

Original Article**Formulation and hypoglycemic activity of pioglitazone-cyclodextrin inclusion complexes**Ahmed Abd Elbary¹, Mahfouz A. Kassem², Mona M. Abou Samra², Rawia M. Khalil^{2,*}¹ Faculty of Pharmacy, Cairo University, Cairo, Egypt;² Department of Pharmaceutical Technology, National Research Centre, Dokki, Cairo, Egypt.

ABSTRACT: Pioglitazone is a thiazolidinedione derivative used for the treatment of type 2 diabetes. The drug's poor aqueous solubility and slow dissolution rate are the main causes of its limited therapeutic action in some cases. The aim of the present study is to formulate a more soluble product of pioglitazone at physiological pH. The potential interaction of pioglitazone with cyclodextrins and water-soluble polymers was investigated to enhance the drug's bioavailability and improve its efficacy. The interaction of pioglitazone with β -cyclodextrin, HP- β -cyclodextrin, and dimethyl- β -cyclodextrin was evaluated by spectrophotometric and solubility methods. Both methods revealed the formation of 1:1 inclusion complexes. The phase solubility diagram of pioglitazone-cyclodextrin systems with or without water-soluble polymers was classified as the AL type. The solubilization strength of cyclodextrins and the apparent stability constant of systems increased upon addition of water-soluble polymers. Inclusion complexes of pioglitazone in cyclodextrins with or without water-soluble polymers were prepared by the kneading method. Binary systems were characterized and confirmed by IR spectroscopy, X-ray diffractometry, and thermogravimetric analysis. The dissolution rates of pioglitazone, pioglitazone-cyclodextrin physical mixtures, pioglitazone-cyclodextrin complexes, and ternary systems containing water-soluble polymers were determined using a USP dissolution tester; results revealed enhanced dissolution properties of cyclodextrin complexes compared to drug and physical mixtures, and all of the ternary systems displayed higher dissolution efficiency than corresponding binary systems. The permeation of pioglitazone and pioglitazone-cyclodextrin complexes through a cellulose membrane with and without water-soluble polymers (PVP and HPMC) present increased and the release

pattern follows the kinetics of a Higuchi equation. Assessment of the hypoglycemic effect of the free drug and its cyclodextrin complexes in normal rats *via* measurement of blood glucose levels (BGL) after administration of a single oral dose revealed that the hypoglycemic effect of pioglitazone-cyclodextrin complexes was greater than that of the free drug and that a pioglitazone-DM β -cyclodextrin complex had the greatest effect. In conclusion, the physicochemical and biological properties of pioglitazone improved as a result of complexation with cyclodextrins, and the improvement of physicochemical properties was more prominent after water-soluble polymers were associated with pioglitazone-cyclodextrin systems.

Keywords: Cyclodextrins, Water-soluble polymers, Dissolution rate, Complexation, Solubility

1. Introduction

Type 2 diabetes is the most prevalent form of diabetes mellitus. Approximately 90% of diabetic cases are the non-insulin-dependent phenotype (1). Impaired insulin secretion and resistance to the action of insulin, rather than an absolute insulin deficiency, characterize patients with type 2 diabetes.

Thiazolidinediones (TZDs) are a new class of oral antidiabetic agents that act to improve insulin sensitivity and decrease the insulin resistance of peripheral tissues (2). Newer TZDs such as pioglitazone are now available and have been approved by health authorities worldwide for use in patients with inadequate glycemic control (3). Pioglitazone decreases insulin resistance in the periphery and in the liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Pioglitazone is a potent and highly selective agonist for peroxisome proliferator-activated receptor gamma (PPAR γ). PPAR receptors are found in tissues critical to insulin action such as adipose tissue, skeletal muscle, and the liver. Activation of PPAR γ nuclear receptors modulates the transcription

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of a number of insulin-responsive genes involved in the control of glucose and lipid metabolism (4,5).

Pioglitazone is a drug that is essentially water-insoluble. The poor aqueous solubility and slow dissolution rate of the drug may have a negative impact on its bioavailability and subtherapeutic plasma drug levels and may lead to therapeutic failure in some cases.

Drug-cyclodextrin complexation is an important approach for development of oral drug delivery systems. The addition of cyclodextrins increases the water solubility of several poorly water-soluble substances; in most cases this results in improving a drug's bioavailability and increasing its pharmacological effect, allowing a reduction in the dose of the drug administered and decrease in side effects (6). Cyclodextrins have been used to optimize the bioavailability of many oral anti-diabetic drugs (7,8).

Moreover, the complexation efficiency and solubilizing effect of cyclodextrins in aqueous solutions have been increased by adding water-soluble polymers (9). This strategy is a useful way to decrease the amount of cyclodextrin needed in oral dosage form and decrease cost and toxicity, thus increasing the pharmaceutical usefulness of cyclodextrins.

The main goal of this study was to improve the biological performance of pioglitazone by inclusion complexation of the drug in cyclodextrins.

2. Materials and Methods

2.1. Materials

Pioglitazone, Hera, Hyderabad, India was kindly donated by Medical Union Pharmaceutical, Abu-Sultan, Ismailia, Egypt. β -cyclodextrin (β -CD), Heptakis (2,6-Di-*o*-methyl)- β -cyclodextrin (DM- β -CD, MW 1331.4), hydroxypropyl- β -cyclodextrin (HP- β -CD, MW 1380), and hydroxypropyl methylcellulose were purchased from Sigma-Aldrich, St. Louis, USA. Polyvinylpyrrolidone (K-30) was purchased from Sisco Research Laboratories (Bombay, India). Methanol, sodium dihydrogen phosphate, citric acid, and hydrochloric acid were all analytical grade.

2.2. Elucidation of the stoichiometric ratio of Pioglitazone cyclodextrin complexes

Using spectrophotometric measurements, the stoichiometric ratio of pioglitazone-cyclodextrin complexes was determined using the continuous variation method (10).

2.3. Effect of cyclodextrins on the solubility of pioglitazone with and without water-soluble polymers

Phase solubility studies for both binary and ternary systems were carried out according to the method

of Higuchi and Connors (11). An excess amount of pioglitazone was added to different concentrations of cyclodextrins in distilled water with or without a fixed PVP concentration of 0.25% (w/v) or fixed HPMC concentration of 0.1% (w/v). All glass containers were sealed; for ternary systems, containers were heated in an autoclave at 120°C for 20 min. All suspensions obtained were shaken at $25 \pm 0.5^\circ\text{C}$ until reaching equilibrium (about 72 h). Filtration of all suspensions was carried out through Millipore filters (0.45 μm), and filtrates were spectrophotometrically analyzed for drug content at 266 nm.

2.4. Preparation of pioglitazone-cyclodextrin physical mixtures

In accordance with the stoichiometric ratio of the complexes (1:1), a homogenous blend of pioglitazone and cyclodextrin derivative was prepared by mixing using a porcelain mortar. The mixture was then sieved through a 250- μm sieve.

2.5. Preparation of pioglitazone-cyclodextrin complexes

Using the kneading method (12), complexes of pioglitazone in cyclodextrins were prepared; pioglitazone was added to cyclodextrin in a molar ratio equivalent to its corresponding stoichiometric ratio in the complex (1:1) and then kneaded thoroughly with a minimal amount of water to obtain a paste. The paste was then dried under vacuum at room temperature in the presence of phosphorus pentoxide as a drying agent. The dried mass was pulverized and sieved through a 250- μm sieve.

2.6. Preparation of pioglitazone-cyclodextrin-polymer complexes

Ternary systems consisting of pioglitazone, cyclodextrin, and a water-soluble polymer were prepared by the kneading method. Two water soluble polymers were used, namely PVP and HPMC in respective concentrations of 0.25% and 0.1% (w/v). Pioglitazone and cyclodextrins were used in a molar ratio of 1:1. The three components were kneaded thoroughly with a minimal amount of water. The paste formed was then dried under vacuum at room temperature in the presence of phosphorus pentoxide as a drying agent. The dried mass was pulverized and sieved through a 250- μm sieve.

2.7. Characterization of pioglitazone-cyclodextrin complexes

2.7.1. X-ray diffractometry

The x-ray diffractometer patterns of pioglitazone, physical mixtures, and complexes were obtained using

a Diano X-ray diffractometer equipped with CoK α . The tube operated at 45 kV, 9 mA.

2.7.2. Infrared spectroscopy

Infrared spectra of pioglitazone, cyclodextrin derivatives, physical mixtures, and complexes were determined as KBr discs using a Shimadzu 435 U-04 IR spectrophotometer.

2.7.3. Thermal measurement

Using a thermogravimetric technique (TGA), the stability and thermal behavior of pioglitazone, physical mixtures, and complexes were determined. A TGA scan was carried out using a computerized Perkin Elmer TGA series under a dynamic N₂ purging gas atmosphere at a constant rate of 50 mL/min and a heating rate of 5°C/min.

2.8. In vitro dissolution studies

Using the USP Dissolution Tester Apparatus 1 (rotating basket), the dissolution of pioglitazone was assessed; dissolution media were 900 mL of 0.1 N HCl (pH 1.2 simulating gastric pH), citrate, or phosphate buffers (pH 4.6 and 6.8 simulating duodenal and intestinal pH values) at a rotation rate of 50 rpm. Powder samples containing 30 mg of pioglitazone or an equivalent amount of complex or physical mixtures with cyclodextrins in were prepared using transparent hard gelatin capsules (number 0). Aliquots of 5 mL each were withdrawn from the dissolution medium at intervals of 15, 30, 60, 90, and 120 min 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 content by measuring its absorbance at 266 nm using fresh dissolution medium as a blank. Experiments for each dissolution study were carried out in triplicate.

2.9. Effect of cyclodextrins on the permeation of pioglitazone through a cellulose membrane with and without water-soluble polymers present

The permeation of pioglitazone and its cyclodextrin complexes with or without water-soluble polymers through a semipermeable cellulose membrane (dialysis tubing, high retention seamless cellulose tubing, 12,000 daltons) was investigated. The cellulose membrane was placed in Franz-type diffusion cells; the surface area of membrane in the diffusion cells was 5 cm². The receptor phase consisted of 60 mL phosphate buffer solution (PBS) (pH 7.4) containing cyclodextrin (0.5%, w/v) as a solubilizing agent for permeated pioglitazone. The receptor phase was sonicated under vacuum prior to usage to remove dissolved air. The membrane of diffusion cells was stirred with a magnetic bar and kept at 37°C by circulating water through an external

jacket. The donor phase consisted of a 10 mL aqueous suspension or solution of 30 mg pioglitazone or its equivalent amount of pioglitazone-cyclodextrin complexes with or without water-soluble polymers. The aqueous solutions contained pioglitazone-cyclodextrin complexes and 0.25% (w/v) PVP or 0.1% (w/v) HPMC. Water-soluble polymers were heated in an autoclave (120°C for 20 min) before use. Samples of receptor fluid (2 mL) were withdrawn at various intervals up to 4 h and replaced with fresh buffer solution. The samples were analyzed spectrophotometrically at 266 nm.

2.10. Effect of complexation with cyclodextrins on the hypoglycemic efficacy of pioglitazone in normal rats

2.10.1. Animals

Male Albino rats (150-250 g) purchased from the animal house of the National Research Center were used in the experiment. They were kept for one week in the laboratory before the experiment for acclimatization to laboratory conditions and were given a standard diet and water. Prior to experimental treatments, animals fasted overnight but were allowed free access to water. Ten animals were used for each study group.

2.10.2. Determination of the blood glucose levels

Blood glucose concentrations (mg/100 mL) were determined using a glucometer (Dr-gluco) with electrochemical biosensors. Blood samples were collected from the orbital sinus of the animal.

2.10.3. Protocol

The fasting blood glucose level of each animal was determined at the beginning of the experiment, *i.e.* after overnight fasting with free access to water. Animals in the control group received 0.5% carboxy-methyl cellulose (CMC) only. The test groups of animals were treated with the test samples suspended in the same vehicle in a parallel group design. Intra-gastric tubing was used to administer a single dose of 10 mg/kg of the free drug or the equivalent amount of complexes. Blood samples were collected at 2, 4, 6, 8, 10, 24 h after the oral administration of test samples.

Hypoglycemic response was evaluated as a percentage decrease in blood glucose levels (BGL) calculated as follows:

$$\% \text{ decrease in BGL} = \frac{[\text{BGL at } t = 0] - [\text{BGL at } t]}{[\text{BGL at } t = 0]} \times 100$$

The pharmacodynamic parameters taken into consideration were the maximum percentage decrease in BGL, time of maximum percentage decrease in BGL (t_{max}), and area under the percentage decrease in BGL versus time curve (AUC_{0-24 h}), which was calculated using the trapezoidal rule (13). Other parameters were also considered when assessing the duration of drug action such as mean residence time

(MRT) and time of half-peak percentage decrease in BGL ($t_{1/2p}$).

Statistical analysis of the results was performed using one-way analysis of variance (ANOVA), followed by a least-significance test (LSD). This statistical analysis was computed with SPSS software.

3. Results and Discussion

3.1. Elucidation of the stoichiometric ratio of pioglitazone-cyclodextrin complexes

The absorbance values of solutions with a fixed total concentration (0.048 mM) and containing different mole fractions of pioglitazone and β -CD, HP- β -CD, and DM- β -CD were measured at 266 nm. Results revealed that the absorbance values of these solutions were not equivalent to the sum of the corresponding values of their components. This serves as evidence of complex formation between pioglitazone and these cyclodextrins. The calculated absorbance differences were plotted against mole fractions (Figure 1). For constant total concentration of the two species of complex, the complex is in its greatest concentration at the point where the two species are combined at the ratio in which they occur in the complex. Figure 1 shows that maximum extent of sharp changes in absorbance occurs at a mole fraction of 0.5, indicating the formation of an equimolar complex (1:1) between pioglitazone and β -CD, HP- β -CD, and DM- β -CD.

3.2. Effect of cyclodextrins on the solubility of pioglitazone with and without water-soluble polymers present

The effect of cyclodextrins, namely, β -CD, HP- β -CD, and DM- β -CD, on the solubility of pioglitazone in distilled water at 25°C was investigated. Figures 2 and 3 reveal that the aqueous solubility of pioglitazone increased linearly as a function of increasing cyclodextrin concentration. The phase-solubility diagram was classified as an AL type, which indicates the formation of 1:1 pioglitazone-cyclodextrin complexes over the investigated range of cyclodextrin concentration. The slope values in all diagrams were less than one, suggesting the formation of 1:1 complexes in solution and allowing the calculation of apparent stability constants of the drug complexes. The apparent stability constants (K_c) of these complexes were calculated from the slopes and intercept of the straight lines of the phase-solubility diagram according to the following equation (11):

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

where S_0 is the solubility of pioglitazone in water at 25°C ($2 \text{ M} \times 10^{-4}$) with no cyclodextrins present.

The stability constant values for the investigated cyclodextrin complexes were computed and found to be 464.92 M^{-1} , 381.44 M^{-1} , and 567.00 M^{-1} for β -CD, HP- β -CD, and DM- β -CD, respectively. Equimolar complexes with DM- β -CD were found to be the most stable, followed by β -CD and HP- β -CD. The lower value for the complex stability constant observed for HP- β -CD than for β -CD suggested that the presence of a hydroxypropyl substituent could interfere with the inclusion of the drug into the cyclodextrin cavity

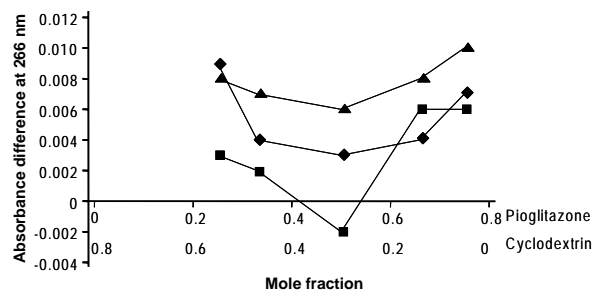


Figure 1. Elucidation of the stoichiometric ratio of pioglitazone-cyclodextrin complexes spectrophotometrically: Pioglitazone- β -CD complex (\blacklozenge); Pioglitazone-HP- β -CD complex (\blacksquare); Pioglitazone-DM- β -CD complex (\blacktriangle).

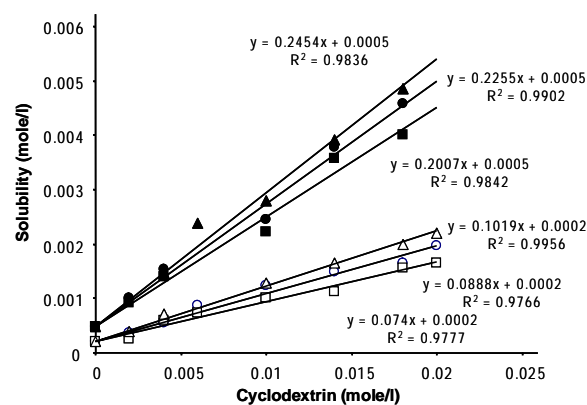


Figure 2. Effect of cyclodextrins on the solubility of pioglitazone in absence or presence of (HPMC): Pioglitazone- β -CD system (\circ); Pioglitazone-HP- β -CD system (\square); Pioglitazone-DM- β -CD system (Δ); pioglitazone- β -CD-HPMC system (\bullet); Pioglitazone-HP- β -CD-HPMC system (\blacksquare); Pioglitazone-DM- β -CD-HPMC system (\blacktriangle).

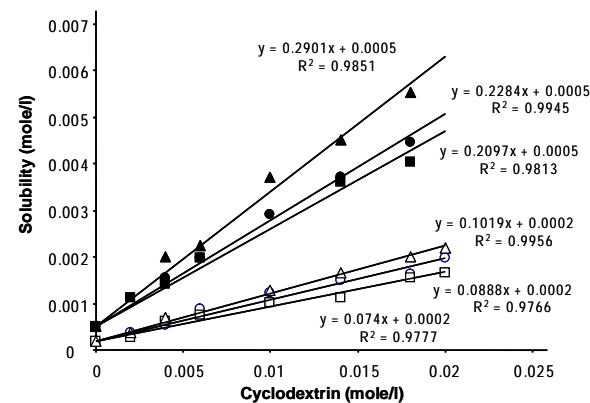


Figure 3. Effect of cyclodextrins on the solubility of pioglitazone in absence or presence of (PVP): pioglitazone- β -CD system (\circ); Pioglitazone-HP- β -CD system (\square); Pioglitazone-DM- β -CD system (Δ); pioglitazone- β -CD-PVP system (\bullet); Pioglitazone-HP- β -CD-PVP system (\blacksquare); Pioglitazone-DM- β -CD-PVP system (\blacktriangle).

because of the partial obstruction of its opening (14). An analogous phenomenon was previously observed for other drugs such as ibuprofen (15), ibuproxam (16), nicardipine hydrochloride (17) and glyburide (18). The increment of pioglitazone solubility seems to be related to the inclusion ability of the cyclodextrin molecules in water. The solubilization strength of the investigated cyclodextrins with respect to the drug, calculated as moles pioglitazone solubilized per mole of cyclodextrin, reveals that the solubilization strength of cyclodextrins decreases in the following order: DM- β -CD > β -CD > HP- β -CD.

Figures 2 and 3 reveal that the addition of water-soluble polymers to the cyclodextrin solution did not change the type of phase solubility diagram (AL type) obtained for binary systems. K_c values in the presence of water-soluble polymers were found to be 592.01, 530.68, and 819.49 M^{-1} for pioglitazone- β -CD, pioglitazone-HP- β -CD, and pioglitazone-DM- β -CD, respectively, in the presence of PVP; these values were 594.19, 512.44, and 663.68 M^{-1} for pioglitazone- β -CD, pioglitazone-HP- β -CD, and pioglitazone-DM- β -CD, respectively, in the presence of HPMC. These results are in agreement with the previous finding

obtained by other authors (19-21) who demonstrated that the addition of small amounts of water-soluble polymers has improved the complexing and solubilizing efficiency of cyclodextrins. The observed enhancement of K_c upon the addition of polymers shows that they are able to interact in a different way with drug-cyclodextrin binary complexes depending on their structures; this is because the polymers can interact differently with cyclodextrin and drug molecules such as hydrophobic bonds, Van der Waals dispersion forces, or hydrogen bonds (22).

3.3. Characterization of pioglitazone-cyclodextrin complexes

3.3.1. X-ray diffractometry (XRD)

Figure 4 shows the powder x-ray diffraction patterns of pioglitazone and its cyclodextrin complexes. The x-ray pattern of free pioglitazone revealed a drug fingerprint with intense and sharp peaks, indicating its crystalline nature.

The diffraction pattern for the complex differs significantly from that of each constituent and constitutes a new solid phase. Some peaks disappeared,

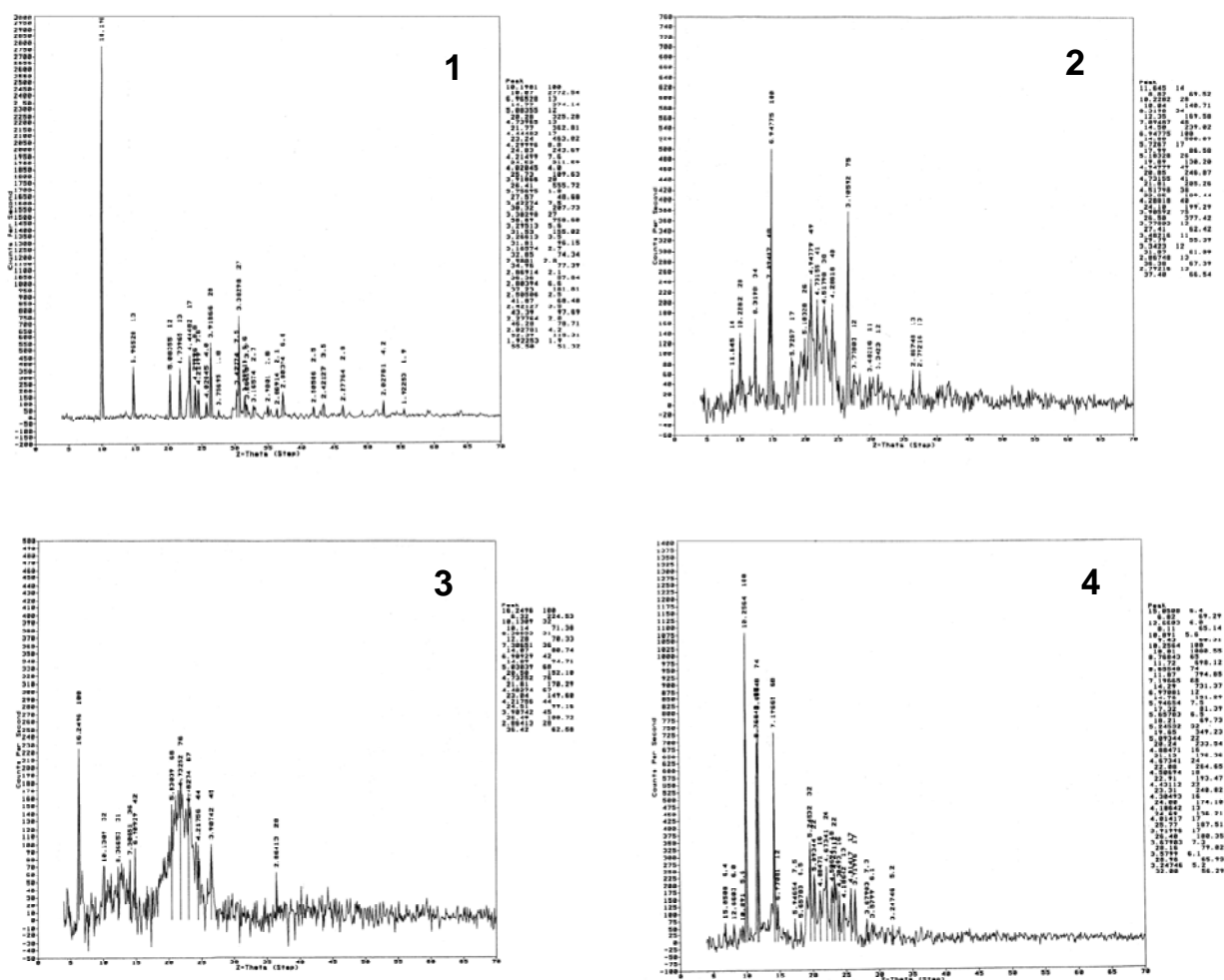


Figure 4. X-ray patterns of pioglitazone and its cyclodextrin complexes. 1, pioglitazone; 2, pioglitazone- β -CD complex; 3, pioglitazone-HP- β -CD complex; 4, pioglitazone-DM- β -CD complex.

some new peaks appeared, and the height of some peaks decreased. A reduction in crystallinity was observed in complexes with all investigated cyclodextrins. Crystallinity was determined by comparing the heights of several representative peaks in the diffraction patterns of the binary systems with those of a reference. The relationship used for calculation of crystallinity was the relative degree of crystallinity (RDC), as indicated by:

$$\text{RDC} = I_s/I_r$$

where I_s is the peak height of the sample under investigation and I_r is the peak height at the same angle for the reference with the highest intensity (23). Free drug peaks at 10.07, 24, and 26.4 (20) were used as a reference to calculate the RDC of kneaded and physical mixture binary systems (Table 1). A reduction in crystallinity was observed in the complexes; all investigated cyclodextrins had reduced diffraction peaks attributable to a new solid phase with low crystallinity, thus indicating inclusion complex formation with these cyclodextrins (more water-soluble) (24). The reduction in RDC values for pioglitazone-cyclodextrin complexes in comparison to the corresponding physical mixtures could be explained by the presence of reciprocal interaction in the solid state between host and guest, namely the formation of mixed particles during the drying process (25).

3.3.2. Infrared spectroscopy

The IR spectrum of pioglitazone (Figures 5-7) reveals the presence of a peak at 3083 cm^{-1} due to N-H stretching while peaks at 2928 and 2741 cm^{-1} corresponded to CH stretching. Strong absorption peaks observed at 1742 and 1685 cm^{-1} were assigned to drug carbonyl stretching vibration (C=O). A peak at 1608 cm^{-1} indicates the aromatic ring and a peak at 1242 cm^{-1} is due to C-O-Ar group. The rest of the fingerprint absorption bands appear at 1176.21, 1148.12, 1038.21, 930.61, 872.6, 850.03, 790.23, 711.8, 738.56, 659.21, 584.91, 602.22, and 519.59 cm^{-1} .

The IR spectrum of the investigated cyclodextrins is characterized by intense bands at $3300\text{-}3500\text{ cm}^{-1}$ due to OH stretching vibrations. The vibration at CH and CH_2 groups appears in the $2800\text{-}3000\text{ cm}^{-1}$ region. As can be seen in the spectral pattern of the physical mixture, it corresponds simply to superposition of the IR spectra of

the two components.

The IR spectra of pioglitazone-cyclodextrins complexes have considerable differences in comparison to those of their corresponding constituents. A decrease in frequency of a specific peak is generally seen on complexation, indicating an ordering of the molecule (26,27). In IR spectrum of pioglitazone-cyclodextrin complexes, the amide-NH stretching vibration at $3,083\text{ cm}^{-1}$ was not detected; a broad band that might be due to co-occurrence of the N-H band of the drug did

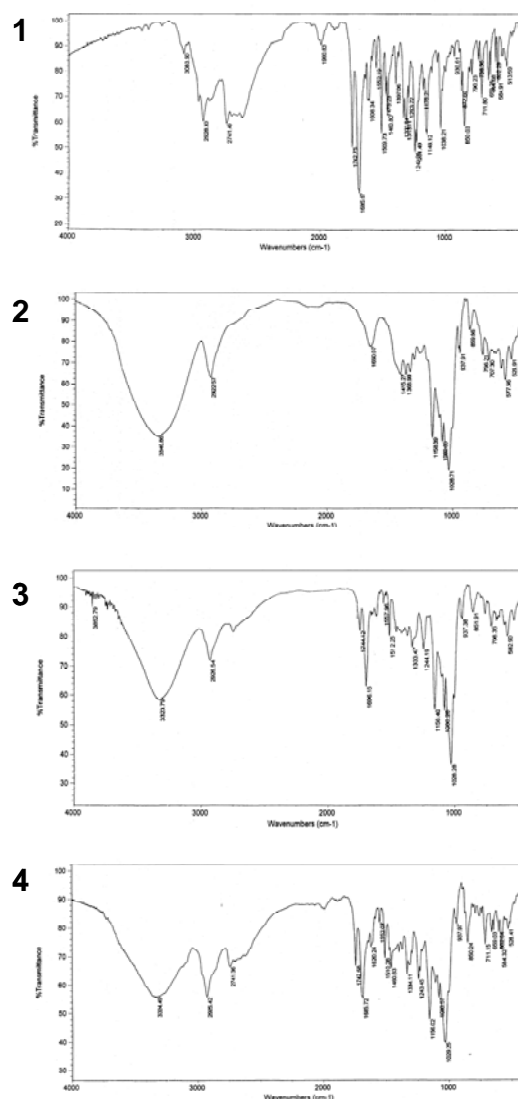


Figure 5. IR spectra of pioglitazone and pioglitazone- β -CD systems. 1, pioglitazone; 2, β -CD; 3, pioglitazone- β -CD complex; 4, pioglitazone- β -CD physical mixture.

Table 1. Relative degree of crystallinity (RDC) of pioglitazone-cyclodextrin complexes and pioglitazone-cyclodextrin physical mixtures

Pioglitazone-cyclodextrin systems	RDC at (2 θ)		
	10.07°	24°	26.4°
Piog- β -CD complex	0.05075	0.818	0.679
Piog-HP- β -CD complex	0.0257	0.407	0.181
Piog-DM- β -CD complex	0.3897	0.714	0.325
Piog- β -CD physical mixture	0.3548	1.310	1.507
Piog-HP- β -CD physical mixture	0.19279	0.607	0.424
Piog-DM- β -CD physical mixture	0.4614	0.805	0.973

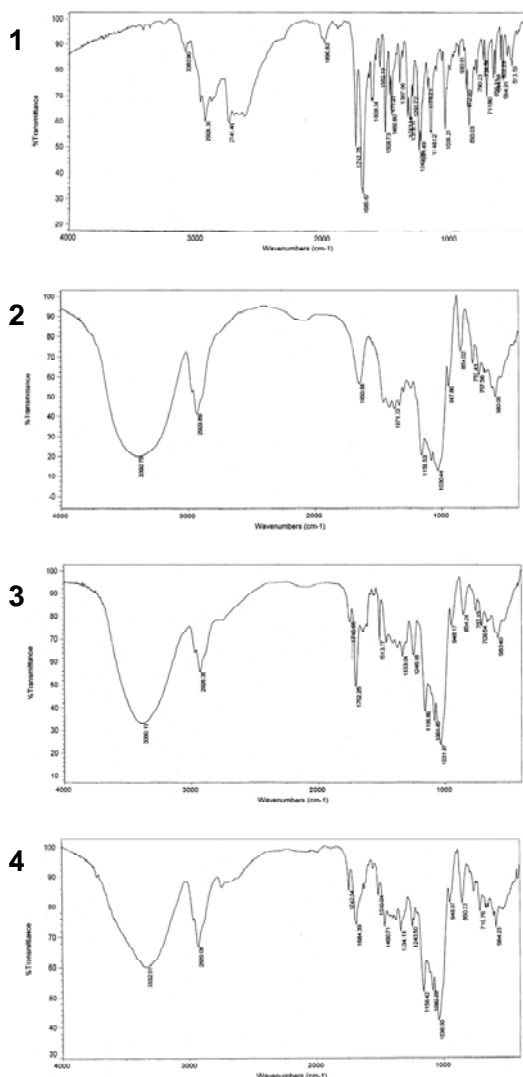


Figure 6. IR spectra of pioglitazone and pioglitazone-HP- β -CD systems. 1, pioglitazone; 2, HP- β -CD; 3, pioglitazone-HP- β -CD complex; 4, pioglitazone-HP- β -CD physical mixture.

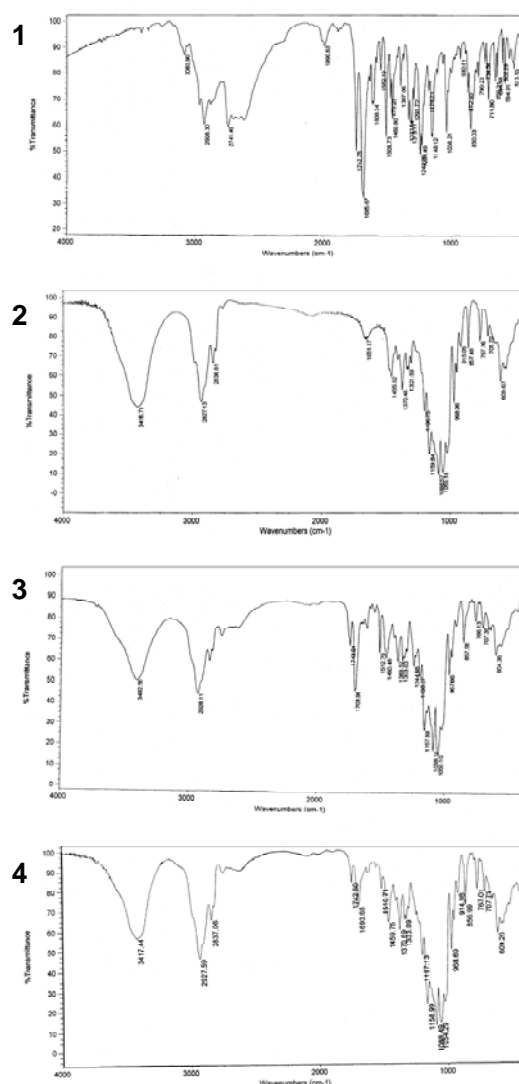


Figure 7. IR spectra of pioglitazone and pioglitazone-DM- β -CD systems. 1, pioglitazone; 2, DM- β -CD; 3, pioglitazone-DM- β -CD complex; 4, pioglitazone-DM- β -CD physical mixture.

appear with the OH intensified band of cyclodextrins at $3327\text{-}3425\text{ cm}^{-1}$. This indicates a strong interaction and complex formation of pioglitazone with cyclodextrins. The absorption band that appears at 1685.07 cm^{-1} due to carbonyl groups of pioglitazone broadened and shifted to a higher wave number for pioglitazone-CD complexes as follows: 1696.15 cm^{-1} for pioglitazone- β -CD, 1702.25 cm^{-1} for pioglitazone-HP- β -CD, and 1703.96 cm^{-1} for pioglitazone-DM- β -CD. The intensities of the bands appearing at 1724.75 , 1608.34 , 1509.73 , 1460.80 , and 1242 cm^{-1} were also affected by such an interaction.

All these modifications clearly indicate the presence of host-guest interaction and the formation of stable hydrogen bonds between pioglitazone and cyclodextrins; this is because spectral changes always involve the C-OH, CH_2 , and CH groups of the cyclodextrins (28,29).

3.3.3. Thermo gravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) was carried out

to investigate the thermal stability of pioglitazone-cyclodextrin complexes (Figures 8-10). As is clear from TGA thermo-grams of the cyclodextrins investigated, the weight change due to water loss was 13.294% for β -CD, 4.56% for HP- β -CD, and 0.503% for DM- β -CD and occurred up to a temperature of 130°C . This water content within the cavity of the cyclodextrins molecules stabilizes their ring structure (30).

Figures 8-10 show the TGA curves of free pioglitazone; in these curves a maximum rate of weight loss was observed at 308.96°C ; this indicated a thermal event due to melting and was associated with a 67.49% loss in weight. A second thermal event was observed at 504°C . For pioglitazone-cyclodextrins complexes, weight change due to water loss from the cyclodextrins cavities occurs up to 130°C . The complexation of pioglitazone with cyclodextrins was accompanied by weight loss, and the temperature of the maximum weight loss differed from that of the drug.

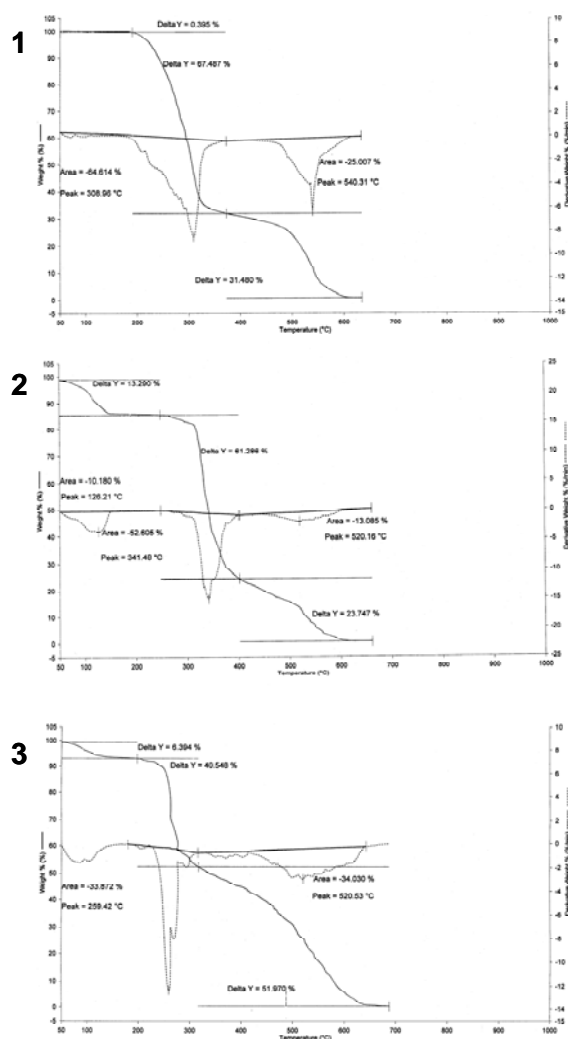


Figure 8. TGA thermograms of pioglitazone and pioglitazone- β -CD systems. 1, pioglitazone; 2, β -CD; 3, pioglitazone- β -CD complex.

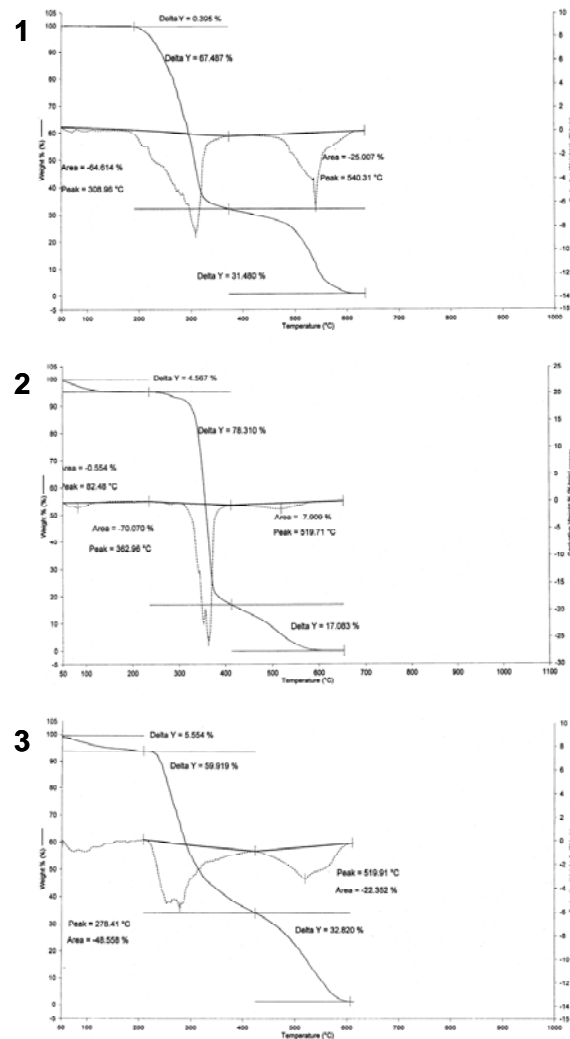


Figure 9. TGA thermograms of pioglitazone and pioglitazone-HP- β -CD systems. 1, pioglitazone; 2, HP- β -CD; 3, pioglitazone-HP- β -CD complex.

For a pioglitazone- β -CD complex, a maximum rate of weight loss occurred at 259.4°C (40.53% weight loss). Additional thermal events are observed within the temperature range of 278°C up to 300°C. For pioglitazone-HP- β -CD, a maximum rate of weight loss appeared at 278.41°C (weight loss: about 59.911%). For DM- β -CD, a maximum rate of weight loss appeared at 401.44°C (79.35% weight loss). This indicates that complexation induced changes in the thermal behavior of the drug.

Based on this information, pioglitazone decomposed at 308.96°C before it was included in DM- β -CD but an inclusion complex in DM- β -CD decomposed at 401.44°C. These findings show that the thermal stability of pioglitazone improved when it was included in DM- β -CD. The complexation of pioglitazone with cyclodextrins was accompanied by a change in the rate of decomposition of the drug, which clearly indicates the existence of new compounds in the solid state and points to inclusion of the drug in cyclodextrins.

3.4. Effect of cyclodextrins on the dissolution rate of pioglitazone with and without water-soluble polymers present

Figure 11 shows that the dissolution of pioglitazone in acidic medium (0.1 N HCl, pH 1.2) was incomplete even after 120 min. All the binary systems with cyclodextrins displayed better dissolution properties than pioglitazone alone, being immediately dispersed and completely dissolved within 15 min. As expected, Figures 12 and 13 demonstrate that all preparations had a lower percentage of drug dissolved at duodenal and intestinal pH values (citrate buffer, pH 4.6, and phosphate buffer, pH 6.8) in comparison to gastric fluid 0.1 N HCl (pH 1.2). The dissolution rate decreased with the increasing pH (4.6 to 6.8) of the dissolution medium. Evident from Figures 12 and 13 is the fact that the dissolution rate of the drug is enhanced in presence of cyclodextrins and that the dissolution rate of the prepared complexes is higher than that of the

corresponding physical mixtures. In all cases, DM- β -CD had a greater effect on enhancing the dissolution rate of the drug. The inclusion complexation of pioglitazone in cyclodextrins increased the dissolution rate of the drug in the following order: DM- β -CD > HP- β -CD > β -CD. This result coincides with the water solubility of these cyclodextrins (31).

The heightened effectiveness of DM- β -CD can be explained on the basis of its greater water solubility and greater ability to amorphize, wet, solubilize, and complex pioglitazone in a solid state. The increase in drug dissolution rate observed for physical mixtures may be mainly attributed to the hydrophilic effect of the carriers, which can reduce the interfacial tension between the poorly soluble drug and the dissolution medium, thus leading to a higher dissolution rate (32). As is well known, the mere presence of a hydrophilic carrier cannot improve the dissolution properties of the drug, also requiring the participation of several factors such as increase in particle surface, formation of a soluble complex, changes in crystallinity or

amorphization, and a significant dispersion degree in a hydrophilic carrier.

Thus, negligible improvement was registered for the physical mixtures, whereas the kneading method

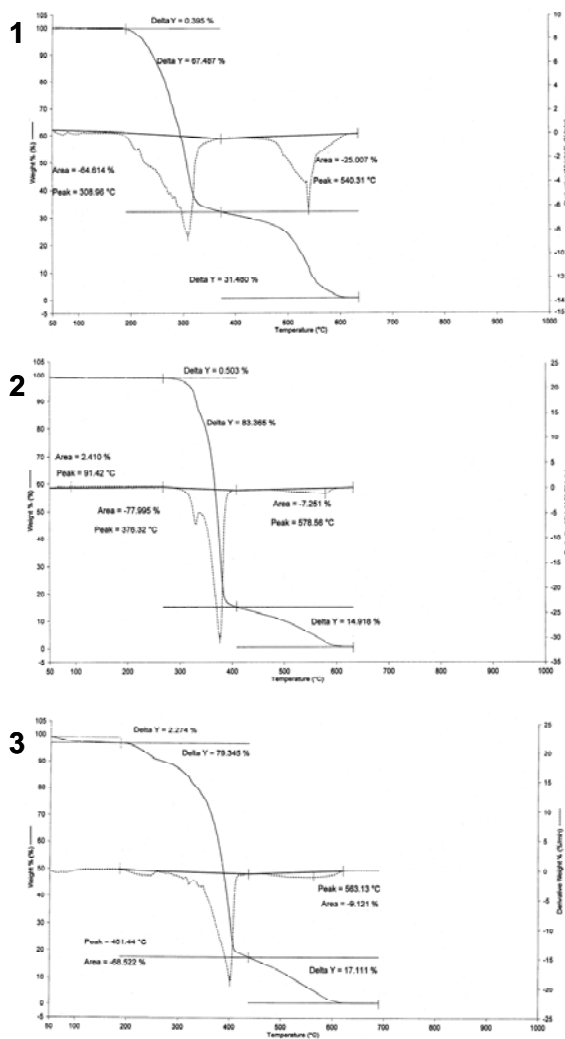


Figure 10. TGA thermograms of pioglitazone and pioglitazone-DM- β -CD systems. 1, pioglitazone; 2, DM- β -CD; 3, pioglitazone-DM- β -CD complex.

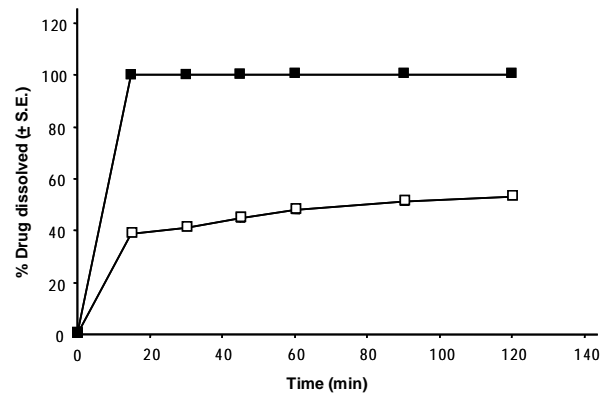


Figure 11. Effect of cyclodextrins on the dissolution rate of pioglitazone in pH 1.2: Free pioglitazone (□); Piog- β -CD complex (■); Piog- β -CD physical mixture (■); Piog-HP- β -CD complex (■); Piog-HP- β -CD physical mixture (■); Piog-DM- β -CD complex (■); Piog-DM- β -CD physical mixture (■).

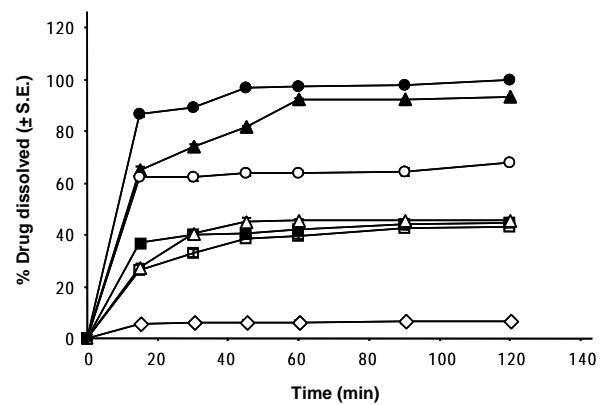


Figure 12. Effect of cyclodextrins on the dissolution rate of pioglitazone in pH 4.6: Free pioglitazone (◇); Piog- β -CD complex (■); Piog- β -CD physical mixture (□); Piog-HP- β -CD complex (▲); Piog-HP- β -CD physical mixture (△); Piog-DM- β -CD complex (●); Piog-DM- β -CD physical mixture (○).

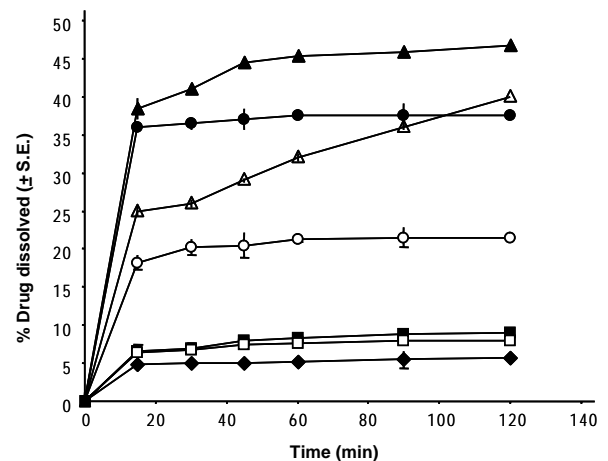


Figure 13. Effect of cyclodextrins on the dissolution rate of pioglitazone in pH 6.8: Free pioglitazone (◆); Piog- β -CD complex (■); Piog- β -CD physical mixture (□); Piog-HP- β -CD complex (●); Piog-HP- β -CD physical mixture (○); Piog-DM- β -CD complex (▲); Piog-DM- β -CD physical mixture (△).

enhanced the dissolution rate of the drug somewhat.

The dissolution profiles of pioglitazone and pioglitazone-cyclodextrin ternary systems containing water-soluble polymers, namely (0.25% PVP or 0.1% HPMC) at simulated intestinal pH (4.6 and 6.8), are shown in Figures 14-17. As is evident from the figures, the addition of hydrophilic polymers markedly enhanced the dissolution rate of pioglitazone compared to the binary system in all cases. DM- β -CD ternary systems had the greatest effect on enhancing the dissolution rate of the drug, followed by HP- β -CD ternary systems. The dissolution rate of the pioglitazone decreases with the increase in pH of the dissolution medium from 4.6 to 6.8. The order of hydrophilic polymers in terms of enhancing the dissolution rate of pioglitazone-cyclodextrin complexes was: PVP > HPMC. Thus, inclusion of hydrophilic polymers in the cyclodextrin complexes yielded rates of dissolution higher than those for pioglitazone or its complexes with cyclodextrins. This result is in agreement with the previous investigation performed by Chowdary and Srinivas (20), which demonstrated that inclusion of a

hydrophilic polymer in a celecoxib-HP- β -CD complex yielded a higher dissolution rate than that of celecoxib and its complexes with HP- β -CD. The increase in the dissolution rate of the drug in presence of these water-soluble polymers could be interpreted to mean that in ternary preparations the molecules of pioglitazone-cyclodextrin complex are supposed to be present in a more or less intimate dispersed state within the polymer matrix through interaction between the exterior of the complex and the polymer (19).

In the current study, the presence of hydrophilic polymers increased the dissolution efficiency of pioglitazone-cyclodextrin complexes. The differences in the dissolution efficiencies of ternary systems might be due to different complexation efficiency of the cyclodextrins in presence of these polymers. This may be due to different types of linkages established with the polymers and the drug (21).

3.5. In vitro permeation studies

3.5.1. Effect of cyclodextrins on the permeation of

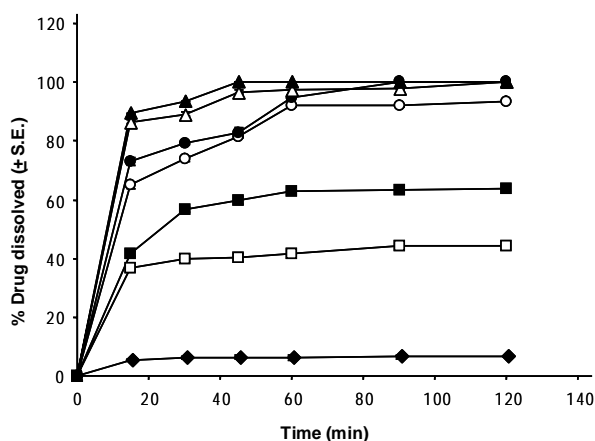


Figure 14. Effect of PVP on the dissolution rate of pioglitazone-cyclodextrin complexes in pH 4.6: Free pioglitazone (♦); Piog- β -CD PVP complex (■); Piog- β -CD complex (□); Piog-HP- β -CD PVP complex (●); Piog-HP- β -CD complex (○); Piog-DM- β -CD PVP complex (▲); Piog-DM- β -CD complex (Δ).

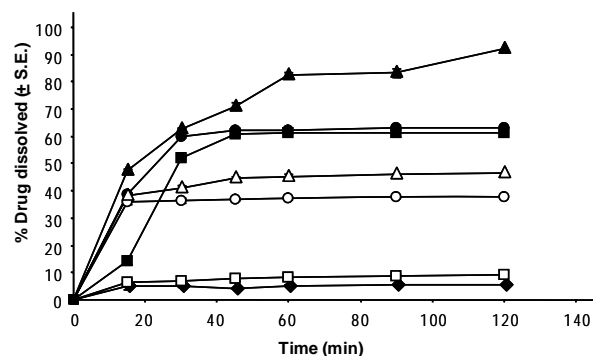


Figure 15. Effect of PVP on the dissolution rate of pioglitazone-cyclodextrin complexes in pH 6.8: Free pioglitazone (♦); Piog- β -CD PVP complex (■); Piog- β -CD complex (□); Piog-HP- β -CD PVP complex (●); Piog-HP- β -CD complex (○); Piog-DM- β -CD PVP complex (▲); Piog-DM- β -CD complex (Δ).

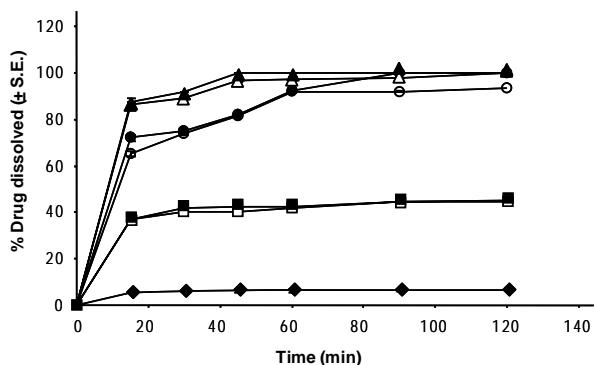


Figure 16. Effect of HPMC on the dissolution rate of pioglitazone-cyclodextrin complexes in pH 4.6: Free pioglitazone (♦); Piog- β -CD HPMC complex (■); Piog- β -CD complex (□); Piog-HP- β -CD HPMC complex (●); Piog-HP- β -CD complex (○); Piog-DM- β -CD HPMC complex (▲); Piog-DM- β -CD complex (Δ).

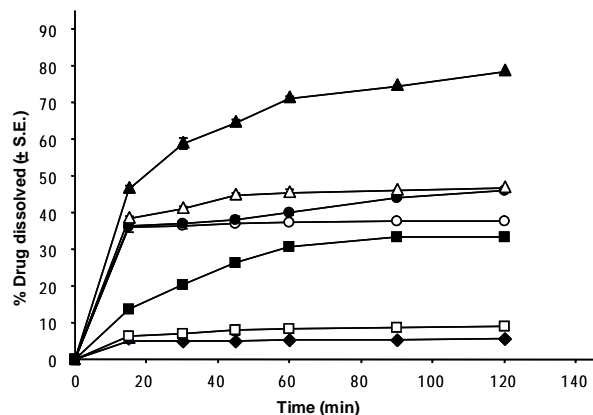


Figure 17. Effect of HPMC on the dissolution rate of pioglitazone-cyclodextrin complexes in pH 6.8: Free pioglitazone (♦); Piog- β -CD HPMC complex (■); Piog- β -CD complex (□); Piog-HP- β -CD HPMC complex (●); Piog-HP- β -CD complex (○); Piog-DM- β -CD HPMC complex (▲); Piog-DM- β -CD complex (Δ).

pioglitazone through a cellulose membrane with and without water-soluble polymers present

To evaluate the effect of complexation on the oral bioavailability of the included pioglitazone, an *in vitro* study was performed to measure the permeation through a cellulose membrane as a barrier model. In this study, permeation were performed using pioglitazone- β -CD, pioglitazone-HP- β -CD, and pioglitazone-DM- β -CD complexes in 1:1 molar ratios in comparison to uncomplexed pioglitazone. Table 2 shows that there was a significant increase in total pioglitazone that permeated through the membrane when the drug was complexed with cyclodextrins. This trend may due to rapid dissolution of the complex in comparison to pioglitazone alone, thus enhancing the availability of the drug in solution at the absorption site. The extent of enhancement of drug permeation was found to be dependent on the type of cyclodextrin; DM- β -CD had the highest rate of drug permeation. The enhancement of drug permeation was in the order of DM- β -CD > β -CD > HP- β -CD. This ranking was the same as that found for the effect of cyclodextrins on the solubility of pioglitazone. The data presented in Table 2 were mathematically processed using a Higuchi equation (33) and zero-order and first-order kinetics. Results indicated that the permeation pattern follows the kinetics of the Higuchi equation (33):

$$Q = RT_{1/2}$$

where Q is the amount of drug that permeated per unit area at time T and R is the rate of drug permeation; this is indicated by the very high correlation coefficient for most formulations. General opinion is that cyclodextrin molecules do not penetrate biological membranes but act as a carrier by keeping the hydrophobic drug in solution, delivering it to the surface of the biological membranes where it is inserted into the barrier (34,35). Cyclodextrins act as penetration enhancers by assuring constant high concentration of dissolved drug at the membrane surface (36).

The current results parallel those of Uekama *et al.* (37) which proved that the permeation of oral benzodiazepine through a cellulose membrane was significantly increased by complexation with γ -cyclodextrin.

Table 2 also shows that the presence of water-soluble polymers increased the amount of pioglitazone transferred across the cellulose membrane. The release pattern was found to follows the kinetics of a Higuchi equation (33). The permeation as measured by the flux improved significantly when pioglitazone was complexed with cyclodextrins, and this improvement was more prominent in the presence of water-soluble polymers. There appear to be few interpretations of this result other than that the free pioglitazone concentration in these systems was greater than that for pioglitazone solubility in a phosphate buffer solution at 37°C. In addition, preheating pioglitazone suspensions at 120°C should easily result in a higher free pioglitazone concentration as the aqueous solubility of organic compounds is generally temperature-dependent. As the temperature is allowed to drop back to 37°C, PVP, a well-known nucleation and crystal growth inhibitor, can sustain the supersaturated state for long periods of time. The current results are in agreement with the previous investigation performed by Shaker *et al.* (38) which demonstrated that the addition of PVP increased the flux of corticosterone from suspensions in the presence of HP- β -CD after autoclaving at 120°C. That work suggested that the increased flux is related to an increased corticosterone-CD complexation binding constant.

3.6. Effect of complexation with cyclodextrins on the hypoglycemic efficacy of pioglitazone for normal rats.

Figure 18 represents the mean % decrease in BGL after administration of free pioglitazone and pioglitazone-cyclodextrin complexes as a function of time. As is evident from the figure, there was a marked difference between the mean % decrease in BGL over time for

Table 2. *In vitro* permeation characteristics of pioglitazone and its cyclodextrin complexes in presence or absence of water soluble polymers through cellulose membrane (values \pm S.E.)

Time (min)	Percentage drug permeated from:									
	Free Piog.	Pioglitazone			PVP			HPMC		
		β -CD	HP- β -CD	DM- β -CD	Piog. β -CD	Piog.HP- β -CD	Piog.DM- β -CD	Piog- β -CD	Piog-HP- β -CD	Piog.DM- β -CD
15	2.23 \pm 0.63	9.35 \pm 0.13	9.80 \pm 1.17	9.20 \pm 1.54	11.11 \pm 1.46	9.30 \pm 0.69	9.20 \pm 0.93	11.00 \pm 0.79	10.61 \pm 0.73	8.56 \pm 0.33
30	2.53 \pm 0.50	11.40 \pm 0.25	10.53 \pm 0.87	10.45 \pm 1.25	11.78 \pm 1.56	10.00 \pm 0.70	10.45 \pm 0.61	11.83 \pm 1.07	10.76 \pm 0.72	9.72 \pm 0.12
60	3.30 \pm 0.75	12.51 \pm 0.22	11.00 \pm 0.83	11.50 \pm 1.31	12.79 \pm 2.07	10.50 \pm 0.46	12.96 \pm 1.66	13.32 \pm 1.82	12.06 \pm 1.10	12.16 \pm 0.79
90	3.57 \pm 0.37	13.48 \pm 0.25	11.64 \pm 1.09	12.65 \pm 0.55	13.95 \pm 2.22	12.00 \pm 1.57	13.98 \pm 2.03	13.47 \pm 1.20	13.16 \pm 1.06	14.64 \pm 1.18
120	3.66 \pm 0.14	13.85 \pm 0.29	12.00 \pm 0.98	13.43 \pm 0.02	14.22 \pm 1.82	13.00 \pm 0.4	14.81 \pm 2.38	14.90 \pm 1.60	13.36 \pm 0.82	14.27 \pm 0.84
150	3.90 \pm 0.54	14.48 \pm 0.43	12.80 \pm 1.04	14.50 \pm 0.72	15.70 \pm 1.86	13.70 \pm 1.34	15.31 \pm 1.94	15.99 \pm 1.50	14.00 \pm 1.14	15.10 \pm 0.28
180	4.10 \pm 0.94	14.74 \pm 0.49	13.00 \pm 1.11	15.44 \pm 1.08	16.70 \pm 1.13	14.20 \pm 1.72	17.01 \pm 1.66	16.08 \pm 1.40	15.00 \pm 2.03	16.50 \pm 0.36
210	4.50 \pm 0.64	15.60 \pm 0.09	13.66 \pm 1.33	16.50 \pm 0.57	16.99 \pm 1.06	14.30 \pm 2.08	18.69 \pm 1.34	16.70 \pm 1.48	15.16 \pm 1.54	17.06 \pm 0.59
240	4.90 \pm 0.54	16.05 \pm 0.21	14.02 \pm 1.14	17.46 \pm 0.66	17.01 \pm 0.42	14.70 \pm 1.57	19.18 \pm 1.42	16.82 \pm 0.52	15.66 \pm 0.54	17.48 \pm 0.78
R*	0.012	0.030	0.021	0.041	0.033	0.029	0.050	0.031	0.027	0.047

R*(mg \cdot cm² \cdot min^{1/2}) = rate of drug released

pioglitazone and pioglitazone-cyclodextrin complexes as well as for the control. The maximum decrease in BGL for all the investigated systems appears to occur 6 h post-dosing. The effect on BGL fades in about 24 h, while the effect was maintained to different degrees at this time point for the complexes. Figure 18 shows that pioglitazone-cyclodextrin complexes have a greater effect on decreasing BGL in comparison to uncomplexed pioglitazone. The greatest peak increase occurs after administration of a pioglitazone-DM- β -CD complex.

Table 3 shows the effect of pioglitazone-cyclodextrin complexes on the maximum percentage decrease in BGL compared to the control and free pioglitazone. The maximum % decrease in BGL is the least for the control, followed by the free drug. The pioglitazone-cyclodextrin complexes behave differently, not only from the control but also from the free drug. Pioglitazone-DM- β -CD complex displayed the highest maximum decrease in BGL. The difference in the maximum percentage decrease in BGL between the pioglitazone-DM- β -CD complex and all investigated systems is very highly statistically significant ($p < 0.001$). These results point to a statistically significant increase in the intensity of action of pioglitazone when present in the form of a pioglitazone-DM- β -CD complex.

Table 3 shows that t_{max} is reached for the free drug earlier than for the pioglitazone-cyclodextrin complexes. Pioglitazone-DM- β -CD complex had the greatest delay in maximum response.

Statistical analysis of the data on the time of maximum percentage decrease in BGL indicated that the differences in t_{max} for most of the investigated systems are insignificant ($p > 0.05$). The difference between the free drug and pioglitazone-DM- β -CD

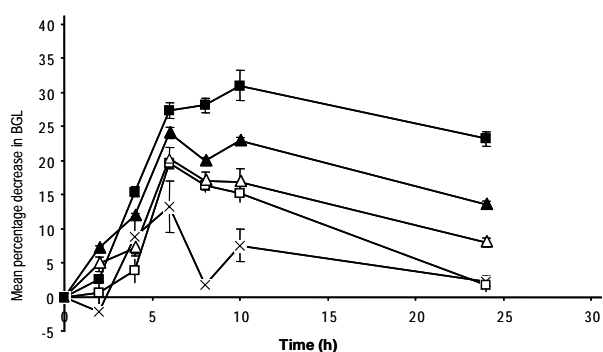


Figure 18. Mean percentage decrease in blood glucose levels of normal rats after administration of free pioglitazone and its complexes: Control (x); Free pioglitazone (\square); Piog-HP- β -CD complex (Δ); Piog- β -CD complex (\blacktriangle); Piog-DM- β -CD complex (\blacksquare).

is statistically significant ($p < 0.05$). This indicates that the time of the maximum effect of drug action is delayed for a pioglitazone-DM- β -CD complex.

The AUC_{0-24h} values for all the investigated systems are higher than for the control and the free drug and are highest for a pioglitazone-DM- β -CD complex. The differences in AUC_{0-24h} values between the different test groups are in most cases very highly significant ($p < 0.001$). This indicates a pronounced and statistically significant augmentation of the bioavailability of pioglitazone when prepared in the form of pioglitazone-cyclodextrin complexes and especially when prepared as a pioglitazone-DM- β -CD complex.

The relative bioavailability was calculated according to the following equation:

$$\text{Relative bioavailability} = \frac{\text{Mean } AUC_{0-24h} (\text{Complex})}{\text{Mean } AUC_{0-24h} (\text{Drug})} \times 100$$

The values indicate an increase in relative bioavailability of the drug *via* complexation. The increased relative bioavailability was more prominent for pioglitazone-DM- β -CD complex, with the mean value reaching 261% in comparison to 190% and 135% for pioglitazone- β -CD complex and pioglitazone-HP- β -CD complex, respectively. These results indicated that pioglitazone was not bioequivalent to any of its complexes according to FDA standards and that a dose correction was required for the complexes.

Table 3 shows the values of $t_{1/2p}$ for the prepared pioglitazone-cyclodextrin complexes was higher than those for the free drug and the control. Pioglitazone-DM- β -CD complex had the highest $t_{1/2p}$ value. Statistical analysis of the data on $t_{1/2p}$ showed that the difference between the free drug and pioglitazone-HP- β -CD is not significant ($p > 0.05$). At the same time, the differences between the free pioglitazone and pioglitazone- β -CD and pioglitazone-DM- β -CD are very highly significant ($p < 0.001$). These results point to the longer duration of drug action when formulated as pioglitazone- β -CD or pioglitazone-DM- β -CD complexes.

Quotient R was calculated for the investigated groups according to the following equation:

$$R\Delta (39) = \frac{\text{Mean } t_{1/2p} (\text{complex})}{\text{Mean } t_{1/2p} (\text{drug})}$$

The values indicated an increase in the duration of the drug action *via* complexation as revealed by the values of R Δ being higher than unity. The increase in duration of drug action is more prominent for pioglitazone-DM-

Table 3. Pharmacodynamic parameters for free pioglitazone and pioglitazone-cyclodextrin complexes (value \pm S.E.)

Formulations	Max % decrease in BGL	T_{max} (h)	AUC_{0-24h}	$t_{1/2h}$	MRT (h)
Control	17.716 \pm 2.52	6.333 \pm 0.62	145.983 \pm 29.90	7.133 \pm 1.18	8.602 \pm 0.73
Free pioglitazone	19.788 \pm 0.71	6.800 \pm 0.44	213.119 \pm 12.14	9.040 \pm 0.75	8.587 \pm 0.38
Pioglitazone- β -CD complex	24.607 \pm 0.56	8.000 \pm 0.66	404.781 \pm 5.43	20.640 \pm 1.37	9.567 \pm 0.04
Pioglitazone-HP- β -CD complex	23.391 \pm 1.25	7.200 \pm 0.53	289.242 \pm 18.72	10.640 \pm 1.37	9.186 \pm 0.26
Pioglitazone-DM- β -CD complex	33.099 \pm 1.84	8.600 \pm 0.60	558.021 \pm 24.73	29.600 \pm 1.80	10.127 \pm 0.11

β -CD complex where the mean value is equal to 3.27 in comparison to 2.28 for pioglitazone- β -CD complex and 1.17 for pioglitazone-HP- β -CD complex.

Table 3 shows that the greatest augmentation in MRT was observed for pioglitazone-cyclodextrin complexes, and pioglitazone-DM- β -CD displayed the highest MRT value, followed by pioglitazone- β -CD complex. Statistical analysis of the data showed that the difference between the free drug and pioglitazone-DM- β -CD is highly significant ($p < 0.01$) while other differences are insignificant ($p > 0.05$). These results point to the augmentation of the duration of drug action for a pioglitazone-DM- β -CD complex.

In conclusion, the bioavailability, intensity, and duration of drug action increased when pioglitazone was prepared in the form of pioglitazone-cyclodextrin complexes, and the greatest increase took place with a pioglitazone-DM- β -CD complex.

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