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Research Article

Preformulation Study of the Inclusion Complex Irbesartan-β-Cyclodextrin

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Abstract. The aim of the present work was to improve the solubility and dissolution profile of Irbesartan (IRB), a poorly water-soluble drug by formation of inclusion complex with β-cyclodextrin (βCD). Phase solubility studies revealed increase in solubility of the drug upon cyclodextrin addition, showing A_L—type of graph with slope less than one indicating formation of 1:1 stoichiometry inclusion complex. The stability constant (K_s) was found to be 104.39 M^{-1}. IRB–βCD binary systems were prepared by cogrinding, kneading using alcohol, kneading using aqueous alcohol, and coevaporation methods. Characterization of the binary systems were carried out by differential scanning calorimetry, Fourier transform infrared spectroscopy, scanning electron microscopy, X-ray diffraction, and proton nuclear magnetic resonance. The dissolution profiles of inclusion complexes were determined and compared with those of IRB alone and physical mixture. Among the various methods, coevaporation was the best in which the solubility was increased and dissolution rate of the drug was the highest. The study indicated the usefulness of cyclodextrin technology to overcome the solubility problem of IRB.

KEY WORDS: β-cyclodextrin; dissolution; inclusion complex; Irbesartan; solubility.

INTRODUCTION

Irbesartan (IRB), 2-butyl-3-[[2_-(1*H*tetrazole-5-yl)(1,1_-biphenyl)-4yl]methyl]-1,3 diazaspiro[4,4]non-1-en-4-one, is a nonpeptide specific competitive antagonist of the angiotensin II receptor (AT_1 subtype) used orally for treatment of hypertension (1,2). The drug exhibits low bioavailability related to its poor water solubility (3). IRB is a class II compound, i.e., water-insoluble, lipophilic, and highly permeable according to Biopharmaceutical Classification System. Therefore, IRB bioavailability can be improved by increasing its solubility (4). Cyclic oligosaccharides like beta-cyclodextrins (βCD) are regarded as most functional and enabling excipients which via dynamic complex formation interact with many water insoluble drugs or their lipophilic moieties thereby handling undesirable physicochemical properties including low aqueous solubility, poor dissolution rate, and limited drug stability (5,6).

The aim of present work was to improve the solubility of IRB by complexation with βCD and to compare the effect of various complexation methods on drug dissolution profile. Different methods employed to prepare binary mixtures were cogrinding (CG), kneading (K), and coevaporation (CE). The solubility curve type and stability constant of the complex was established according to phase solubility studies. The dissolution studies of the inclusion complexes were carried out and compared with IRB alone and its physical mixture with βCD. Characterization of the binary systems was performed by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and proton nuclear magnetic resonance (^{1}H NMR).

EXPERIMENTAL

Materials

Irbesartan was kindly supplied by Aarti Drugs Ltd, Mumbai, India as a gift sample. β-Cyclodextrin (M.W. 1135) was gifted by Signet, India. All the reagents used were of analytical grade. Freshly prepared distilled water was used throughout the experiments.

Phase Solubility Studies

Phase solubility studies were performed in distilled water in triplicate according to Higuchi and Connors method (7). An excess amount of IRB (20 mg) was added to 5 ml of aqueous solutions containing various concentrations of βCD (0–0.01 M) in glass vials which were subsequently tightly closed and mechanically shaken at 25±2°C for 48 h. Aliquots were withdrawn, filtered, and spectrophotometrically analyzed (Shimadzu UV 1601 Japan) at 244 nm for IRB content. The presence of βCD did not interfere with the spectropho-

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ABBREVIATIONS: βCD, beta-cyclodextrins; CE, coevaporation; CG, cogrinding; DSC, differential scanning calorimetry; FTIR, Fourier transform infra-red spectroscopy; ^{1}H NMR, nuclear magnetic resonance; HCl, hydrochloric acid; IRB, Irbesartan; K_s, apparent stability constant; K, kneading; PM, physical mixture; SEM, scanning electron microscopy; TMS, tetramethylsilane; XRD, X-ray diffraction.

tometric assay of the drug. The apparent stability constant K_s was calculated from the slope of the linear plot of the phase solubility diagram according to Eq. 1.

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

where S_0 is the solubility of drug in absence of βCD.

Preparation of Solid Binary Systems

The following binary systems of IRB with βCD were prepared in 1:1 molar ratio.

Physical Mixture

Physical mixture (PM) of drug and βCD were prepared by homogeneous mixing of individual components that had been previously sieved through no. 80 sieve (180 μm).

Cogrinding Method

CG samples were prepared by the method reported by Zingone and Rubessa (9). Drug and βCD were ground in a ceramic mortar with a pestle for 45 min. The mixture was then passed through no. 80 sieve.

Kneading Method

IRB and βCD were mixed in ceramic mortar with a pestle for 15 min and the required amount of solvent (ethanol for kneading using alcohol [K1] and ethanol–water 1:1 mixture for kneading using aqueous alcohol [K2]) just to make a smooth paste was added. The paste was then further kneaded for about 45 min. A similar method was reported by Fernandes *et al.* (10) where prazosin hydrochloride was kneaded with either βCD or HPβCD. During this process, an appropriate quantity of the solvent was added in order to maintain a suitable consistency. Further, the product was dried at 50°C for 24 h. The dried mass was then passed through no. 80 sieve.

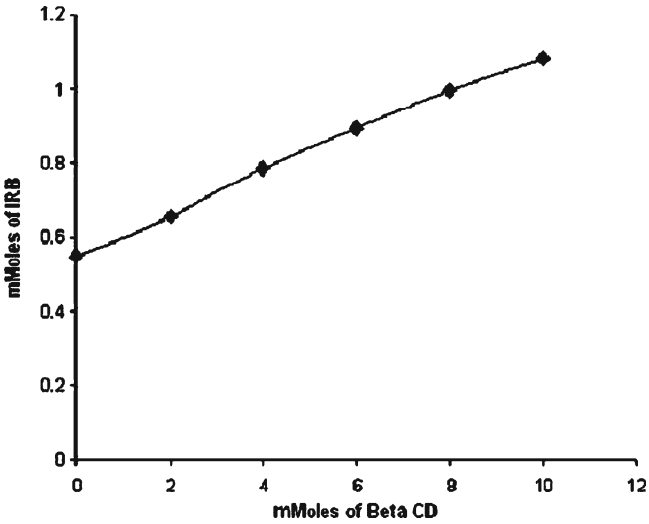

Fig. 1. Phase solubility diagram IRB–βCD system in water at 25±2°C (n=3)

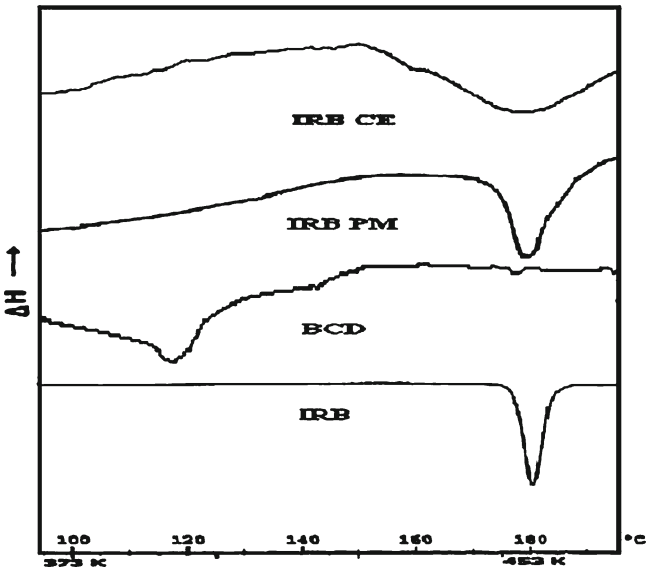

Fig. 2. DSC spectra of IRB–βCD systems

Coevaporation Method

Liu and his coworkers (11) used the coevaporation method for preparation of inclusion complex of nicardipine and βCD. Weighed amount of drug and βCD were dissolved in required amount of ethanol–water 50% *v/v* mixture. The suspension was further sonicated. The clear solution was kept for stirring on a magnetic stirrer till all the solvent got evaporated. The mass obtained was dried at 50°C and further sieved through no. 80 sieve.

Differential Scanning Calorimetry

The DSC curves of drug, βCD, their PM, and CE were recorded on METTLER TOLLEDO-DSC-822^e (USA) model of differential scanning calorimeter. The thermal behavior was studied by heating all samples (5–10 mg of drug or its equivalent) in sealed aluminum pans, using an empty sealed pan as reference, over a temperature range of 30–200°C at heating rate of 10°C/min.

Fourier Transform Infrared Spectroscopy

FT-IR spectra were recorded on Jasco-700 FT-IR (UK) spectrophotometer using KBr discs. The instrument was operated under dry air purge and the scans were collected at scanning speed of 2 mm/s with resolution of 4 cm^{-1} over the region of 4,000–400 cm^{-1}.

X-Ray Diffraction

Powder XRD patterns were recorded using Phillips P Analytical X'Pert PRO powder X-Ray diffractometer (The Netherlands) using Ni-filtered, CuKα radiation, a voltage of 40 kV, and a current of 30 mA. The scanning rate employed was 1° per min and samples were analyzed between 2θ angles of over 5–45°.

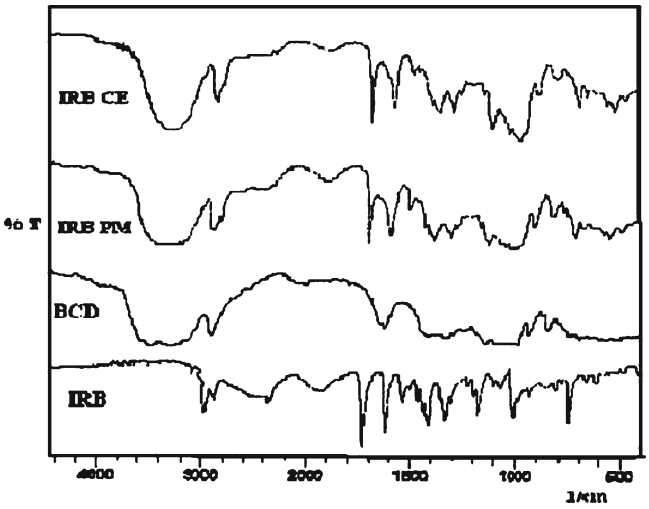

Fig. 3. FTIR spectra of IRB–βCD systems

Scanning Electron Microscopy

The surface morphology of the drug, physical mixture, and coevaporate were examined by a Philips 500 scanning electron microscope (Japan). The samples were fixed on a brass stub using double-sided tape and made electrically conductive by coating with a thin layer of gold by sputter coater Palaron E 5100. The photographs were taken at an electric voltage of 20 kV and a magnification of ×500 and ×2,500.

Proton Nuclear Magnetic Resonance

The H^+ spectra were taken at 25°C on a Variant Mercury Plus model (Japan) operating at a proton frequency 400 MHz using a 5-mm sample tubes. Dimethyl sulfoxide [2.5 ppm from tetramethylsilane (TMS)] was used as solvent. Chemical shifts were expressed in parts per million downfield from the signal (0 ppm) of TMS.

Saturation Solubility Studies

In order to analyze the improvement in solubility by various complexation methods, saturation solubility studies were performed as per Li *et al.* (8) in distilled water in triplicate. Excess of pure drug, physical mixture, and inclusion complexes were added to 5 ml of distilled water in glass vials which were subsequently tightly closed and shaken for 24 h in a mechanical shaker at room temperature to achieve the equilibrium. In preliminary studies, it was found that equilibrium solubility was achieved in 24 h and therefore, samples were shaken for 24 h. Appropriate aliquots were then withdrawn, filtered, diluted, and were analyzed spectrophotometrically at 244 nm.

Dissolution Studies

The dissolution studies were performed in triplicate in dissolution apparatus (Model: Veego VDA-6DR tablet dissolution test apparatus, India) using the paddle method (US Pharmacopeia type II). Dissolution studies were carried out using 1,000 ml of 0.1 N hydrochloric acid (pH 1.2) at 37± 0.5°C at 50 rpm. IRB 75 mg or its equivalent amount of IRB–βCD complex was added to 1,000 ml of 0.1 N HCl. Samples of 5 ml were withdrawn at time intervals of 5, 10, 20, 30, 45, 60, 90, 120, and 180 min. The volume of dissolution medium was adjusted to 1,000 ml by replacing with 5 ml of fresh 0.1 N HCl. The solutions were immediately filtered through 0.45 μm membrane filter, suitably diluted,

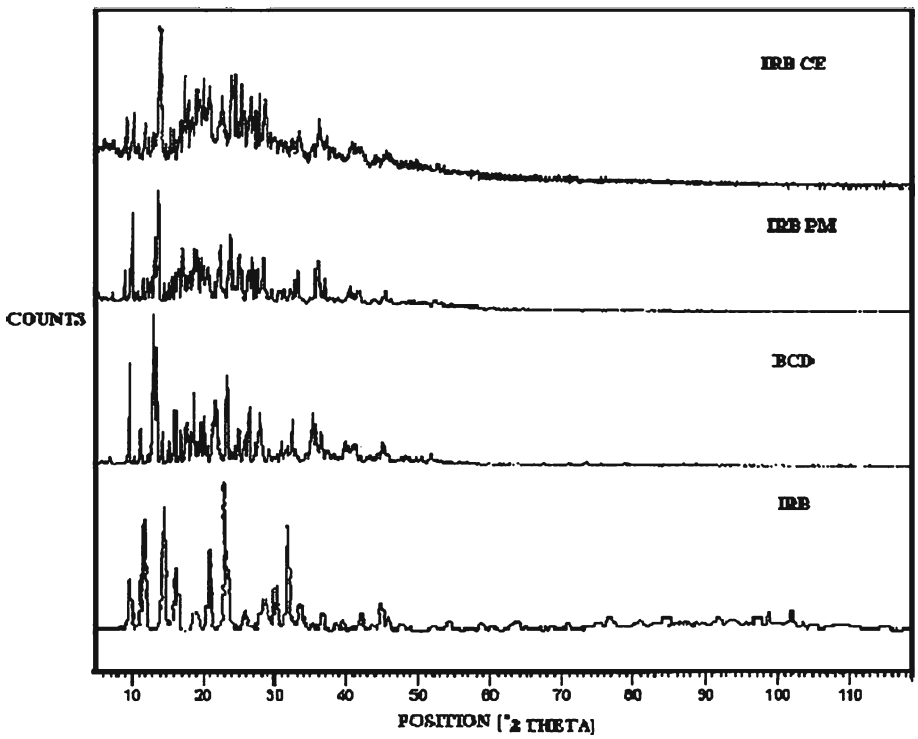

Fig. 4. XRD spectra of IRB–βCD systems

and the concentrations of IRB in samples were determined spectrophotometrically at 244 nm. The dissolution profile was constructed by plotting the percent drug dissolved against time.

RESULTS AND DISCUSSIONS

Phase Solubility Studies

The phase solubility profile of IRB–βCD is presented in Fig. 1. The solubility of IRB increased with increasing concentration of βCD and hence, phase solubility diagram could be classified as A_L type according to Higuchi and Connors (7). The linear host–guest correlation coefficient R^2 0.9963 and slope of 0.0542 indicated that a complex of 1:1 molar ratio was formed. IRB showed solubility enhancement of 42.27% at 10 mM concentration of βCD with 104.39 M^{-1} as the apparent stability constant (K_s).

Differential Scanning Calorimetry

The DSC curve for IRB alone and its binary mixture with the carrier are shown in Fig. 2. The thermal curve of IRB showed a sharp melting endothermic peak at 182°C, corresponding to the melting point of the drug (3). The DSC thermogram of βCD showed a broad endotherm in the range of 90–120°C (9). In the thermal curve of physical mixture of drug and cyclodextrin, the endothermic peak for drug is slightly broadened. The comparison of DSC curves of the pure components and the respective drug-carrier system prepared by coevaporation method revealed that sharp endothermic peak of drug was broadened to a greater extent. As explained by Zingone and Rubessa (9), broadening of the peak suggested that inclusion complex was formed between drug and CD. Hence, broadened drug endothermic peak may be a strong indication of the formation of amorphous entity along with interaction between IRB and βCD (10).

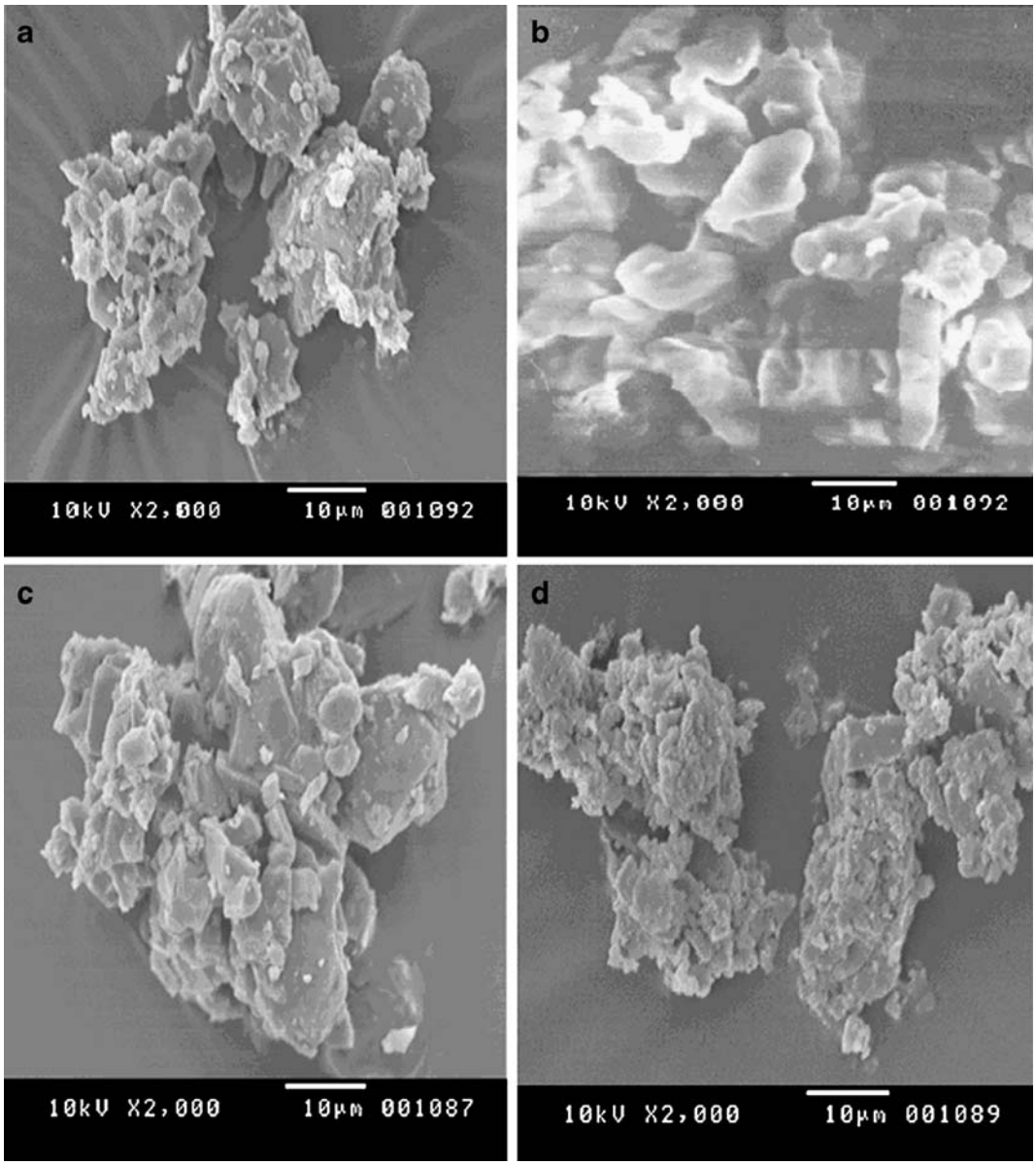

Fig. 5. Scanning images of **a** Irbesartan, **b** β-cyclodextrin, **c** physical mixture, and **d** coevaporate

Table I. 1H NMR Chemical Shifts Corresponding to IRB in the Absence and Presence of βCD (Coevaporate)

βCD	δ_{free}	$\delta_{complexed}$	$\Delta\delta$ (ppm)	Comment
H_1	4.673	4.668	0.005	Downfield
H_3	3.498	3.537	0.039	Downfield
H_4	3.579	3.560	−0.019	Up field
H_5	3.791	3.749	−0.042	Up field

δ_{free} ^{1}H NMR chemical shifts corresponding to IRB in the absence of βCD, $\delta_{complexed}$ ^{1}H NMR chemical shifts corresponding to IRB in the presence of βCD, *Δδ (ppm)* difference in chemical shifts values corresponding to IRB in the absence and presence of βCD, *βCD* β-cyclodextrin

Fourier Transform Infrared Spectroscopy

The FTIR spectra of IRB, βCD, their physical mixture, and IRB–βCD inclusion complex are illustrated in Fig. 3. IR spectrum of IRB was characterized by strong absorption peaks at 1,731 and 1,622 cm^{-1} assigned to C=O and C–N stretch, respectively. In IR spectra of CE, peak at 1,731 cm^{-1} was shifted to a lower frequency of 1,711 cm^{-1} suggesting formation of hydrogen bonds between the carbonyl groups of IRB and βCD, during inclusion complexation. These findings were in full agreement with earlier author Fernandes *et al.* (10) who reported similar behavior for nicardipine–βCD complex. A similar observation was also made for prazosin HCl–βCD complex by Liu and Zhu (11). Peak at 1,622 cm^{-1} was shifted to a higher frequency of 1,641 cm^{-1} indicating an interaction between IRB and βCD.

X-Ray Diffraction

The powder diffraction patterns of IRB, βCD, PM, and CE are shown in Fig. 4. The XRD pattern of drug was characterized by presence of sharp peaks at 9.277°, 12.68°, 17.77°, 19.48°, 21.34°, 32.13°, and 34.94° (2θ) indicative of the crystalline nature of drug (3). The crystalline nature of βCD was evident from presence of sharp peaks at 7.31°, 10.38°, 12.38°, 14.487°, and 22.838° (2θ). The physical mixture was characterized by presence of combined peaks of drug as well as βCD, however, with slightly reduced intensities. The diffractogram of CE complex was found to be devoid of characteristic peaks at 9.277°, 17.77°, 19.48°, 21.34°, and 34.94° (2θ) of drug indicating loss of its crystalline nature. The sharpness of peaks as well as the number of sharp peaks existing with plain drug was found to be significantly diminished in case of complex which may mainly be due to the existence of drug in a totally different form other than crystalline as a result of processing during the formation of inclusion complex (8,10).

Table II. Solubility Profile of Plain IRB, Physical Mixture, and Inclusion Complex

SAMPLE	Solubility in distilled water at 25°C (n=3; μg/ml)
IRB	37.8
PM	64.2
CG	70.2
K1	105.0
K2	103.2
CE	137.6

IRB Irbesartan, *PM* physical mixture, *CG* cogrinding, *K1* kneading using alcohol, *K2* kneading using aqueous alcohol, *CE* coevaporation

Scanning Electron Microscopy

From SEM scans as seen in Fig. 5a, pure IRB particles appeared as irregular shaped crystals (10–30 μm), βCD particles (Fig. 5b) consisted of crystals of irregular sizes. Microscopic examination of IRB–βCD PM (Fig. 5c) showed the presence of IRB crystals mixed and adhered on the surface of CD particles revealing no apparent interaction between both species in the solid state. CE products as seen in Fig. 5d showed small and irregular pieces with a change from crystalline to amorphous nature. Fernandes *et al.* (10) have found that a modification in the shape of drug particles was indicative of a new solid state. Thus, changes in the morphology of CE complex as compared to drug showed interaction between IRB and βCD.

Proton Nuclear Magnetic Resonance

The ^{1}H NMR studies on IRB alone and in the presence of βCD (CE) were performed since the NMR technique is known to yield useful information on the nature of the interactions between CDs and guest molecules. In the CD cavity, the hydrogen atoms (H_3 and H_5) are located in the interior of the cavity and hydrogen atoms (H_1, H_2, H_4, and H_6) are located on the outer surface of the cyclodextrin molecule cavity. When any guest molecule gets incorporated in the CD cavity, the hydrogen atoms located inside the cavity experience significant changes in the δ (parts per million)

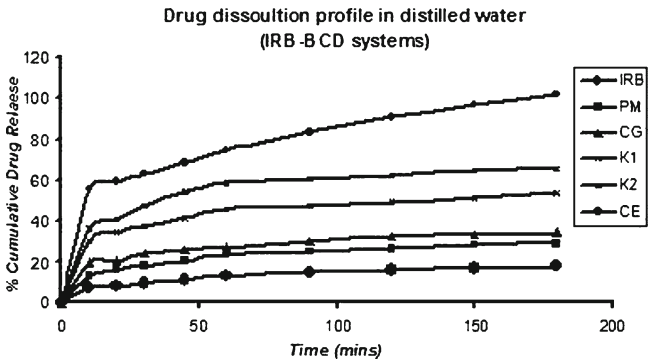

Fig. 6. Dissolution profile of IRB–βCD systems at 37±0.5°C (n=3): *open diamond* IRB, *open square* physical mixture, *open triangle* cogrinding, *multiplication symbol* kneading using alcohol, *closed square* kneading using aqueous alcohol, *closed diamond* coevaporation

values. But in case of association of guest molecule with CD the hydrogen on the exterior surface shows shifts in δ (parts per million) values (12).

The differences in chemical shift values of IRB in free and complexed state was as presented in Table I, showing changes in δ (parts per million) values for interior as well as exterior hydrogen of βCD. This suggested that there was association as well as complexation of drug with βCD. Since the $\Delta\delta$ (parts per million) values for H_3 and H_5 were significantly higher than that of the $\Delta\delta$ (parts per million) values of H_1, H_2, H_4, and H_6 hydrogen, it could be concluded that the extent of inclusion complex formation was comparatively higher than the association of drug with βCD (13).

Saturation Solubility Studies

The solid binary systems of IRB and βCD showed increase in aqueous solubility as compared to the pure drug alone with CE complex showing the highest solubility than all other binary systems as shown in Table II. The stability constant, 104.39 M^{-1}, showed that IRB and βCD have affinity toward each other to form stable inclusion complex and hence attributed to the enhancement of the solubility of drug. The solubility of PM and CE was increased by 41.12% and 72.5%, respectively, as compared to plain drug. The enhancement in aqueous solubility from kneading product of IRB can be explained in terms of wetting property of βCD with simultaneous reduction in the crystallinity of the drug and in CE product, by inclusion into the hydrophobic cavity of βCD.

Dissolution Studies

The dissolution curves of all the binary systems are shown in Fig. 6. According to these results, PM and CG binary systems gave better drug release than plain drug. Binary systems prepared by kneading with alcohol [K1] and aqueous alcohol [K2] showed higher dissolution rates as compared to PM and CG. A very high increase of the drug dissolution rate was found in the case of coevaporation method probably due to formation of soluble inclusion complex, amorphization of drug confirmed by XRD and DSC studies, and better wettability of the drug. The improvement in the dissolution was as follows: IRB < PM < CG < K1 < K2 < CE, suggesting that the dissolution rate was influenced by the preparation method of the binary systems.

CONCLUSION

IRB interacts with βCD to form inclusion complex in 1:1 molar ratio resulting in an increased solubility. Preparation methods strongly influenced the ability of cyclodextrin to include the drug. All binary systems with βCD displayed better dissolution profile compared to IRB alone with CE method giving best solubility and dissolution. Characterization by DSC, FTIR, XRD, SEM, and NMR confirmed the formation of inclusion complex. Enhancement of the solubility of IRB was 72.5% with a 3.4-fold increase in its dissolution rate. Thus, the IRB–βCD CE complex having improved solubility and dissolution rate can be incorporated into suitable dosage form which will have improved bioavailability.

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