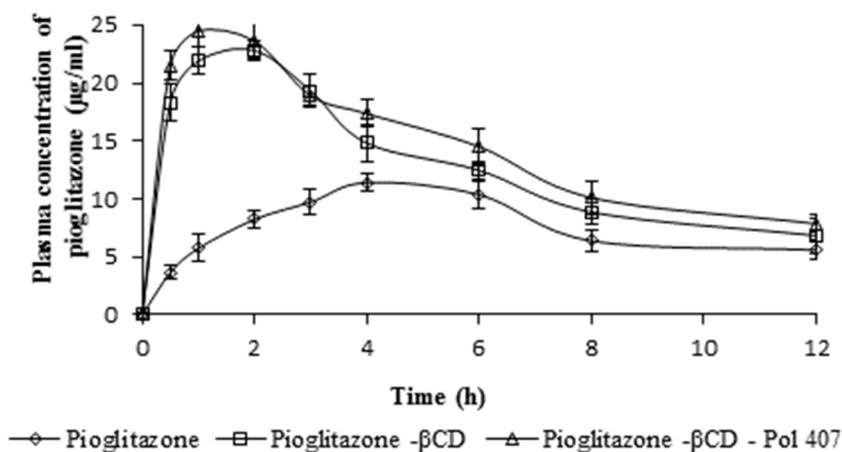


Fig. 5: Plasma concentrations of pioglitazone following the oral administration of pioglitazone and its βCD complexes in rabbits (n=6, Data presented as mean ±SD)

## CONCLUSION

Combination of βCD with Poloxamer 407 and PVP K30 resulted in a much higher enhancement (13.85-7.06 fold) in the solubility of pioglitazone than the βCD alone. Though PVP K30 has given highest enhancement (9.54 fold) in the solubility of pioglitazone, it gave only a marginal increase in the dissolution rate,  $K_1$  (1.96 fold) and  $DE_{30}$  (1.34 fold). The  $t_{1/2}$  value of pioglitazone estimated (6.92 h-7.46 h) in the present study is in good agreement with the literature reported value of 6-10 h. The elimination characteristics of pioglitazone have not changed when it was administered as βCD complexes. Pioglitazone was absorbed slowly when given orally with an absorption rate constant ( $K_a$ ) of  $0.629\text{ h}^{-1}$  and a peak plasma concentration ( $C_{max}$ ) of  $11.40 \pm 0.7\text{ µg/ml}$  was observed at 4.0 h after administration. A 3.43 and 4.67 fold increase in the absorption rate ( $K_a$ ) and a 1.49 and 1.67 fold increase in  $(AUC)_{0-\infty}$  was observed respectively with pioglitazone-βCD (1:2) and pioglitazone-βCD (1:2)-Poloxamer 407 (2%) complexes when compared to pioglitazone pure drug. Pioglitazone-βCD (1:2)-Poloxamer 407 (2%) solid inclusion complexes exhibited markedly higher rates and extent of absorption of pioglitazone when compared to pioglitazone alone and pioglitazone-βCD (1:2) complexes. Combination of βCD with Poloxamer 407 gave higher rates of absorption and bioavailability of pioglitazone than is possible with βCD alone. Hence the combination of βCD with Poloxamer 407 is recommended to enhance the absorption and bioavailability of pioglitazone, a BCS class II drug.

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## AUTHORS CONTRIBUTIONS

All the author have contributed equally

## CONFLICTS OF INTERESTS

Authors have no conflict of interest

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