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Formulation and biological evaluation of glimepiride–cyclodextrin–polymer systems

H.O. Ammar [a,*], H.A. Salama [a], M. Ghorab [b], A.A. Mahmoud [a]

[a] *Department of Pharmaceutical Technology, National Research Center, Dokki, Cairo, Egypt*
[b] *Faculty of Pharmacy, Cairo University, Cairo, Egypt*

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Abstract

Glimepiride is one of the third generation sulfonylureas used for treatment of type 2 diabetes. Poor aqueous solubility and slow dissolution rate of the drug lead to irreproducible clinical response or therapeutic failure in some cases due to subtherapeutic plasma drug levels. Consequently, the rationale of this study was to improve the biological performance of this drug through enhancing its solubility and dissolution rate. Inclusion complexes of glimepiride in β-cyclodextrin (β-CyD), hydroxypropyl-β-cyclodextrin (HP-β-CyD) and sulfobutylether-β-cyclodextrin (SBE-β-CyD), with or without water soluble polymers were prepared by the kneading method. Binary systems were characterized by thermogravimetric analysis, IR spectroscopy and X-ray diffractometry. Phase solubility diagrams revealed increase in solubility of the drug upon cyclodextrin addition, showing A_p type plot indicating high order complexation. All the ternary systems containing β-CyD or HP-β-CyD showed higher dissolution efficiency compared to the corresponding binary systems. The hypoglycemic effect of the most rapidly dissolving ternary system of glimepiride–HP-β-CyD–PEG 4000 was evaluated after oral administration in diabetic rats by measuring blood glucose levels. The results indicated that this ternary system improves significantly the therapeutic efficacy of the drug. In conclusion, the association of water soluble polymers with glimepiride–CyD systems leads to great enhancement in dissolution rate, increased duration of action and improvement of therapeutic efficacy of the drug.

Keywords: Glimepiride; Cyclodextrins; Water soluble polymers; Complexation

1. Introduction

Glimepiride is one of the third generation sulphonylurea drugs (Massimo, 2003; Kouichi et al., 2005) useful for control of diabetes mellitus, type 2. Preclinical investigations of glimepiride suggested a number of potential benefits over sulfonylureas currently available including lower dosage, rapid onset, longer duration of action and lower insulin C-peptide levels, possibly due to less stimulation of insulin secretion and more pronounced extrapancreatic effects (Geinsen, 1988; Muller et al., 1994).

Glimepiride is practically insoluble in water; this poor aqueous solubility and slow dissolution may lead to irreproducible clinical response or therapeutic failure in some cases due to subtherapeutic plasma drug levels (Thummel, 1997; Frick et al., 1998; Massimo, 2003).

From an economic point of view, low oral bioavailability results in wasting of a large portion of an oral dose and adds to the cost of drug therapy, especially when the drug is an expensive one (Aungst, 1993). The approach of complexation has been frequently used to increase the aqueous solubility and dissolution rate of water insoluble and slightly soluble drugs in an effort to increase oral bioavailability. However, in certain instances, this approach can be used to increase drug stability, particularly esters, control drug release rate, improve organoleptic properties and maximize the gastrointestinal tolerance by reducing drug irritation after oral administration. Generally speaking, cyclodextrins are potential carriers for achieving such objectives (Higuchi and Kristiansen, 1970).

For a variety of reasons including cost, production capability and toxicology, the amount of cyclodextrin incorporated into a drug formulation is limited. It is, therefore, important to develop methods, which can be applied in order to enhance the efficiency of drug–cyclodextrin complexation (Loftsson, 1998). The complexation efficiency and solubilising effect of cyclodextrins in

* Corresponding author. Tel.: +20 124472851.
E-mail address: husseinammar@hotmail.com (H.O. Ammar).

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aqueous solutions have been increased by addition of water soluble polymers (Loftsson et al., 1994, 1996; Fridriksdottir et al., 1997). This might be a useful strategy to decrease the amount of cyclodextrin needed in oral dosage forms and, therefore, to increase the pharmaceutical usefulness of cyclodextrins in solid oral dosage forms (Savolainen et al., 1998).

Consequently, the rationale of this study was to improve the therapeutic efficacy of glimepiride utilizing the approach of inclusion complexation of the drug in cyclodextrins in presence of water soluble polymers.

2. Materials and methods

2.1. Materials

Glimepiride was kindly provided by Delta Pharma. Co. (Tenth of Ramadan City, Egypt). β-Cyclodextrin, hydroxypropyl-β-cyclodextrin (MW 1380), streptozotocin and hyroxypropyl methylcellulose were purchased from Sigma Chemical Company (St. Louis, USA). Sulfobutylether-β-cyclodextrin sodium salt (MW 2160) was kindly provided by Cydex L.C. (Overland Park, KS, USA). Polyethylene glycol 4000 and polyvinylpyrrolidone (K-30) were purchased from Sisco Research Laboratories Pvt. Ltd. (Bombay, India). Polyethylene glycol 6000 was purchased from Fluka (Germany). Sodium dihydrogen phosphate was supplied from s. d. fine-chem. Ltd. (Mumbai, India) and disodium hydrogen phosphate was purchased from BDH Laboratory Supplies (Poole England).

2.2. Methods

2.2.1. Elucidation of the stoichiometric ratio of glimepiride–cyclodextrin complexes

The continuous variation method (Martin et al., 1993) was utilized to determine the stoichiometric ratio of glimepiride–cyclodextrin complexes by spectrophotometric measurements.

2.2.2. Preparation of glimepiride–cyclodextrin complexes

Inclusion complexes of glimepiride in cyclodextrins under investigation were prepared by the kneading method (Uekema et al., 1988), whereby glimepiride was added to cyclodextrin in a molar ratio equivalent to its corresponding stoichiometric ratio in the complex, kneaded thoroughly with least amount of water to obtain a paste which was then dried under vacuum at room temperature in presence of phosphorus pentoxide as a drying agent.

2.2.3. Characterization of glimepiride–cyclodextrin complexes

2.2.3.1. Infrared spectroscopy. I.R. spectra of glimepiride–cyclodextrin physical mixtures and complexes were monitored as KBr disc using a Shimadzu 435 U-O4 IR spectrophotometer.

2.2.3.2. Thermal measurements. The stability and thermal behaviour of glimepiride and its physical mixtures and complexes with cyclodextrins were traced by thermogravimetric (TGA) technique. The TGA scan was carried out using a computerized Perkin-Elmer TGA series under a dynamic N_2 purging gas atmosphere at a constant rate of 50 cc/min and a heating rate of 5 °C/min.

2.2.3.3. X-ray diffractometry. X-ray diffraction patterns were obtained by using a Diano X-ray diffractometer equipped with Co Kα. The tube operated at 45 kV, 9 mA.

2.2.4. Effect of cyclodextrins on the solubility of glimepiride

Solubility measurements were carried out according to the method of Higuchi and Connors (1965). An excess of glimepiride was added to phosphate buffer solutions (pH 6.8) containing different concentrations of cyclodextrins. The suspensions were shaken at 25 °C for 72 h and then filtered through a Millipore filter (0.45 μ). An aliquot portion of the filtrate was analyzed for its drug content by measuring its extinction at 226 nm against blank solution containing the same concentration of cyclodextrin.

2.2.5. Preparation of glimepiride–cyclodextrin–polymer systems

Ternary systems consisting of glimepiride, cyclodextrin and a water soluble polymer were prepared by the kneading method. Four water soluble polymers, viz., HPMC, PVP, PEG 4000 and PEG 6000, in a concentration of 5% (w/w), were investigated. The three components were kneaded thoroughly with least amount of water; the paste formed was then dried under vacuum at room temperature in presence of phosphorus pentoxide as a drying agent.

2.2.6. In vitro dissolution studies

Dissolution of glimepiride was assessed at 37 °C by the USP Dissolution Tester, Apparatus I (Rotating basket), using 900 ml of phosphate buffer (pH 6.8) as the dissolution medium and at a rotation rate of 75 rpm (Frick et al., 1998). Aliquots, each of 5 ml, from the dissolution medium were withdrawn at time intervals of 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min and replenished by an equal volume of fresh dissolution medium. The samples were withdrawn through sintered glass filter and analyzed for glimepiride content by measuring its absorbance at 226 nm using phosphate buffer (pH 6.8) as a blank. Replicate batches of each binary and ternary complex were used for dissolution studies.

2.2.7. Assessment of therapeutic efficacy

These studies were performed on free drug, the suggested formula selected on the basis of the dissolution studies data (glimepiride–HP-β-CyD–5% PEG 4000) and the innovated drug product (Amaryl® 3 mg). This study was of a single dose and parallel group design using diabetic rats. Male Wistar rats weighing 180–240 g were kept on standard diet, fasted for 24 h and then intraperitoneally injected with 50 mg/kg streptozotocin (Stepensky et al., 2001; Pepato et al., 2002) 10–14 days prior to the study for inducing diabetes. These rats were divided into three groups, each of 12 rats. Fasting blood glucose level was assessed using FastTake glucometer (SmartScan®) (Gabra and Sirois, 2005). FastTake glucometer provides rapid, accurate

and reproducible results in both laboratory and clinical settings (Albertson et al., 1998).

The blood glucose level (BGL) after administration, by intragastric tubing, of a single dose of 10 mg/kg (Ladriere et al., 1997) of the drug or its equivalent amount of the drug–cyclodextrin–polymer system or innovated drug product was measured at different time intervals, up to 24 h. Blood was withdrawn from the orbital sinus of the animal (Varma and Panchagnula, 2005; Zhang et al., 2000). Each animal served as its own control and hence, the hypoglycemic response was evaluated as percentage decrease in blood glucose level 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 maximum percentage decrease in blood glucose level, time for maximum response (t_{max}), time at which half peak percentage decrease in BGL prevails ($t_{1/2\,p}$) and area under percentage decrease in BGL versus time curve ($AUC_{0-24\,h}$) which was calculated adopting the trapezoidal rule (Wagner, 1975).

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

3. Results and discussion

3.1. Elucidation of the stoichiometric ratio of glimepiride–cyclodextrin complexes

The absorbance values of fixed total concentration (0.072 mmol/l) of glimepiride and each of β-CyD, HP-β-CyD and SBE-β-CyD were measured at 226 nm. It was found that the absorbance values of these solutions were not the same as the sum of the corresponding values of their components. This can be considered as an evidence for complex formation between glimepiride and these cyclodextrins. The calculated absorbance difference was plotted against mole fraction (Figs. 1–3). For a constant total concentration of the two species of a complex, the complex is in its highest concentration at the point where the two species are combined in the ratio in which they occur in the complex. Figs. 1 and 2 show that abrupt changes in absorbance difference exist at 0.33 drug mole fraction indicating formation of complexes between glimepiride and each of β-CyD and HP-β-CyD in the ratio of 1:2. For SBE-β-CyD, the maximum absorbance difference was evident at 0.25 drug mole fraction indicating formation of 1:3 complex (Fig. 3).

3.2. Characterization of glimepiride–cyclodextrin complexes

Microcrystalline complexes of glimepiride with cyclodextrins were examined by infrared spectroscopy, thermal analysis (TGA) and X-ray diffractometry.

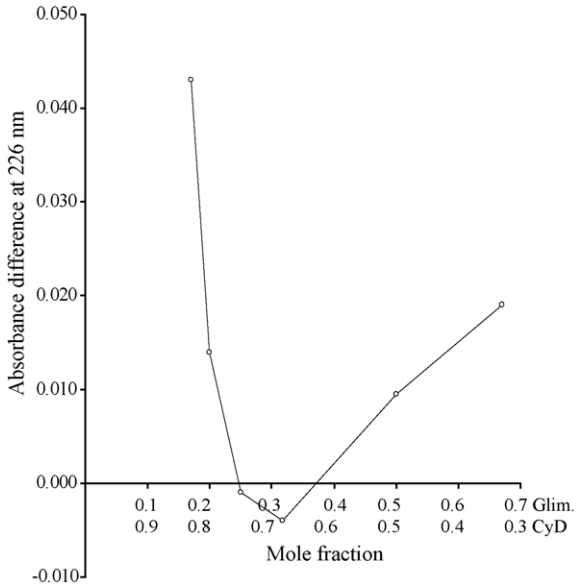

Fig. 1. Elucidation of the stoichiometric ratio of glimepiride–β-cyclodextrin complex spectrophotometrically.

3.2.1. Infrared spectroscopy

The IR spectrum of glimepiride (Figs. 4–6) reveals the presence of peaks at 3369 and 3288 cm^{-1} due to N–H stretch for urea, peaks at 1345 and 1153 cm^{-1} corresponding to the sulphonamide group and peaks at 1708 and 1674 cm^{-1} corresponding to the carbonyl group.

The IR spectrum of the investigated CyDs is characterized by intense bands at 3300–3500 cm^{-1} due to O–H stretching vibrations. The vibration of the –CH and CH_2 groups appears in the 2800–3000 cm^{-1} region.

The spectrum patterns of the physical mixtures correspond simply to superposition of the IR spectra of the two components.

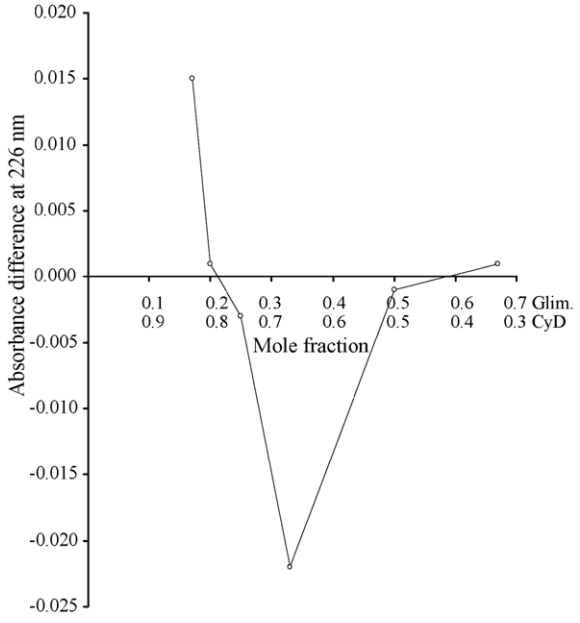

Fig. 2. Elucidation of the stoichiometric ratio of glimepiride–HP-β-cyclodextrin complex spectrophotometerically.

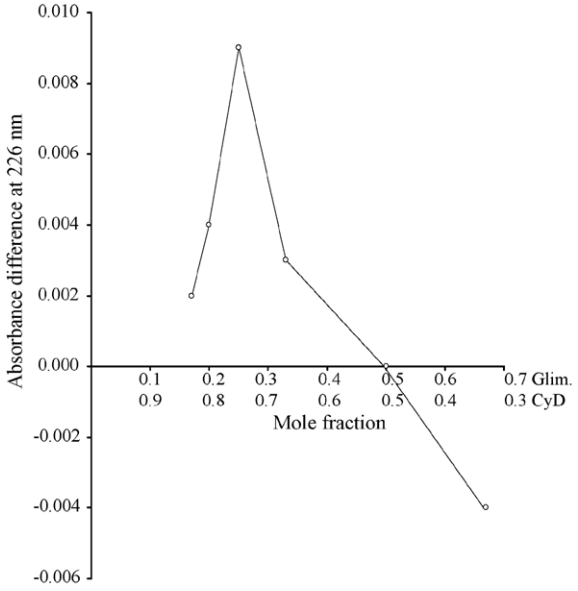

Fig. 3. Elucidation of the stoichiometric ratio of glimepiride–SBE-β-cyclodextrin complex spectrophotometerically.

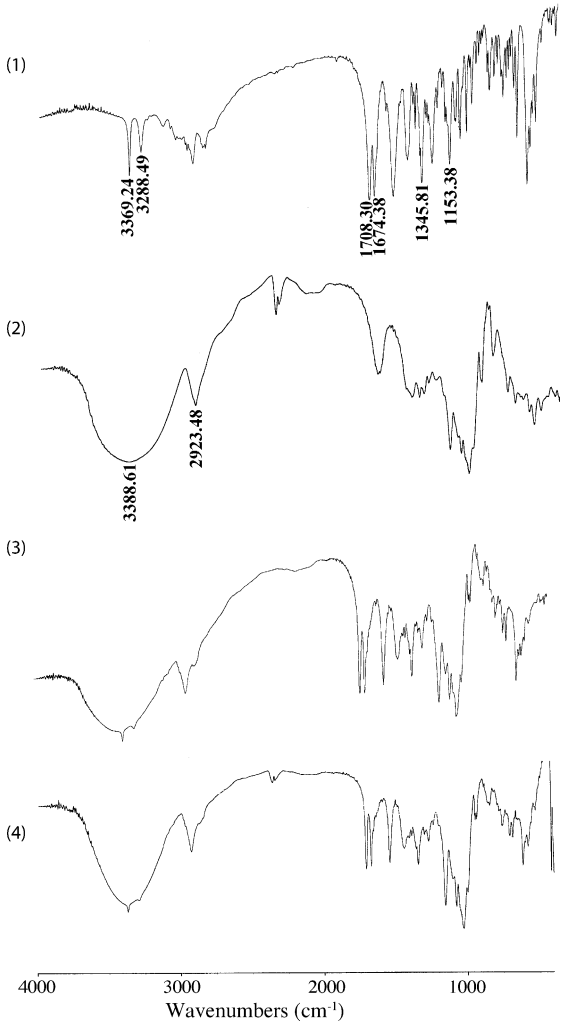

Fig. 4. IR spectra of glimepiride and glimepiride–β-CyD complex: (1) glimepiride; (2) β-CyD; (3) glimepiride–β-CyD physical mixture; (4) glimepiride–β-CyD complex.

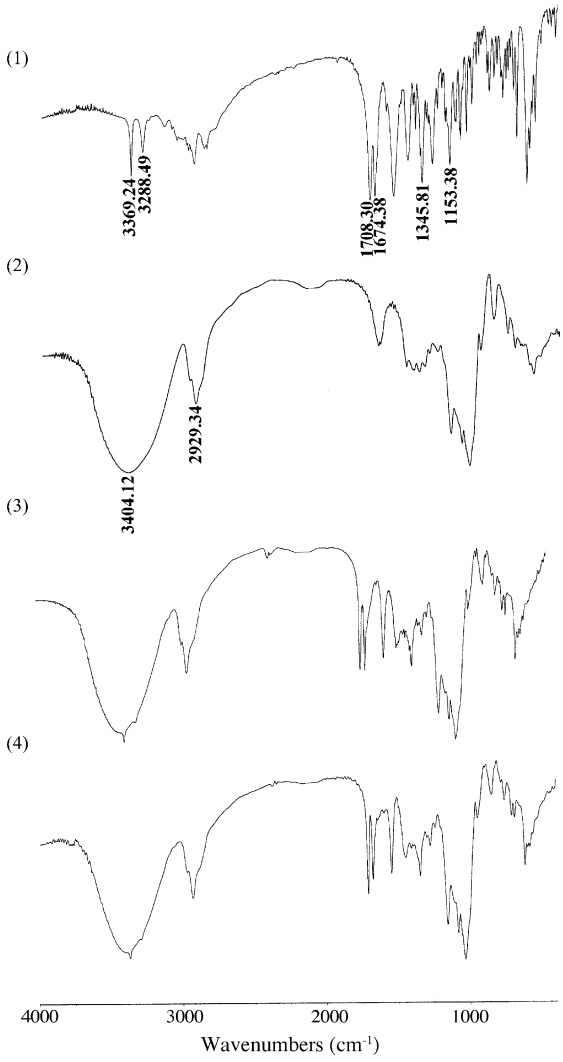

Fig. 5. IR spectra of glimepiride and glimepiride–HP-β-CyD systems: (1) glimepiride; (2) HP-β-CyD; (3) glimepiride–HP-β-CyD physical mixture; (4) glimepiride–HP-β-CyD complex.

The IR spectra of glimepiride–CyD complexes show considerable differences when compared with those of their corresponding constituents. A decrease in frequency of a specific peak is generally seen on complexation (Kurozumi et al., 1975), which indicates an ordering of the molecule (Winters et al., 1997). The vibrations of sulphonylurea groups were shifted towards higher frequencies (Figs. 5–7), suggesting that after the formation of the inclusion complexes, existing bonds were broken and also reduced in their intensities (Özkana et al., 2000). Similarly the N–H stretching modes of the amide exhibited a broadening when the inclusion complexes were formed. These modifications clearly indicate the presence of host–guest interactions and suggest the formation of stable hydrogen bonds between glimepiride and CyDs (Fernandes et al., 2002; Jug and Becirevic-Lacan, 2004).

3.2.2. Thermal measurements

The TGA thermogram of the drug showed 65.87% weight loss at 190.00 °C corresponding to its melting point (Figs. 7–9).

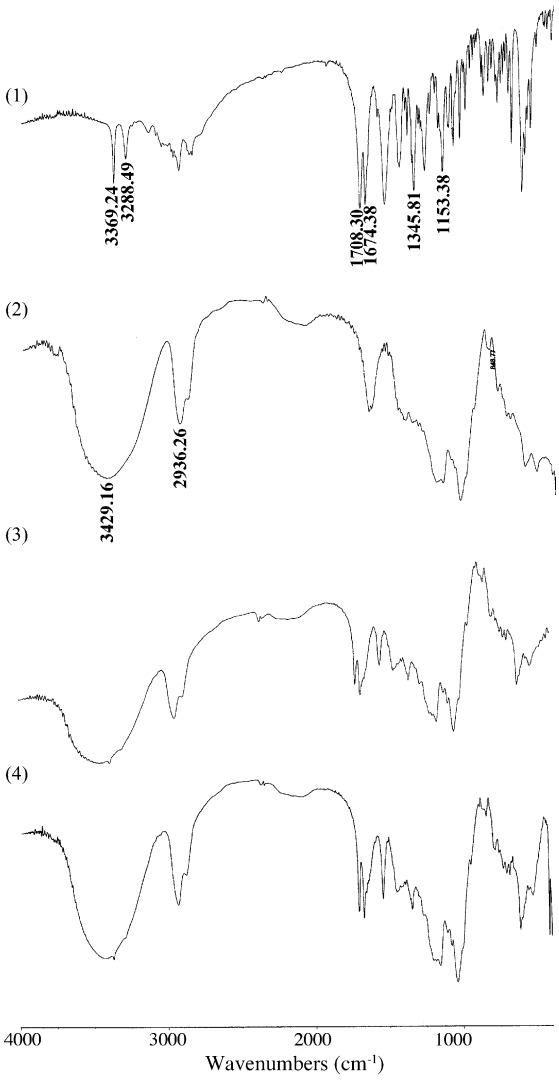

Fig. 6. IR spectra of glimepiride and glimepiride–SBE-β-CyD systems: (1) glimepiride; (2) SBE-β-CyD; (3) glimepiride–SBE-β-CyD physical mixture; (4) glimepiride–SBE-β-CyD complex.

These values were changed to 40.48% at 200.00 °C, 63.57% at 266.18 °C and 40.28% at 262.21 °C, for β-CyD, HP-β-CyD and SBE-β-CyD, respectively. This would indicate formation of more stable complexes between glimepiride and each of these cyclodextrins compared to the drug as evidenced by the obvious decrease in percentage of weight loss and the elevation of the melting point of the drug.

TGA shows that the complexes of glimepiride with the investigated cyclodextrins contained less water, in the range of 70–130 °C, compared to that of the corresponding physical mixtures (Table 1). Water is present within the cavity of the cyclodextrin molecule to stabilize the ring structure (Furo et al., 1987). The decomposition temperature of the cyclodextrin was found to remain unchanged on complexation indicating that the integrity of the cyclodextrin ring is maintained. Hence, the decrease in water content of the complex, compared to the physical mixture, may result from glimepiride occupying the position of some of the water molecules associated with torus of the cyclodextrin (Winters et al., 1997).

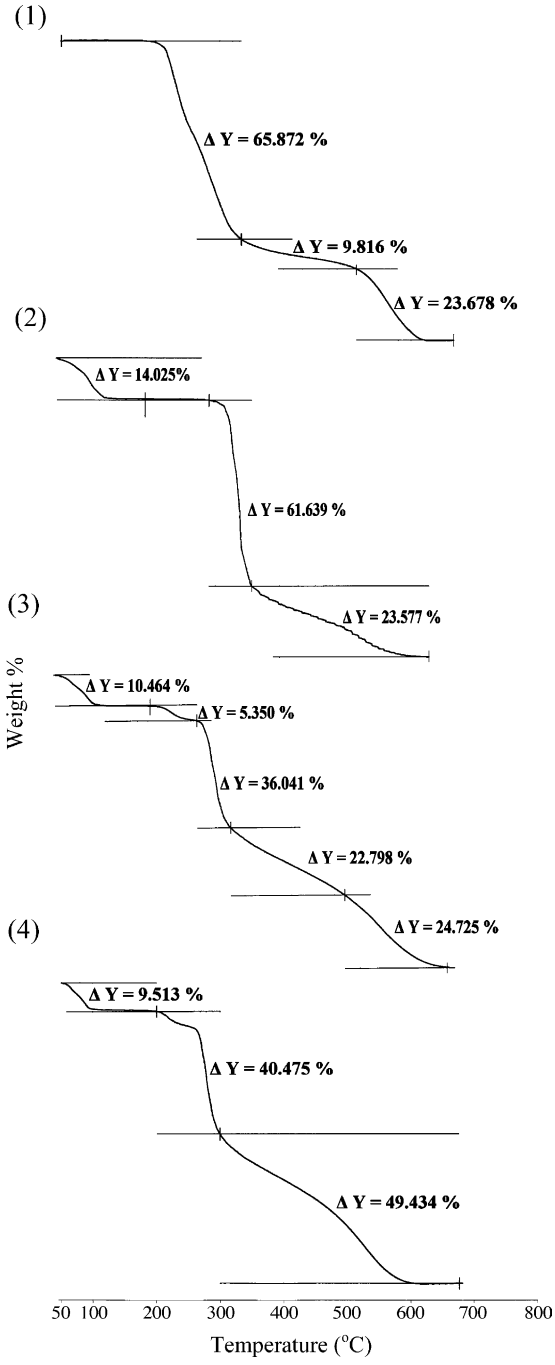

Fig. 7. TGA thermograms of glimepiride and glimepiride–β-CyD systems: (1) glimepiride; (2) β-CyD; (3) glimepiride–β-CyD physical mixture; (4) glimepiride–β-CyD complex.

3.2.3. X-ray diffractometry

Fig. 10 shows the powder X-ray diffraction patterns of glimepiride and its cyclodextrin complexes. Based on the method used, it is verified that glimepiride forms inclusion complexes in solid phase with all cyclodextrins tested. Crystallinity can be determined by comparing some representative peak heights in the diffraction patterns of the binary systems with those of a reference. The relationship used for the calculation of crystallinity was relative degree of crys-

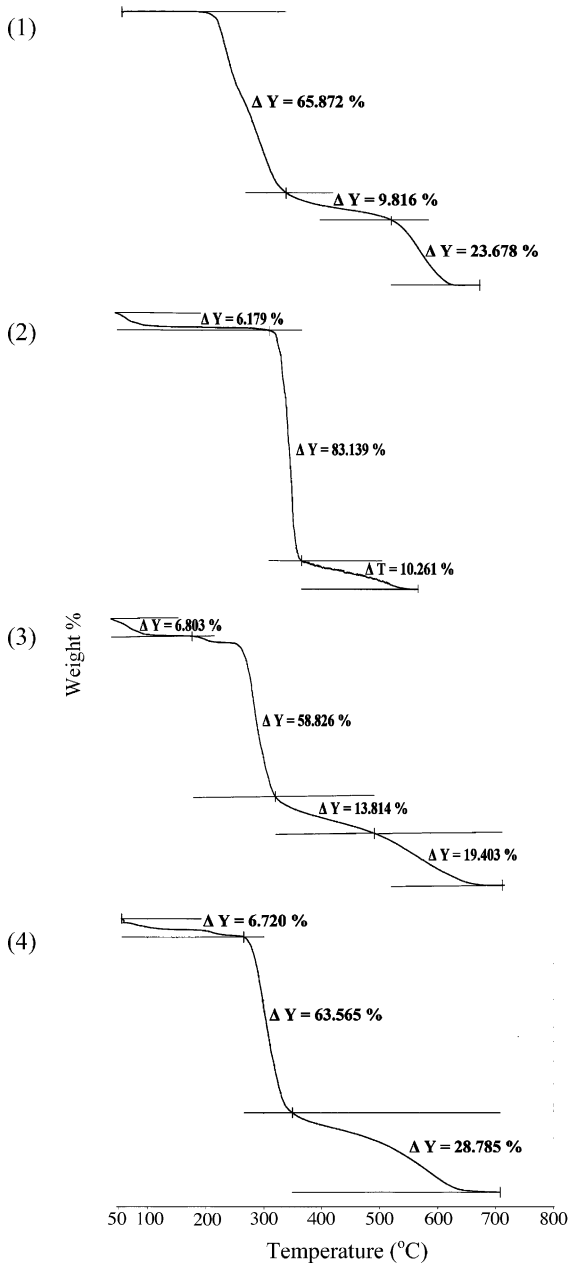

Fig. 8. TGA thermograms of glimepiride and glimepiride–HP-β-CyD systems: (1) glimepiride; (2) HP-β-CyD; (3) glimepiride–HP-β-CyD physical mixture; (4) glimepiride–HP-β-CyD complex.

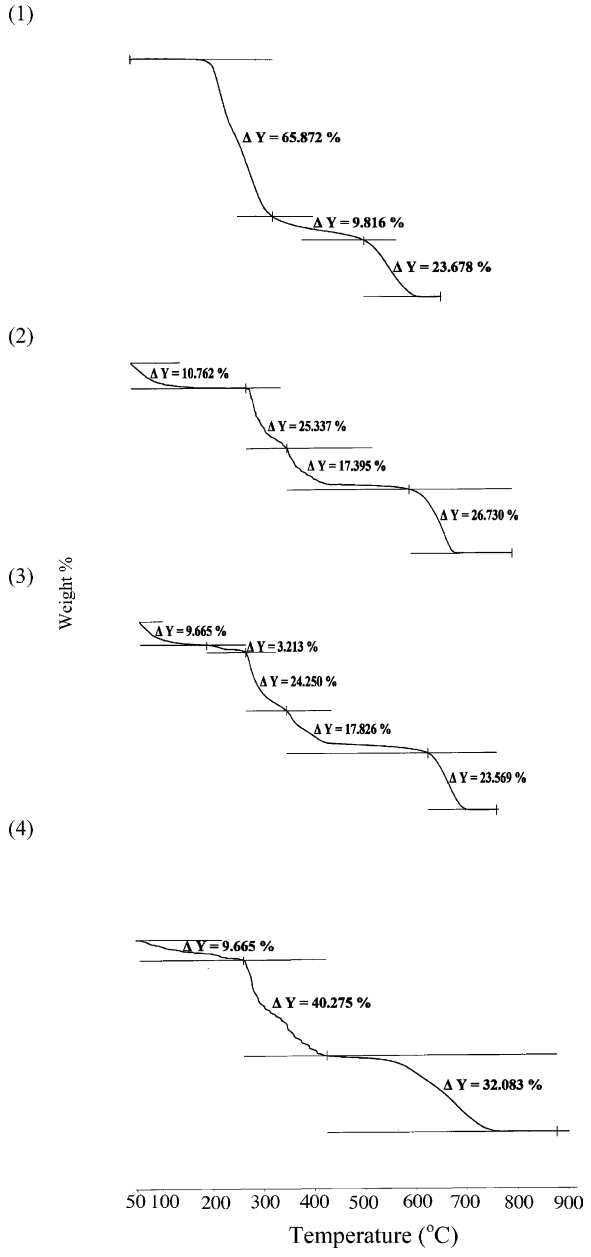

Fig. 9. TGA thermograms of glimepiride and glimepiride–SBE-β-CyD systems: (1) glimepiride; (2) SBE-β-CyD; (3) glimepiride–SBE-β-CyD physical mixture; (4) glimepiride–SBE-β-CyD complex.

Table 1
Data from TGA diagrams of glimepiride–cyclodextrin systems

System	Water loss (%)
β-CyD	11.77
Glimepiride–β-CyD physical mixture	9.24
Glimepiride–β-CyD complex	6.26
HP-β-CyD	2.73
Glimepiride–HP-β-CyD physical mixture	2.67
Glimepiride–HP-β-CyD complex	2.12
SBE-β-CyD	5.65
Glimepiride–SBE-β-CyD physical mixture	4.58
Glimepiride–SBE-β-CyD complex	3.42

tallinity (RDC). RDC = I_{sam}/I_{ref}, where I_{sam} is the peak height of the sample under investigation and I_{ref} is the peak height at the same angle for the reference with the highest intensity (Ryan, 1986).

Pure drug peak at 24.5° (2θ) was used for calculating RDC of binary systems. The RDC-values of the complexes were less than that of the drug and can be arranged in the following order: β-CyD > HP-β-CyD > SBE-β-CyD. Furthermore, a reduced number of signals were noticeable in the complexes, of remarkably lowered intensity, indicating a greater amorphousness of the inclusion compounds, compared to the free molecules (Calabrò et al., 2004).

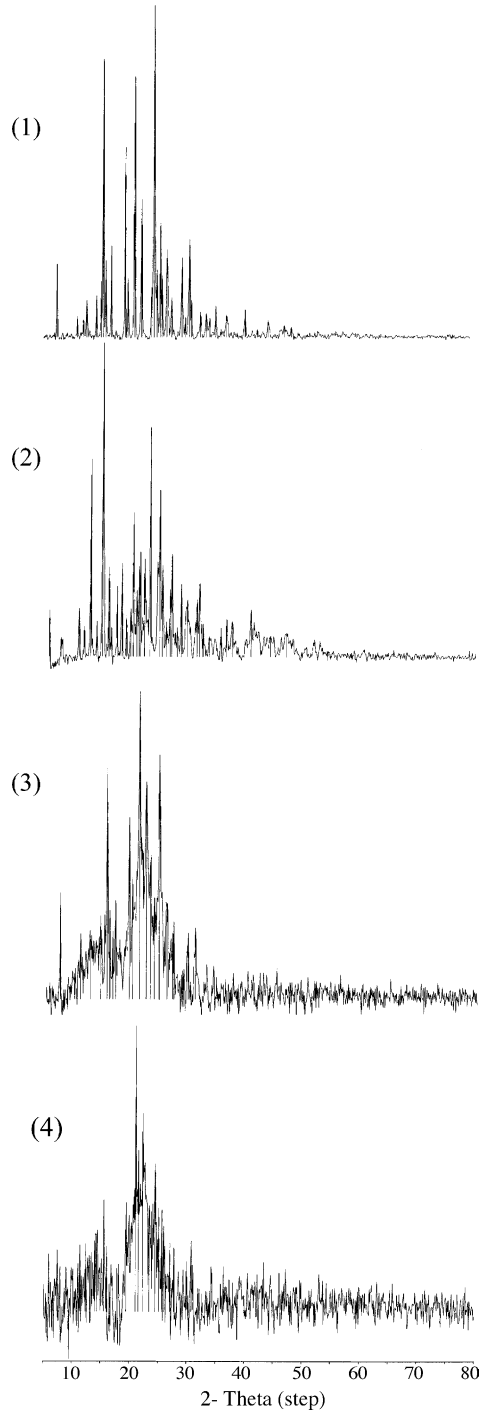

Fig. 10. X-ray diffraction of glimepiride and glimepiride–CyDs complex: (1) glimepiride; (2) glimepiride–β-CyD complex; (3) glimepiride–HP-β-CyD complex; (4) glimepiride–SBE-β-CyD complex.

3.3. Effect of cyclodextrins on the solubility of glimepiride

The effect of cyclodextrins on the solubility of glimepiride in phosphate buffer solutions was investigated at 25 °C (Fig. 11). It is evident that the solubility of glimepiride was increased markedly by complexation with β-CyD or its derivatives. The solubilizing power of the investigated cyclodextrins towards the drug, calculated as mole glimepiride solubilized per one mole of cyclodextrin, revealed that the solubilizing power of CyDs follows the order: β-CyD (0.003) > HP-β-CyD (0.002) > SBE-β-CyD (0.001). Cyclodextrins are known to solubilize lipophilic entities through molecular encapsulation. Our results are comparable to the data of other authors (Uekama et al., 1980; Özkana et al., 2000; Sridevi et al., 2000; Veiga et al., 2000; Aggarwal et al., 2002) regarding improvement of the solubility of other sulfonylurea drugs as gliquidone, gliclazide, glibenclamide, tolbutamide and acetohexamide by cyclodextrins.

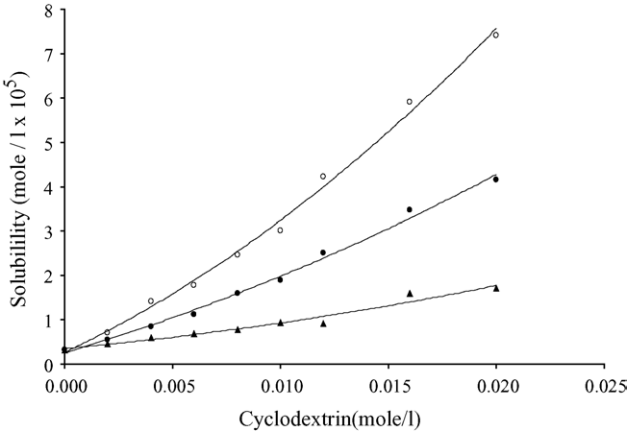

Fig. 11. Effect of cyclodextrins on the solubility of glimepiride: β-CyD (◯); HP-β-CyD (●); SBE-β-CyD (▲).

Fig. 11 reveals the existence of a phase solubilizing diagram of the A_p type indicating the formation of high order complexes between the drug and the cyclodextrins under investigation at high cyclodextrin concentrations (Del Valle, 2004). Such high order systems are characterized by stepwise binding constants, e.g., that 1:2 complex is formed by association of the 1:1 complex with one additional cyclodextrin molecule (Loftsson et al., 2002). Cyclodextrins are able to form both inclusion and non-inclusion complexes. In addition, cyclodextrins and their complexes form water soluble aggregates in aqueous solutions and these aggregates are able to solubilize lipophilic water insoluble drugs through non-inclusion complexation or micelle-like structures (Loftsson et al., 2004).

3.4. In vitro dissolution studies

3.4.1. Effect of complexation with cyclodextrins on the dissolution rate of glimepiride

Fig. 12 shows that inclusion complexation of glimepiride in cyclodextrins increased the dissolution rate of the drug. This increase follows the order: SBE-β-CyD > HP-β-CyD > β-CyD. This increase in the dissolution rate of the drug can be attributed to both improvements in drug wettability and formation of readily soluble complexes in the dissolution medium (Corrigan and Stanley, 1982). Inclusion complexation of the drug in SBE-β-CyD enhanced the dissolution rate of the drug to a marked extent; the dissolution efficiency was increased up to 6–7 folds. The high dissolution rate of the complex with SBE-β-CyD compared to that of the β-CyD might be attributed to the higher

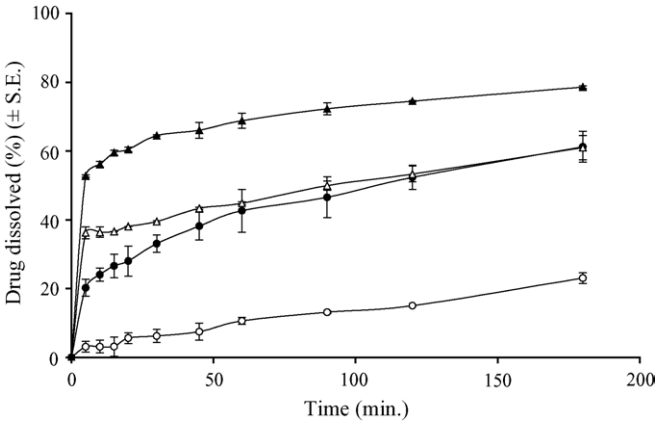

Fig. 12. Effect of cyclodextrins on the dissolution rate of glimepiride: glimepiride (◯); β-CyD (●); HP-β-CyD (△); SBE-β-CyD (▲).

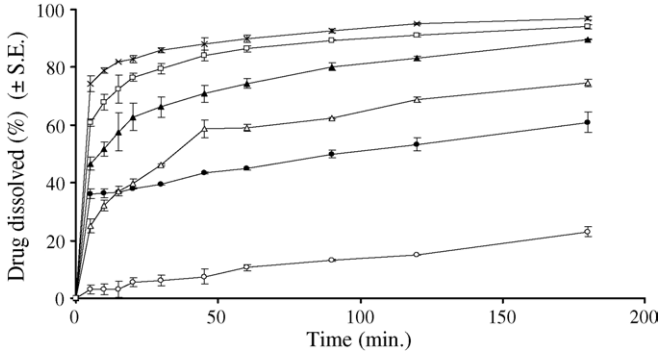

Fig. 14. Effect of polymers on the dissolution rate of glimepiride–HP-β-CyD complex: glimepiride (◯); glimepiride–HP-β-CyD (●); glimepiride–HP-β-CyD + HPMC (△); glimepiride–HP-β-CyD + PVP (▲); glimepiride–HP-β-CyD + PEG 4000 (×); glimepiride–HP-β-CyD + PEG 6000 (□).

inclusion ability of SBE-β-CyD due to its charged groups which are appropriately spaced from the cavity and also the increase in the hydrophobicity around the cavity due to the presence of alkyl chains (Uekema et al., 1988; Mosher and Thompson, 2002).

3.4.2. *Effect of polymers on the dissolution rate of glimepiride–cyclodextrin complexes*

Figs. 13 and 14 illustrate the effect of inclusion complexation of glimepiride in β-CyD and HP-β-CyD in presence of different polymers on the dissolution profile of glimepiride. The dissolution profiles of the ternary systems showed an increase in the dissolution rate of glimepiride compared to the binary systems. The investigated polymers increased the dissolution rate of the drug in the order of PEG 4000 > PEG 6000 > PVP > HPMC. The increase in the dissolution rate of glimepiride in presence of polymers might be related to the increase of complexation efficiency and solubilizing effect of cyclodextrins in presence of water soluble polymers (Loftsson et al., 1994, 1996; Fridriksdottir et al., 1997).

Fig. 15 illustrates the effect of different polymers on the dissolution rate of glimepiride–SBE-β-cyclodextrin complex. It is evident that using HPMC and PVP decreased the dissolution rate of the drug. This might indicate a sort of interaction between these polymers and SBE-β-CyD resulting in formation of polyrotaxanes, where many cyclodextrin molecules are threaded onto a linear polymer. Such inclusion complex formation between CyDs and polymers will reduce the ability of CyD to form complexes with the drug (Fujita et al., 1996; Loftsson and Brewster, 1997). On the other hand, PEG 6000 showed more or less no effect on the dissolution rate of glimepiride, while PEG 4000 enhanced markedly the dissolution rate of the drug.

The differences in the dissolution efficiency of the studied binary systems in presence of the water soluble polymers might be due to different complexation efficiency of the CyDs in presence of these polymers. This may be due to structural and polarity differences of the CyD molecules that could be in the origin of different type of linkings established with the polymers and the drug (Ribeiro et al., 2003).

On the basis of the above results, the dissolution of glimepiride from the ternary system glimepiride–HP-β-CyD–PEG 4000 was higher than from the other systems. Therefore, this ternary system was selected for the subsequent therapeutic efficacy studies.

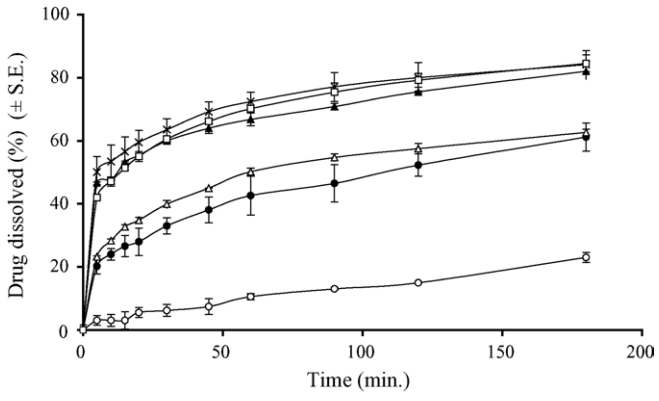

Fig. 13. Effect of polymers on the dissolution rate of glimepiride–β-CyD complex: glimepiride (◯); glimepiride–β-CyD (●); glimepiride–β-CyD + HPMC (△), glimepiride–β-CyD + PVP (▲); glimepiride–β-CyD + PEG 4000 (×); glimepiride–β-CyD + PEG 6000 (□).

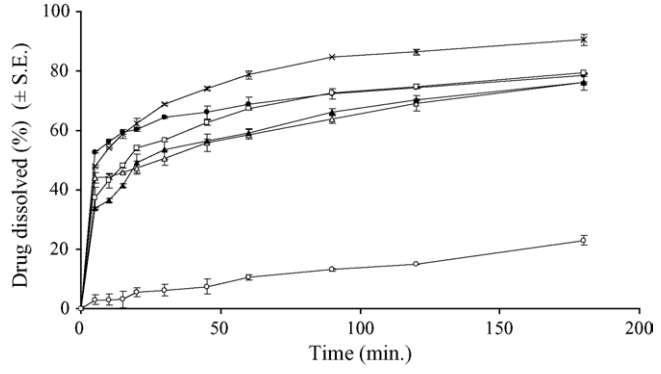

Fig. 15. Effect of polymers on the dissolution rate of glimepiride–SBE-β-CyD complex: glimepiride (◯); glimepiride–SBE-β-CyD (●); glimepiride–SBE-β-CyD + HPMC (△); glimepiride–SBE-β-CyD + PVP (▲); glimepiride–SBE-β-CyD + PEG 4000 (×); glimepiride–SBE-β-CyD + PEG 6000 (□).

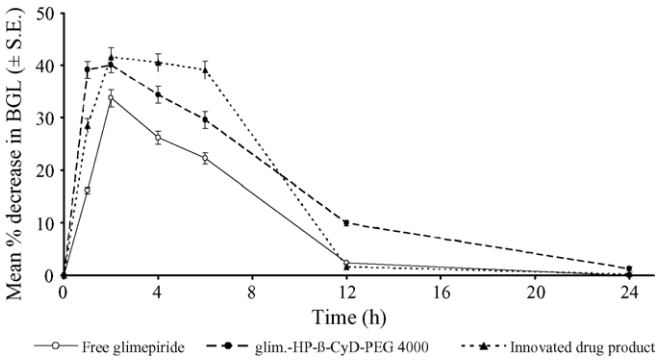

Fig. 16. Mean percentage decrease in blood glucose level of diabetic rats after administration of free glimepiride, glimepiride–HP-β-CyD–PEG 4000 system and the innovated drug product.

3.5. Assessment of therapeutic efficacy

The mean percentage decrease in BGL of diabetic rats after administration of free drug, the suggested formula and the innovated drug product was computed and the data are presented in Fig. 16. It is evident that the percentage decrease in BGL–time profiles for the investigated systems are quite different.

The percentage decrease in BGL, 12 and 24 h post administration, of the suggested formula is higher ($p < 0.001$) than the corresponding values of the innovated drug product or the free drug. This indicates that the duration of action of the suggested formula is markedly longer than that of the innovated drug product or the free drug (Fig. 16).

It is evident that both the suggested formula and the innovated drug product exhibit more or less similar mean maximum percentage decrease in BGL which is higher than that of the free drug (Fig. 16). The difference between the mean maximum percentage decrease in BGL of the suggested formula and that of the innovated drug product is not significant ($p > 0.05$), while the difference between both values and that of the free drug is very highly significant ($p < 0.001$).

With respect to the time for maximum percentage decrease in BGL (t_{max}), it is evident from Table 2 that t_{max} of the suggested formula has the lowest value followed by the free drug and then the innovated drug product. This would indicate that the suggested formula exhibits a faster onset of action compared to that of the free drug or the innovated drug product. The difference between the value of t_{max} of the suggested formula and that of the innovated drug product is very highly significant. On the other hand, the difference between the value of t_{max} of the suggested formula and that of the free drug is not significant. The difference between the t_{max}-value of the innovated drug product and that of the free drug is highly significant ($p < 0.01$).

Table 2
Pharmacodynamic parameters for free glimepiride, glimepiride–HP-β-CyD–PEG 4000 system and the innovated drug product (value ± S.E.)

	t_{max} (h)	$t_{1/2\,p}$ (h)	$AUC_{0-24\,h}$
Free glimepiride	2.2 ± 0.2	6.5 ± 0.3	229.4 ± 8.0
Glimepiride–HP-β-CyD–PEG 4000	1.6 ± 0.2	7.7 ± 0.4	382.5 ± 11.4
Innovated drug product	3.3 ± 0.4	7.9 ± 0.2	344.4 ± 11.2

Table 2 shows that the values of $t_{1/2\,p}$ for the innovated drug product and the suggested formula are similar and much higher than that of the free drug. Statistical analysis indicates that there is no significant difference between the value of $t_{1/2\,p}$ of the suggested formula and that of the innovated drug product. On the other hand, there is a high significant difference between the $t_{1/2\,p}$-value of the innovated drug product or the suggested formula and that of the free drug. This would indicate that both the suggested formula and the innovated product have a pronounced longer duration of action compared to that of the drug.

Regarding the area under the percentage decrease in BGL–time curve ($AUC_{0-24\,h}$), it is evident that the suggested formula has the highest value followed by the innovated drug product and then the free drug (Table 2). The difference between the AUC-value for the suggested formula and that of the free drug is very highly significant, while the difference between the AUC-value for the suggested formula and that of the innovated drug product is significant. This would indicate that the suggested formula shows a better therapeutic efficacy compared to that of the free drug or the innovated one.

The above-mentioned results reveal that the ternary system glimepiride–HP-β-CyD–5% PEG 4000 improves significantly the therapeutic efficacy of the drug.

In conclusion, the association of water soluble polymers to glimepiride–CD systems would offer a promising drug delivery system having the great advantage of reducing the dose of the drug and the amount of cyclodextrin needed.

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