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ORIGINAL ARTICLE

Repaglinide-Cyclodextrin complexes: Preparation, Characterization and in vivo evaluation of antihyperglycemic activity

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Abstract The influence of β-cyclodextrin (βCD) and its hydrophilic derivative hydroxypropyl-β-cyclodextrin (HPβCD) on the in vitro dissolution rate and in vivo antihyperglycemic activity of a poorly water soluble drug, repaglinide (RPG) was studied. Solid dispersions of RPG were prepared by various methods such as physical mixing, cogrinding, kneading, and coevaporation. The dispersions were characterized by differential scanning calorimetry (DSC), X-ray diffraction patterns, infrared spectroscopy, nuclear magnetic resonance studies and optical microscopy. The DSC thermogram of HPβCD dispersion indicated complete disappearance of melting endotherm of RPG indicating complete amorphization. The dispersions exhibited faster rates of dissolution compared to that of physical mixtures. The kneaded dispersion with HPβCD exhibited the fastest in vitro dissolution rate when compared to other dispersions. In vivo studies revealed that the kneaded dispersions with βCD and HPβCD showed significant improvement in antihyperglycemic activity as compared to RPG alone, thus confirming the advantage of improved pharmacological activity of RPG when administered as a solid dispersion with cyclodextrin.

Keywords Repaglinide · β-Cyclodextrin · Hydroxypropyl-β-cyclodextrin · In vitro dissolution · In vivo study

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Introduction

Repaglinide (RPG) is an oral prandial glucose regulator agent for the management of type 2 diabetes mellitus [1, 2]. RPG is developed in attempts to overcome the adverse effects associated with existing antidiabetic compounds which include hypoglycemia, secondary failure and cardiovascular side effects [3]. RPG lowers blood glucose levels by stimulating the release of insulin from the pancreas by binding to a receptor site different from that of sulfonylurea. Being a potent molecule it has good therapeutic potential but its low solubility in water with relatively low and variable bioavailability is a limitation [1]. High inter-individual variability in RPG plasma concentrations has been reported in clinical trials [4, 5]. Improvement of aqueous solubility in such a case is a valuable approach to improve the therapeutic efficacy. Sinswat et al. have prepared nanostructured particles containing amorphous RPG by controlled precipitation using different polymers to improve the solubility and thus the dissolution rate [6]. Purvis et al. have used the ultra-rapid freezing technology to produce rapidly dissolving formulation of poorly-water soluble drug RPG [7]. Nicolescu et al. have prepared inclusion complexes of RPG with β-cyclodextrin (βCD), 2-hydroxypropyl-β-cyclodextrin (HPβCD) and randomly methylated β-cyclodextrin and characterized by DSC, IR and NMR in order to evaluate the possibility of enhancing RPG solubility [8]. However, till date the influence of RPG cyclodextrin complexation on its in vitro dissolution and in vivo activity is not yet established. Cyclodextrins are commonly used in drug formulations as solubility enhancers because of their ability to form water soluble inclusion complexes with poorly water-soluble molecules that fit partially or entirely inside the cavity [9, 10]. By complexation, cyclodextrins

can increase the solubility, stability, and bioavailability of the guest molecule [11].

The objective of the present investigation was to explore the potential of βCD and HPβCD in improving the oral delivery of RPG. βCD and HPβCD dispersions were prepared by different methods and were characterized. The ability of βCD and HPβCD to improve the antihyperglycemic activity of RPG was evaluated in diabetic rat model.

Experimental

Materials

RPG was obtained as a gift sample from USV India Ltd., Mumbai, India. βCD (mol. wt 1135) was obtained as a gift sample from Amaizo, USA and HPβCD (mol. wt 1371.5) was obtained as a gift sample from Cerestar, Inc. (IA, USA). All other reagents were of analytical reagent grade purity. Double distilled water was prepared freshly whenever required.

Phase solubility studies

Phase solubility studies were carried out in water according to the method described by Higuchi and Connors [12]. An excess amount of RPG (20 mg) was added to 10 mL of various concentrations (0–15 mM) of cyclodextrins solutions and shaken on an orbital shaker (Orbitek Scigenics) at 25 °C for 72 h. After equilibrium was achieved, the samples were filtered through 0.45 μ filter. The concentration of RPG in the samples was determined using HPLC (Jasco Corporation, Japan) system with detection wavelength of 242 nm. Hi-Q-Sil C18V; 4.6 mm × 250 mm and 5 μm particle size column was used as stationary phase employing acetonitrile and pH 2.5 phosphate buffer (45:55, v/v) as the mobile phase. The retention time of RPG was found to be 11.2 min. The study was carried out in triplicate. The apparent stability constant K_s was calculated from the phase-solubility diagram according to the following equation.

$$K_s = \left(\frac{\text{Slope}}{S_0(1 - \text{Slope})}\right)$$

where, slope is obtained from the graph and S_0 is the equilibrium solubility of RPG in water.

Preparation of cyclodextrin dispersions

Following binary systems of RPG with βCD and HPβCD were prepared in 1:1 M ratio.

Physical mixture (PM)

The physical mixture (PM) was prepared by geometric mixing of RPG with βCD and HPβCD separately without applying pressure.

Coground dispersion (CG)

The coground dispersion (CG) was prepared by mixing and triturating RPG and cyclodextrin for 15–20 min each.

Kneading method (KN)

RPG and cyclodextrins were accurately weighed and mixed in geometric proportion. The mixture of RPG with each of the cyclodextrin was kneaded separately with aqueous ethanol (1:1) to achieve pasty consistency. The kneaded mass was triturated in mortar for about 15–20 min followed by drying in a tray drier at 45 °C.

Co-evaporation method (CE)

BCD was dissolved in water and added to ethanolic solution of RPG. This mixture was evaporated with heating at a temperature not greater than 45 °C on a rotary evaporator (Buchi, Switzerland). The clear solution obtained was further heated until nearly dry.

All the dispersions were prepared in triplicate and were sieved through BSS 85# sieve (Sieve diameter: 180 μm) and stored over anhydrous calcium chloride in a desiccator.

Characterization of solid dispersions

Differential scanning calorimetry (DSC) studies

RPG, βCD, HPβCD and solid dispersions, each weighing in the range of 3–5 mg, were scanned at a rate of 10 °C/min on a Shimadzu DT-40 Thermal Analyzer between 30 and 330 °C under an inert atmosphere of nitrogen.

Infrared (IR) spectroscopic studies

The infrared spectra of RPG, βCD, HPβCD and the dispersions were recorded on a Jasco FT/IR 5300 spectrophotometer by potassium bromide (KBr) pellet method.

Powder X-ray diffraction (PXRD) studies

Powder X-ray diffraction patterns of RPG, βCD, HPβCD and their dispersions were recorded using a Phillips X-ray diffractometer using Ni-filtered, Cu Kα radiation, a voltage of 40 kV and a 25 mA current. The scanning rate

employed was 1° min^{-1} over 10–40° diffraction angle (2θ) range.

Nuclear magnetic resonance (NMR) studies

The ^{1}H NMR spectra of RPG, βCD, HPβCD and kneaded dispersions in D_2O were recorded using Bruker AVANCE 500 DRX (500 MHz) Fourier Transform Nuclear Magnetic Resonance (FTNMR) instrument at 298°K and 500 MHz.

Optical microscopy

Microscopic observation of the dispersions was performed under a microscope (Metzer, India). The samples were mounted on a glass slide and viewed under 45× magnification and pictures were taken with Canon digital camera.

Dissolution studies

Dissolution profile of the dispersions was studied in pH 5.0 buffer containing 0.1% SLS at 37 ± 0.5 °C. The studies were performed in USP type II apparatus (Electrolab, India) rotating at 75 rpm by powder dispersion technique [13]. The samples were withdrawn at fixed time intervals, filtered through 0.45 μ filter and analyzed for drug content at 242 nm by HPLC. Each test was carried out in triplicate (coefficient of variation (CV) < 3%). Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as percentage of the area of the rectangle described by 100% dissolution in the same time [14].

Evaluation of in vivo antihyperglycemic activity

The protocol for animal studies was approved by the Institutional ethical committee of Bombay College of Pharmacy. Neonatal STZ wistar rats in weight range of 150–170 g, 6–8 weeks old were used for the study. The animals were housed in cages in groups of six at a constant temperature of 23 ± 2 °C and 55 ± 5% RH humidity with a light/dark cycle of 12 h. All the animals were given food (pellet diet supplied by Hindustan Lever Ltd) and water ad libitum. Rats were assigned to the groups in a randomized order. Rats were fasted overnight (18 h) and not fed during the study, although they had free access to water during the night and during all the experimental protocols. Oral glucose tolerance test (OGTT) was performed on neonatal STZ rats to evaluate the antihyperglycemic action. Blood was withdrawn from overnight fasted rats followed by oral administration of test samples (RPG, kneaded dispersion of RPG with βCD and kneaded dispersion of RPG with HPβCD) under light anesthesia. Glucose 2 g/kg was administered 60 min post treatment to test samples. Blood samples were collected via retro-orbital plexus at 30, 60 and 120 min after glucose administration. The serum glucose levels were determined using glucose estimation kits (Erba diagnostics, India).

Statistics

Pharmacological data was expressed as mean ± SEM. Data was calculated and analysed for statistical significance using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Values of $p < 0.05$ were considered significant.

Results and discussion

Solubility studies

The phase-solubility graph of RPG with βCD and HPβCD is shown in Fig. 1. The plot showed that aqueous solubility of the drug increased linearly as a function of cyclodextrins concentration. According to Higuchi and Connors, the phase solubility profile can be considered as A_L type. As the slope of line in both the cases was less than unity, it was assumed that the increase in solubility observed was due to the formation of a 1:1 complex. Apparent stability constant $K_{1:1}$ was found to be 227.51 M^{-1} for βCD and 406.81 M^{-1} for HPβCD. The stability constant between the range of 100 and 1000 M^{-1} is considered as an ideal value, smaller values indicate weak interaction between drug and cyclodextrin, while large value indicate incomplete drug release from the inclusion complex [15].

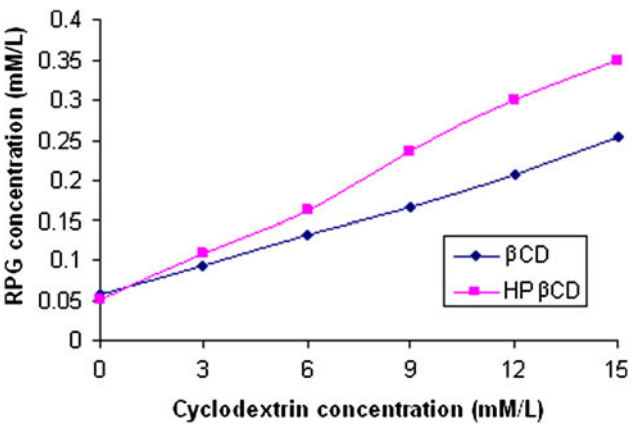

Fig. 1 Phase solubility curve of RPG with βCD and HPβCD at 25 °C. Each point is a mean (±SD) of three determinations

Characterization of solid dispersions

DSC studies

DSC thermogram of RPG showed a sharp melting endotherm at 138 °C indicating the crystalline nature of repaglinide. A shallow endothermic peak at around 86.4 °C was observed for βCD (Fig. 2b), which is related to loss of water. The βCD dispersions showed a reduction in the intensity of the melting endotherm, indicating partial amorphisation of RPG. Thermogram of HPβCD did not show any thermal event (Fig. 2c). The coground dispersion with HPβCD depicted reduction in the height and broadening of the melting endotherm whereas melting endotherm of RPG was not evident in thermogram of kneaded dispersion with HPβCD (Fig. 2j) indicating complete amorphization and stronger interaction with HPβCD.

Infrared (IR) spectroscopic studies

IR spectra of βCD dispersions did not show any significant changes in the characteristic peaks when compared with spectra of RPG and βCD (data not shown). In case of HPβCD dispersions, the peak at 3,308 cm^{-1} was not evident and could have been masked by the broad signal observed at 3,406 cm^{-1}. There are reports indicating limited use of this technique in identifying complexation with cyclodextrins [16, 17].

Powder X-ray diffraction (PXRD) studies

The PXRD spectra of RPG and its solid dispersions with βCD and HPβCD are shown in Fig. 3. It is evident from the figure that RPG has crystalline nature as indicated by the sharp peaks. The PXRD pattern of βCD also revealed

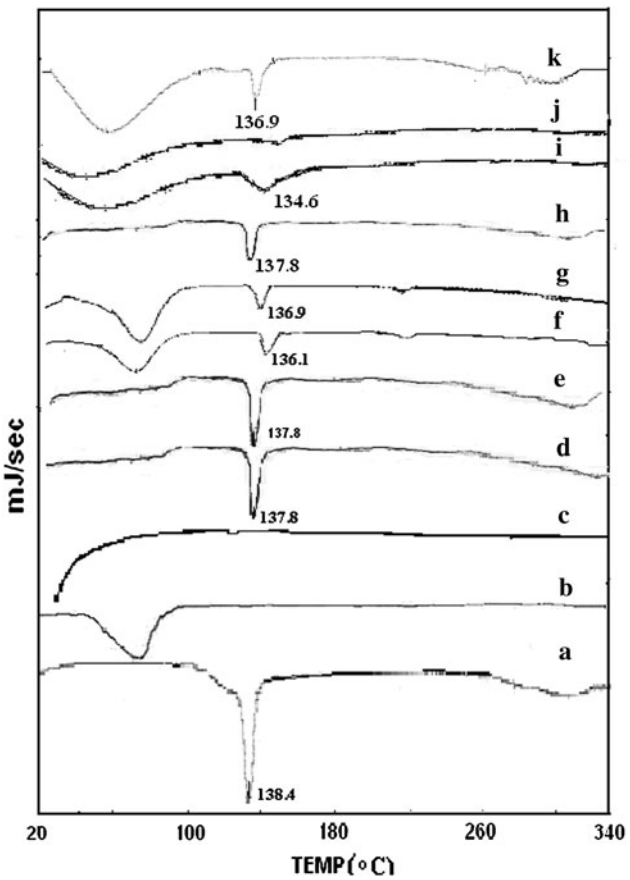

Fig. 2 DSC thermograms of: **a** RPG, **b** βCD, **c** HPβCD, **d** RPG-βCD physical mixture, **e** RPG-HPβCD physical mixture, **f** RPG-βCD coground dispersion, **g** RPG-βCD kneaded dispersion, **h** RPG-βCD coevaporated dispersion, **i** RPG-HPβCD coground dispersion, **j** RPG-HPβCD kneaded dispersion, and **k** RPG-HPβCD coevaporated dispersion

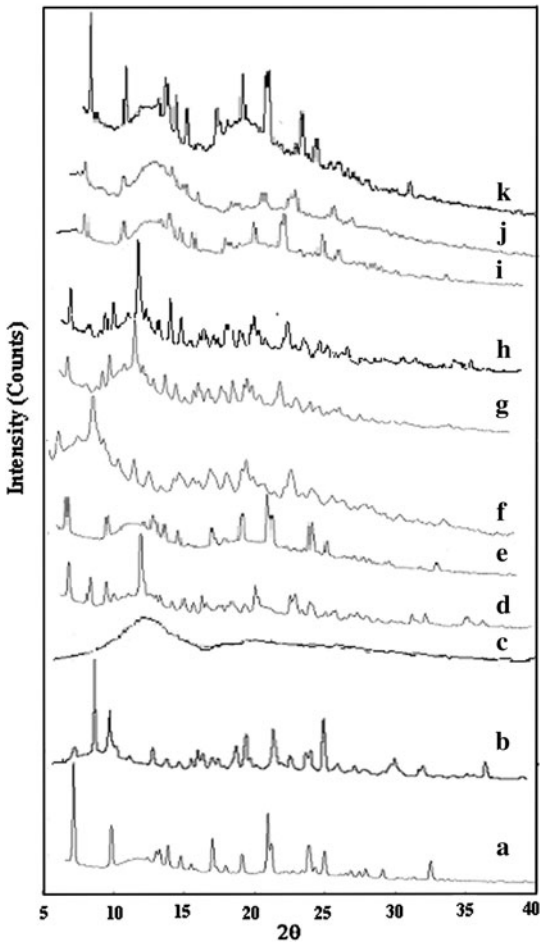

Fig. 3 PXRD patterns of: **a** RPG, **b** βCD, **c** HPβCD, **d** RPG-βCD physical mixture, **e** RPG-HPβCD physical mixture, **f** RPG-βCD coground dispersion, **g** RPG-βCD kneaded dispersion, **h** RPG-βCD coevaporated dispersion, **i** RPG-HPβCD coground dispersion, **j** RPG-HPβCD kneaded dispersion, and **k** RPG-HPβCD coevaporated dispersion

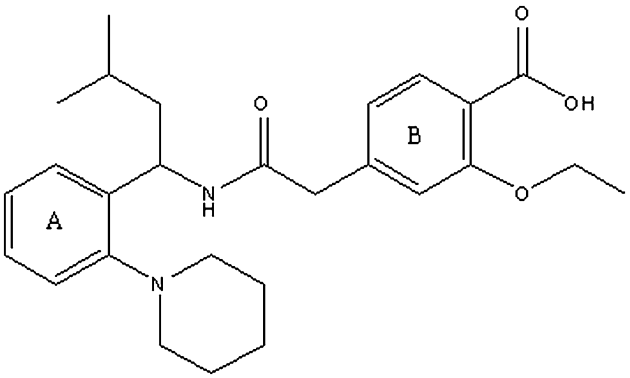

Fig. 4 Structure of RPG

several diffraction peaks attributed to its crystalline nature while absence of any peak in diffraction patterns of HPβCD indicated its amorphous state. The dispersions with βCD and HPβCD showed reduction in the intensity of prominent peaks of RPG. This may be due to partial loss of crystalline nature of drug during the process of dispersion preparation. Moreover, peaks with remarkably lower intensity were observed for coground and kneaded dispersions with HPβCD. This is indicative of transformation of RPG from crystalline to amorphous state which might be due to its inclusion in the cyclodextrin cavity.

Nuclear magnetic resonance (NMR) Studies

In NMR analysis, formation of inclusion complexes of CDs is normally evidenced by changes in chemical shifts of both the host and guest molecules [18]. These chemical shift changes may provide an insight into the inclusion mode of the complex Fig. 4 gives the structure of RPG. ^{1}H NMR spectra of RPG, βCD, RPG- βCD complex, HβCD and RPG- HβCD complex are shown in Fig. 5. Table 1 shows the chemical shift values of βCD protons in presence and absence of RPG. The H3 and H5 protons located inside the cavity were found to experience highest upfield shift in comparison to other protons. In case of kneaded dispersion with βCD, a significant change was observed in the peak shape in the region 7.2–7.3 ppm which corresponds to ring B i.e. the aromatic ring attached to carboxylic group (Fig. 6) of RPG. Due to change in peak shape it was difficult to calculate the chemical shift values. This is indicative of an interaction between ring B and βCD.

In NMR spectrum of RPG in the presence of HPβCD, appreciable chemical shift displacements were observed due to some conformational changes having occurred via complexation. The protons H3 and H5 located within the cavity were markedly affected with $\Delta\delta$ values of -0.024 and -0.134 ppm, respectively (Table 2). The signals of protons H1, H4 and H6 on the outer surface changed slightly whereas for the proton H2, the $\Delta\delta$ is -0.057. A distinct change was observed in the NMR spectrum of

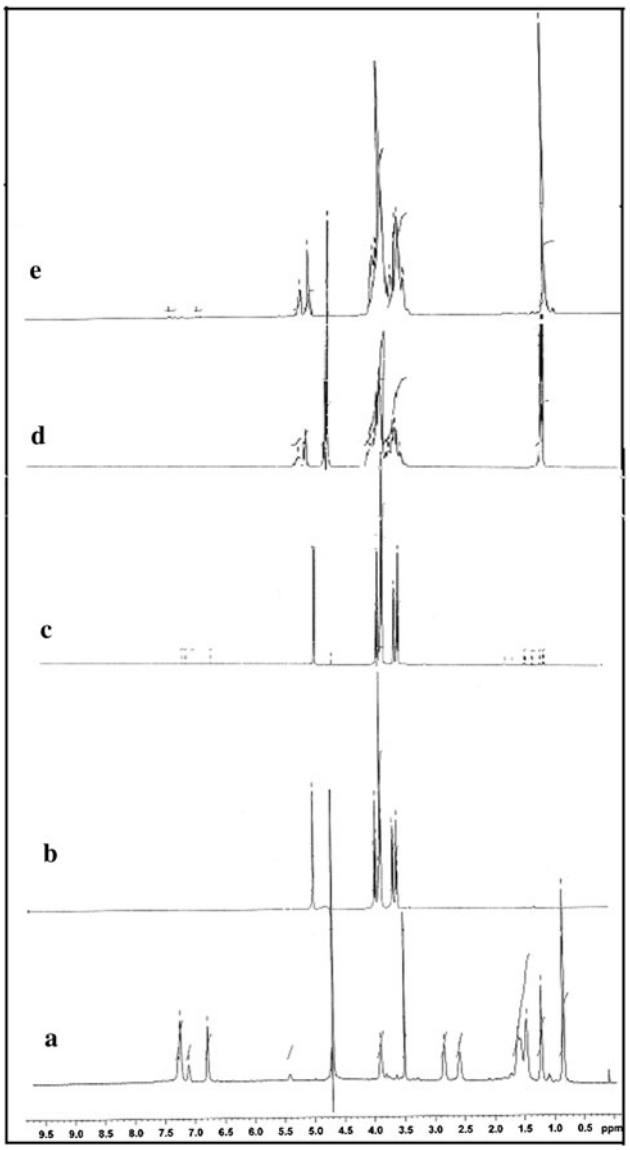

Fig. 5 ^{1}H NMR spectra in D_2O of **a** RPG, **b** βCD, **c** RPG-βCD kneaded dispersion, **d** HPβCD, and **e** RPG-HPβCD kneaded dispersion

Table 1 Chemical shift values of βCD protons in presence and absence of RPG

βCD protons	δ_{Free}	$\delta_{Complex}$	$\Delta\delta_{ppm}$
H-1	5.005	4.996	−0.009
H-2	3.585	3.576	−0.009
H-3	3.9	3.886	−0.014
H-4	3.519	3.509	−0.01
H-5	3.791	3.779	−0.012
H-6	3.818	3.813	−0.004

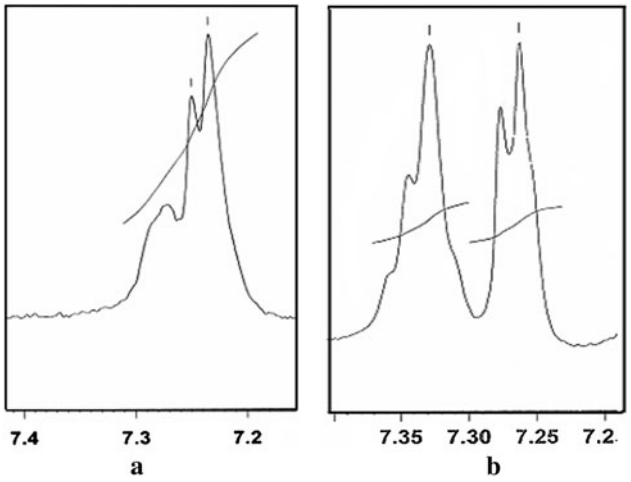

Fig. 6 [1]HNMR spectra of **a** RPG and **b** RPG-βCD dispersion

Table 2 Chemical shift values of HPβCD protons in presence and absence of RPG

HPβCD protons	δ_{Free}	$\delta_{Complex}$	$\Delta\delta_{ppm}$
H-1	5.013	5.000	−0.013
H-2	3.611	3.554	−0.057
H-3	3.897	3.873	−0.024
H-4	3.409	3.426	0.017
H-5	3.785	3.651	−0.134
H-6	3.812	3.83	0.018

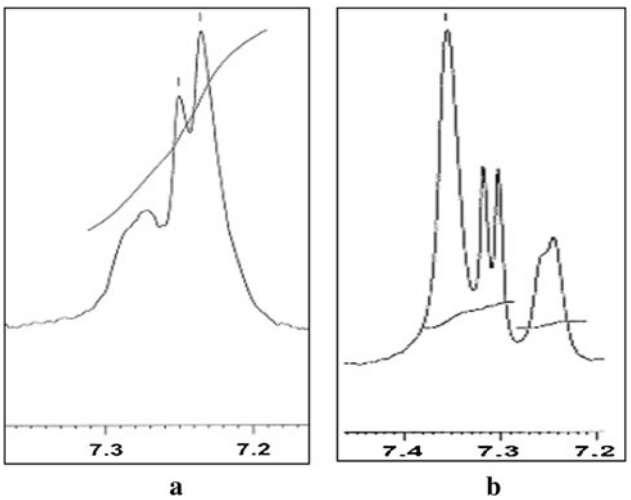

Fig. 7 [1]HNMR spectra of **a** RPG and **b** RPG-HPβCD dispersion

ring B as was observed in case of βCD dispersion when compared with NMR spectrum of the drug (Fig. 7). Therefore, we can infer that a part of the molecule is included in the cavity and the other part interacts with the outer surface especially with the H2 proton.

It is reported in literature that in certain cases, chemical shifts displacements of external protons (H1, H2, and H4) can be observed. Distinct change was observed for H2 and H4 protons of βCD due to association between βCD and Triton X-100 [19]. This is also observed when the encapsulated molecule has a large size and when it presents one or more aliphatic chains which can remain outside the cavity and interact with the external faces of cyclodextrins [20].

Optical microscopy

Optical microscopy was performed for the RPG, βCD, HPβCD, physical mixtures, βCD and HPβCD solid dispersions in order to investigate the influence of complexation on morphology of particles. In the photomicrograph (Fig. 8), RPG appeared as needle shaped crystals. βCD photomicrograph showed irregular flat plate like structures whereas HPβCD appeared as spherical particles. In physical mixtures, the drug and cyclodextrins existed as two separate entities. The kneaded dispersion with βCD appeared as agglomerate which was totally different from that of the physical mixture. In kneaded, coground and coevaporated dispersion with HPβCD the original morphology of the raw materials disappeared and it was not possible to differentiate between the components. The existence of dispersion in a different form than the physical mixture may be attributed to the different kind of treatment or processing that the binary system undergoes.

Dissolution studies

In order to obtain a discriminating dissolution medium, sodium lauryl sulphate (0.1%) was incorporated in the dissolution medium. The dissolution profile of various RPG dispersions is presented in Fig. 9. The dissolution of RPG dispersions was compared with their physical mixtures. The kneaded dispersion with βCD showed a maximum cumulative release of 78% at the end of one hour. This was followed by co-evaporated dispersion that released 69.52% of RPG. The superior dissolution profiles observed for kneaded and co-evaporated dispersions are attributed to the enhanced interaction of drug with the carrier in the presence of solvent. The presence of solvent during the preparation of solid dispersion allows intimate contact between the drug and the carrier, thus providing better molecular association.

The HPβCD dispersions released the drug faster in comparison to its physical mixture. RPG dissolved rapidly with ~69% release in 10 min from kneaded dispersion and 45% release from both coground and coevaporated dispersion in the same time duration. The significant improvement in the dissolution profile of kneaded

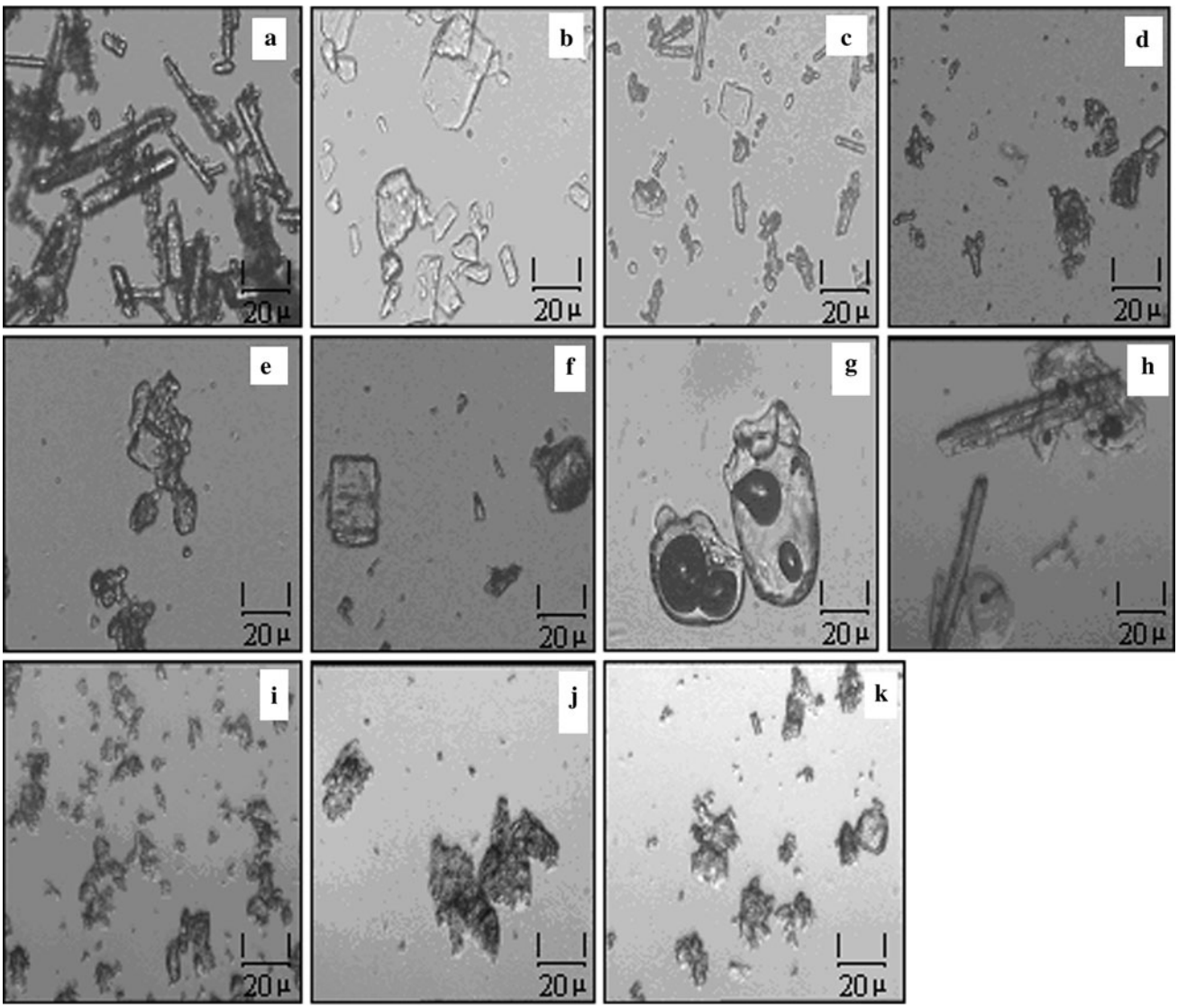

Fig. 8 Optical microscopy at magnification of 45× of **a** RPG, **b** βCD, **c** RPG-βCD physical mixture, **d** RPG-βCD coground dispersion, **e** RPG-βCD kneaded dispersion, **f** RPG-βCD coevaporated dispersion, **g** HPβCD, **h** RPG-HPβCD physical mixture, **i** RPG-HPβCD kneaded dispersion, **j** RPG-HPβCD coground dispersion, and **k** RPG-HPβCD coevaporated dispersion

dispersion is a result of complete drug amorphization with significant molecular dispersion of the drug due to complex formation. This result was found to be in agreement with the observations of DSC and PXRD studies. The coground and co-evaporated dispersions were equivalent in achieving an enhancement in drug dissolution rate (about 45% of drug dissolved in 10 min). The improved solubility of the coevaporated dispersion may be attributed to better interaction of drug with the carrier due to grinding and also due to improved wetting of the drug particles due to presence of soluble carrier leading to better drug dissolution.

The results of dissolution efficiency and percent of active ingredient dissolved at 10 min are presented in Table 3. This provides an insight about the ability of various carriers and dispersion types to solubilize RPG. The dispersion with HPβCD was found to release significant amount of RPG, ∼70% within 10 min in comparison to HPβCD dispersions prepared by other methods and βCD dispersions with RPG. The dissolution efficiency values for kneaded dispersion of repaglinide with βCD and HPβCD was 1.6 and 1.7 times more than their respective physical mixtures. The dissolution efficiency values therefore indicate the ability of the dispersions to enhance the dissolution profile of drug.

Evaluation of in vivo antihyperglycemic activity

The kneaded dispersions of RPG with βCD and HPβCD gave faster drug release hence they were selected for invivo

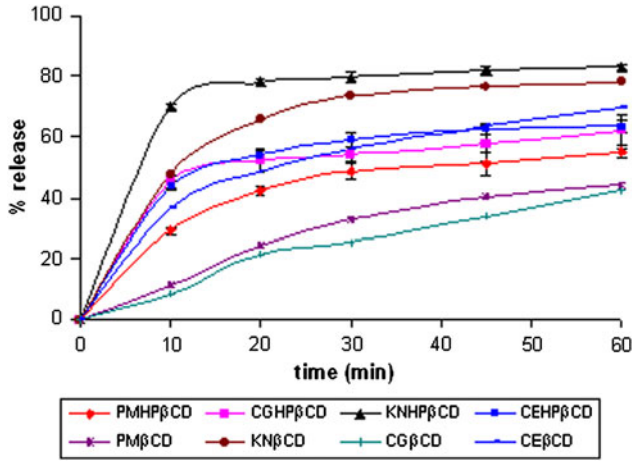

Fig. 9 Comparative dissolution profile of RPG dispersions

Table 3 Dissolution parameters of RPG dispersions with βCD and HPβCD

Dispersion	DP_{10}	DE_{60}
PMβCD	11.58	24.65
CGβCD	10.84	15.29
KNβCD	47.88	40.34
CEβCD	36.90	32.41
PMHPβCD	29.18	25.11
CGHPβCD	45.38	29.98
KNHPβCD	69.95	43.43
CEHPβCD	43.91	31.45

DP_{10} percent drug dissolved at 10 min, DE_{60} dissolution efficiency at $t = 60$ min. Each value is an average of three determinations

evaluation using oral glucose tolerance test. The antihyperglycemic activity of RPG was evaluated on the basis of its ability to prevent the increase in blood glucose levels after administration of glucose load. Figure 10 illustrates the antihyperglycemic activity of the drug and its dispersions.

After 30 min of glucose administration, groups administered with βCD and HPβCD dispersions were found to significantly restrict the increase in blood glucose levels as compared to rats treated with plain drug suspension and vehicle control group ($p < 0.001$). The drug treated group also significantly reduced the glucose levels ($p < 0.05$) when compared to vehicle control group. The blood glucose levels of all animals reached a peak at 60 min after glucose load and thereafter a decrease was observed. The dispersion treated groups at 60 min post glucose load were found to inhibit the increase in glucose levels significantly ($p < 0.001$) in comparison to control group and plain drug treated group. This enhanced activity is due to improved solubility of drug which would lead to faster absorption. Thus, RPG complexes with βCD and HPβCD were found to be more promising as they not only produced an early onset but also an intense hypoglycemic effect due to

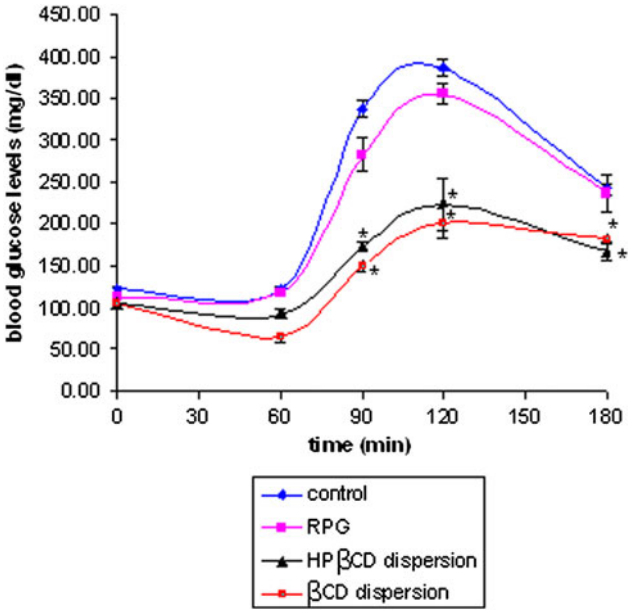

Fig. 10 Antihyperglycemic activity of RPG and its dispersion with βCD and HPβCD prepared by kneading. Each value is expressed as mean ± standard error of mean ($n = 5$). * $p < 0.001$ when compared to corresponding value of control

improved bioavailability of RPG as compared to pure drug powder. These results corroborate the observations of in vitro dissolution study.

Conclusion

In the present study, we proposed the use of βCD and HPβCD to overcome the limitation of poor solubility of RPG ABC-PC. The present study has demonstrated that both βCD and HPβCD are able to interact with RPG in solution and in the solid state. The kneaded dispersions showed a remarkable improvement in the dissolution profile of the drug. Oral administration of dispersions was found to inhibit the rise in blood glucose levels of *n*-STZ diabetic rats to greater extent than RPG. Thus, the utility of cyclodextrins in improving in vitro dissolution rate and in vivo antihyperglycemic activity of RPG on oral delivery has been successfully established.

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