

Effect of method of preparation on pioglitazone HCl- β -cyclodextrin inclusion complexes

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Pioglitazone HCl is an antidiabetic agent with poor aqueous solubility. Inclusion complexes of pioglitazone HCl were prepared with β -cyclodextrin using various methods (physical mixing, kneading method, co-precipitation). The main aim of the present invention is to study the effect of the method of preparation on the dissolution profiles of pioglitazone HCl- β -Cyclodextrin inclusion complexes. The phase solubility profile of pioglitazone HCl with β -cyclodextrin was classified as AL-type and stability constant with 1:1 molar ratio was 115.45, calculated from the phase solubility diagram. Formation of the inclusion complex between pioglitazone HCl and β -cyclodextrin was confirmed by the Fourier Transform Infrared (FT-IR) spectroscopy. The dissolution profile of inclusion complexes were determined and compared with those of pioglitazone HCl alone and its physical mixtures. The dissolution rate of the pioglitazone HCl- β -cyclodextrin inclusion complex prepared by the co-precipitation method was six times higher when compared with the pure drug. The method of complexation of pioglitazone HCl in β -CD increased the dissolution rate of the drug in the following order: Coppt > KM > PM > Drug.

Key words: Inclusion complex, pioglitazone HCl, β -cyclodextrin

INTRODUCTION

Solubilization of a new chemical entity in a pharmaceutically acceptable solvent system remains a major challenge in the development of a solution dosage form.^[1] Cyclodextrins are useful functional excipients, having the ability to interact with poorly water-soluble drugs and drug candidates, resulting in an increase in their apparent water solubility.^[2-4] Cyclodextrins are cyclic oligosaccharides derived from starch, and contain six (α CD), seven (β CD), eight (γ CD), nine (δ CD), ten (ϵ CD) or more (α -1, 4)-linked α -D-glucopyranose units. Due to the chair conformation of the glucopyranose units, the CDs take the shape of a truncated cone or torus, rather than a perfect cylinder.^[3,5,6] The primary hydroxyl group is oriented to the narrow edge of the cone at the exterior and the secondary group to the wider edge. The central cavity of the CD molecule is lined with skeletal carbons and ethereal oxygens of the glucose residue, which gives it a relatively lipophilic character. This cavity enables cyclodextrins to complex the 'guest' drug

molecules and in so doing alters the physicochemical properties of the drug.^[7]

Pioglitazone HCl, belonging to the drug class of thiazolidinedione, is used to decrease insulin resistance. It is an antidiabetic agent to manage a certain type of diabetes, such as, NIDDM (non-insulin-dependent diabetes mellitus, sugar diabetes) called type-II diabetes. It reduces the circulating sugar level. It has poor aqueous solubility (< 1 μ g/mL at a room temperature of 25°C).^[8]

In the present study, inclusion complexes of pioglitazone with β CD were prepared using the methods of kneading and co-precipitation. The formation of inclusion complexes with β -CD in the solid state, were confirmed by infrared spectroscopy, partition coefficient, and thin layer chromatography, and their dissolution rates were determined using the USP basket method. The present invention relates to the study effect of the preparation method on the dissolution profiles of pioglitazone HCl- β -cyclodextrin inclusion complexes.

MATERIALS AND METHODS

Materials

Pioglitazone HCl was a generous gift from Alembic Pharma Ltd and β -CD was purchased from commercial

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suppliers. All chemicals used were of analytical grade and used without further purification.

Phase solubility study

Solubility studies were carried out according to the method of Higuchi and Connors. In this, an excess amount of pioglitazone HCl was added to 10 ml of distilled water containing various concentrations of β -CD (0 – 10 mM), taken in a series of test tubes. The test tubes were shaken on a rotary flask shaker (DBK instrument Mumbai) for 48 hours. The samples were centrifuged using Megafuge 1.0 R at 3000 rpm for 10 minutes. The supernatant was collected and filtered using Whatman filter paper No 40. The filtered samples were suitably diluted and assayed for pioglitazone HCl content by UV analysis against a blank, prepared in the same concentration of β -CD. The experiments were performed in triplicate. The phase solubility diagram was constructed by plotting the dissolved pioglitazone HCl concentration against the respective concentration of β -CD. The binding constant K_s was calculated with the help of the phase solubility diagram, using its slope and intercept value.^[10-12]

$$K_s = \frac{\text{slope}}{S_0 (1 - \text{slope})}$$

S_0 is the solubility of pioglitazone HCl in absence of CDs.

Methods of preparation of binary systems

The preparation of the binary systems of pioglitazone HCl with β -CD was performed by mechanical (physical) mixing, kneading, and co-precipitation methods, as described herewith.

Mechanical (physical) mixing (PM)

Physical mixtures were prepared by the simple intensive mixing of the two components that had previously been sieved through mesh number 80 for 30 minutes in a 1:1 molar ratio.

Kneading method (KM)

Pioglitazone HCl and β -CD were accurately weighed (1:1 molar ratio), placed in the mortar and triturated for 15 minutes. The mixture was then kneaded with acetone for 60 minutes and the resulting paste was kept in desiccators overnight. The dry mass so obtained was powdered and passed through mesh number 80.^[13]

Co-precipitation method (Cppt)

Pioglitazone HCl was dissolved in a sufficient quantity of acetone and added dropwise into a solution of β -CD and a minimum quantity of water, previously maintained at 75°C, while stirring. Stirring was maintained for one hour, at 75°C. Next, it was gradually cooled to room temperature, 25°C, while stirring. The precipitates were then dried and passed through mesh number 80.^[14]

Characterization of pioglitazone HCl- β -Cyclodextrin complexes

Thin layer chromatography

An accurately weighed amount of complex was dissolved in 0.2 M HCl (pH 1.2). The complex and reference standard solution were spotted on previously activated TLC plates at 105°C for one hour. The plates were developed vertically in a rectangular chromatographic chamber saturated with a solvent system comprising of Toulene: Methanol: Ammonia (7:3:0.1).^[15] The plates were exposed to iodine vapors by placing them in closed vessels that were previously saturated with iodine. The distance traveled by a solvent front and solute front in each case was measured, and the R_f values were calculated by using the following formula.^[16]

$$R_f = \frac{\text{Distance traveled by solute front}}{\text{Distance traveled by solvent front}}$$

Partition coefficient determination

The partition coefficient of the drug and complexes between benzene / water were determined at ambient temperature (30±2°C). Ten milliliters each of benzene and distilled water were taken in glass stoppered flasks, to which 10 mg of accurately weighed drug and mixture was added and then shaken with the help of a mechanical shaker for 24 h at a room temperature of 25°C. The mixture was then transferred to a separating funnel and allowed to equilibrate for six hours. The aqueous and benzene phase were separated and filtered, and the drug content in the aqueous phase was analyzed by UV spectrophotometers at 269 nm. The partition coefficient was calculated using the following formula.^[17]

$$K = \frac{C_{aq}}{C_{org}}$$

K = Partition coefficient

C_{aq} = Concentration of solute in aqueous phase

C_{org} = Concentration of solute in organic phase

FT-IR spectroscopy study

Infra red spectra of the prepared complexes were carried out using FT-IR (Jasco FTIR - 410) based on the KBr disc method and compared with that of the pure drug.

Dissolution rate studies

Dissolution studies of the pure drug and the inclusion complexes prepared by different methods were performed in 0.2 M HCl (pH 1.2) using USP eight station dissolution test apparatus-1 (rotating basket) (LABINDIA2000). The stirring speed employed was 50 rpm, and the temperature was maintained at 37°C±0.5°C. Powder samples containing 30 mg of pioglitazone HCl or an equivalent amount of complex or physical mixtures with β -cyclodextrins were filled in transparent hard gelatin capsules (number 0). Aliquots of 5 ml

each were withdrawn from the dissolution medium at intervals of 5, 10, 15, 20, 30, 60, 90, and 120 minutes and replaced by an equal volume of fresh dissolution medium. The samples were filtered through Millipore filters (0.45 μ m) and analyzed for pioglitazone HCl content by measuring its absorbance at 269 nm, using fresh dissolution medium as a blank.^[9,14]

RESULTS AND DISCUSSIONS

Phase solubility studies

The phase-solubility graph of pioglitazone HCl - β -CD is shown in Figure 1. The plot showed that aqueous solubility of the drug increased linearly as a function of β -CD. According to Higuchi and Connors, phase solubility profile could be considered as an AL type. As the straight line had a slope less than unity, thus 1:1 stoichiometry was suggested.^[12] The value of the stability constant was found to be 115.45 M^{-1} . The stability constant between the range of 100 and 1000 M^{-1} was considered as an ideal value, smaller values indicated weak interaction between drug and cyclodextrin, while a large value indicated incomplete drug release from the inclusion complex.

Thin layer chromatography

The R_f value for the pioglitazone HCl, β -CD, and complex were found to be 0.68, 0.56, and 0.63, respectively.

Partition coefficient determination

The partition coefficient of pioglitazone HCl was found to be 1.74 ± 0.46 , while for the co-precipitation complex it was estimated to be 1.29 ± 0.63 . The decrease in the Partition Coefficient value could result from an increase in the hydrophilicity of the drug.

FT-IR spectroscopy study

The formation of the drug polymer complex was confirmed by using FT-IR. Figure 2 illustrates the FT-IR spectra of pioglitazone HCl, β -CD, and their binary mixtures, prepared by different methods, such as, PM, KM, and Co-ppt methods. The various principle absorption peaks for different functional groups in pioglitazone HCl, β -CD and inclusion complexes are given in Table 1.

A: β -CD, B: pioglitazone HCl, C: PM, D: KM, E: Coppt.

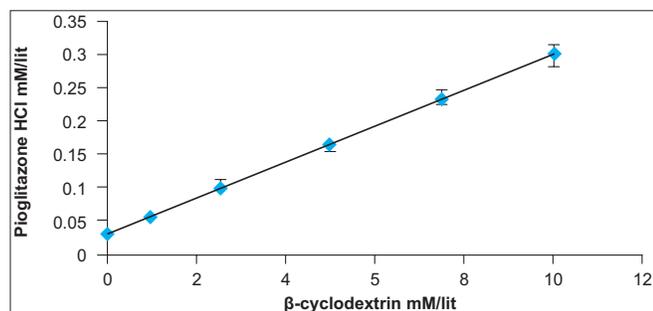


Figure 1: Phase-solubility graph of pioglitazone HCl - β -CD

Table 1: Various principle absorption peaks for different functional groups in pioglitazone HCl, β -CD, and the inclusion complexes

Principle absorption peak (/cm)	Functional group and vibration
A Pioglitazone HCl	
3083.62	N-H stretching
2928.38	CH stretching
2741.32	CH stretching
1742.37	C=O stretching
1693.19	C=O stretching
1615.09	aromatic ring
1242.9	C-O-Ar group
B β-Cyclodextrin	
3382.53	O-H stretching
2923.56	C-H stretching
1643.05	H-O-H bending
1157.08	C-O stretching of COOH
1027.87	C-O-C bending

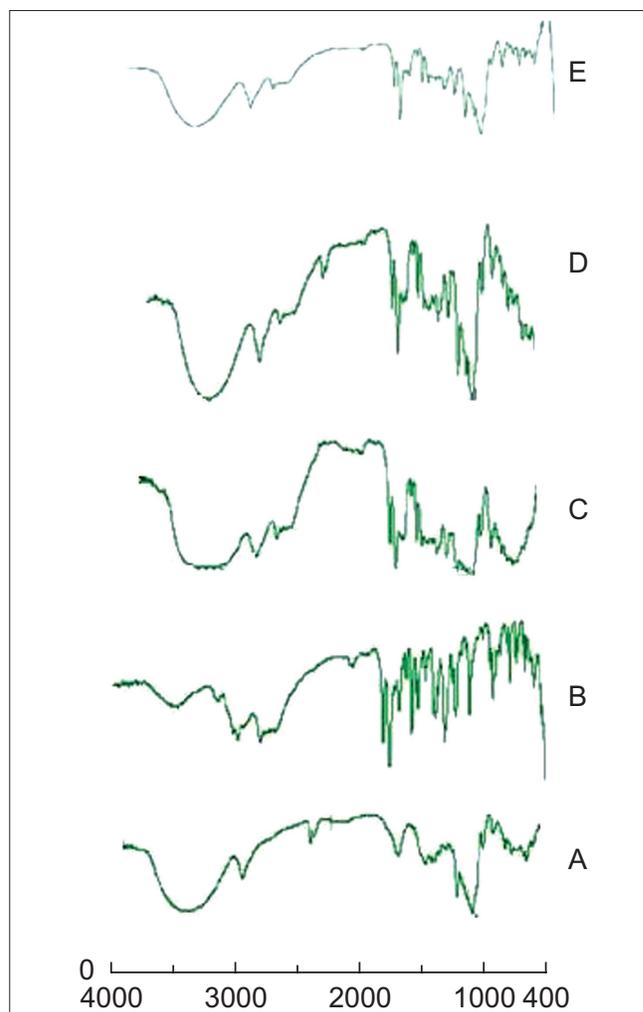


Figure 2: FT-IR spectra of pioglitazone HCl, β -CD, and their binary mixtures

The IR spectra of the binary inclusion complexes had considerable differences in comparison to those of their corresponding constituents. A decrease in frequency of a specific peak was generally seen on complexation, indicating an ordering of the molecule. In the IR spectrum of pioglitazone HCl-cyclodextrin complexes, the amide-NH stretching vibration at 3083.62/cm was not detected on a broad band, which might be because the co-occurrence of the N-H band of the drug appeared with the OH intensified band of β -CD at 3327 – 3425/cm. This indicated a strong interaction and complex formation of pioglitazone HCl with β -CD. The absorption band that appeared at 1693.19/cm due to carbonyl groups of pioglitazone HCl, broadened and shifted to a higher wave number for pioglitazone HCl- β -CD complexes, as follows: 1704/cm Coppt, 1699.94/cm for KM, and 1695.12/cm for PM. Khalil *et al.*,^[9] observed that the absorption band that appeared at 1685.07/cm, due to carbonyl groups of pioglitazone, broadened and shifted to a higher wave number 1696.15/cm for pioglitazone-CD complexes. The IR spectra indicated the presence of a host-guest interaction and the formation of stable hydrogen bonds between pioglitazone HCl and β -CD; this was because spectral changes always involved the C-OH, CH₂, and CH groups of the β -CD.^[18] From these studies it was confirmed that all the methods were effective for the formation of inclusion complexes.

Dissolution rate studies

The aqueous solubility of a drug-cyclodextrin complex can be dramatically different from that of the free drug. This ability of cyclodextrins to form inclusion complexes can occur both in a solution and in the solid state.^[5] The dissolution curves of the pure drug and the inclusion complexes prepared by different methods performed in 0.2 M HCl (pH 1.2) at 37 \pm 0.5 $^{\circ}$ C are shown in Figure 3. The reported values were obtained by calculating the arithmetic mean of three measurements. Figure 3 shows the dissolution profiles of pure pioglitazone HCl, PM, KM, and Coppt. Although the pure drug has poor aqueous solubility, all other powders show improvement in drug solubility. The extent of this enhancement in the solubility varies with the method of complexation. The co-precipitation method gave a better dissolution efficiency than the kneading method.

Figure 3 shows the inclusion complex formed by the co-precipitation method, which shows a higher solubility than that prepared by the kneading method, physical preparation, and pure drug. The inclusion complex prepared by the co-precipitation method released about 96% of the drug in 10 minutes as compared to 90% of the drug in 10 minutes for the inclusion complex prepared by the KM method. The method of complexation of pioglitazone HCl in β -CD increased the dissolution rate of the drug in the following order: Coppt > KM > PM > Drug. The inclusion complex formed by the kneading method showed a drug release profile similar to that of the physical mixture.

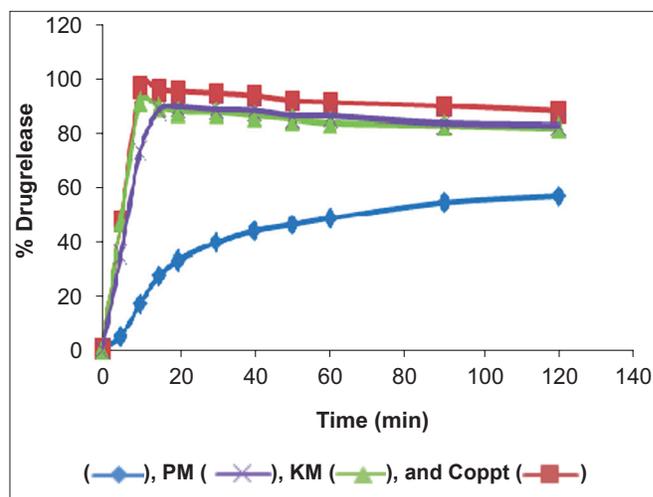


Figure 3 : The dissolution profiles of pure pioglitazone HCl

β -CD acts as a surfactant, by reducing the interfacial tension between the drug and dissolution medium, resulting in a higher dissolution rate.^[14] When molecules having appropriate energies collide with the correct orientation there are chances of inclusion complexes forming. In the case of physical mixtures, the small rise in solubility when compared to pure pioglitazone HCl is due to the rapid formation of inclusion complexes in the dissolution medium or to the wetting effect of β -CD. The kneading method shows lower improvement in solubility as compared to the co-precipitation method, because the energy supplied to molecules during kneading may be insufficient to initiate the collision between molecules resulting in partial complexation. As in the case of the co-precipitation method, inclusion complex formation takes place at the molecular level, and the energy required for the collision of molecules of pioglitazone HCl and β -CD is supplied from heating and stirring. Sapkal *et al.*,^[14] have evaluated some methods for preparing glioclazide- β -Cyclodextrin inclusion complexes and they concluded that inclusion complexes formed by co-precipitation may have a low degree of crystallinity, which enhances dissolution owing to increased drug particle wettability and formation of a readily soluble inclusion complex in the dissolution medium.

CONCLUSION

The phase solubility study of pioglitazone HCl and β -CD indicated the formation of inclusion complexes of 1:1 stoichiometry. The decrease in Partition Coefficient value may result from an increase in the hydrophilicity of the drug. Characterization of binary systems by FT-IR confirmed inclusion complexation by physical mixing, the kneading method, and the co-precipitation method. The improved dissolution rate may be due to an increase in solubility, brought about by the co-precipitation method. Thus from the present invention it can be concluded that the co-precipitation method can be used to prepare pioglitazone HCl- β -CD to improve the dissolution profile of drug.

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REFERENCES

1. Rao VM, Nerurkar M, Pinnamaneni S, Rinaldi F, Raghavan K. Co-solubilization of poorly soluble drugs by micellization and complexation. *Int J Pharm* 2006;319:98-106.
2. Thorsteinn L, Marcus E, Mar M. Role of cyclodextrins in improving oral drug delivery. *Am J Drug Deliv* 2004;2:1-15.
3. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. *Adv Drug Deliv Rev* 2007;59:645-66.
4. Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: An updated review. *AAPS PharmSciTech* 2005;6:E329-57.
5. Bibby DC, Davies NM, Tucker IG. Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems. *Int J Pharm* 2000;197:1-11.
6. Cal K, Centkowska K. Use of cyclodextrins in topical formulations: Practical aspects. *Eur J Pharm Biopharm* 2008;68:467-78.
7. Braibanti A, Fisicaro E, Ghiozzi A, Compari C, Bovis G. Host-guest interactions between β -cyclodextrin and piroxicam. *React Funct Polym* 1998;36:251-5.
8. Rai AK, Rai DK. Spectroscopic studies of some antidiabetic drugs. *Spectrochim Acta A Mol Biomol Spectrosc* 2003;59:1673-80.
9. Khalil R, Elbary A, Kassem M, Samra M. Formulation and hypoglycemic activity of pioglitazone-cyclodextrin inclusion complexes. *Drug Discov Ther* 2008;2:94-107.
10. Waleczek KJ, Marques HM, Hempel B, Schmidt PC. Phase solubility studies of pure (-)- α -bisabolol and camomile essential oil with β -cyclodextrin. *Eur J Pharm Biopharm* 2003;55:247-51.
11. Bhosale A, Hardikar S, Jagtap R, Patil, Dhawale S. Formulation of β -cyclodextrin complexed controlled release matrix tablets of glipizide and its in-vitro evaluation. *Int J PharmTech Res* 2009;1:773-8.
12. Higuchi T, Connors K. Phase-solubility techniques. *Adv Anal Chem Instrum* 1965;4:117-212.
13. Vij M, Garse H, Dand N, Kadam V, Hirlekar R. Effect of preparation method on complexation of cefdinir with β -cyclodextrin. *J Incl Phenom Macrocycl Chem* 2009;11:1-9.
14. Sapkal N, Kilor V, Bhusari K, Daud A. Evaluation of some methods for preparing gliclazide- β -cyclodextrin inclusion complexes. *Trop J Pharm Res* 2007;6:833-40.
15. Karthik A, Subramanian G, Rao M, Bhat K, Musmade P, Surulivelrajan M, Karthikeyan K, Udupa N. Simultaneous determination of pioglitazone and glimepiride in bulk drug and pharmaceutical dosage form by RP-HPLC method. *Pak J Pharm Sci* 2008;21:4215.
16. Suresh S, Shivakumar H, Kumar G. Effect of β -cyclodextrin complexation on solubility and dissolution rate of carbamazepine from tablets. *Indian J Pharm Sci* 2006;68: 301-307.
17. Talegaonkar S, Khan A, Khar R, Ahmad F, Khan Z. Development and characterization of paracetamol complexes with hydroxypropyl- β -cyclodextrin. *Iranian J Pharm Res* 2007;6:95-9.
18. Jug M, Becirevic-Lacan M. Influence of hydroxypropyl- β -cyclodextrin complexation on piroxicam release from buccoadhesive tablets. *Eur J Pharm Sci* 2004;21:251-60.

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