Physicochemical Characterization, In Vitro Dissolution Behavior, and Pharmacodynamic Studies of Rofecoxib-cyclodextrin Inclusion Compounds. Preparation and Properties of Rofecoxib Hydroxypropyl β-cyclodextrin Inclusion Complex: A Technical Note

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INTRODUCTION

Rofecoxib is a nonsteroidal anti-inflammatory drug (NSAID) prescribed for the long-term treatment of musculoskeletal complaints.[1] The major drawback to NSAID drug use is the preponderance of gastrointestinal (GI) side effects. These are generally recognized to be due to interference of the drug with the biosynthesis of prostaglandins and other arachidonic acid metabolites in the gastric mucosa. These side effects can reduce patient compliance and discourage physicians from prescribing them. The most common GI adverse effects include upper GI perforations, ulcerations, and bleeding, which may require hospitalization. There is, therefore, a need for a delivery system for NSAIDs with improved GI tolerability that retains its efficacy.

Complexation with cyclodextrins has been reported to enhance the solubility, dissolution rate, and bioavailability of poorly water soluble drugs. They are known for their ability to moleculary encapsulate a wide variety of drugs into their hydrophobic cavity without the formation of any covalent bonds.[2–5]Cyclodextrins (CDs), especially hydroxypropyl β-cyclodextrin (HPβ-CD), are widely used in the pharmaceutical field owing to their high aqueous solubility and ability to stabilize drug molecules.[6–8] Rofecoxib/β-CD systems have been prepared by a kneading method in a 1:1 molar ratio with a subsequent improvement in dissolution due to amorphization.[9]

Rofecoxib is a selective COX-2 inhibitor with strong anti-inflammatory activity. It is practically insoluble in water (4.6 mg/L)[1] and has been implicated in causing GI ulceration by remaining in contact with stomach mucosa for a longer duration of time than is required, resulting in dangerously high concentration. The present study is an attempt to form an inclusion complex of rofecoxib with HPβ-CD to improve the aqueous solubility of the drug, thus enhancing its dissolution rate, thereby leading to a faster onset of action and less GI mucosal toxicity.

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MATERIALS AND METHODS

Materials

Rofecoxib was obtained as a gift sample from Ranbaxy Laboratories Pvt Ltd (New Delhi, India). Rofebax 12.5 mg conventional tablets (Ranbaxy Laboratories Pvt Ltd, New Delhi) were purchased from the market. HPβ-CD was purchased from Sigma Aldrich (St. Louis, MO). Other reagents and chemicals were of analytical reagent grade.

Preliminary Studies

Solubility Studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors.[10] An excess amount of rofecoxib was added to the aqueous solution of HPβ-CD solution (molecular weight = 1380) at various concentrations (2 to 10 mM/L). The contents were stirred for 72 hours at 30°C ± 1°C. After equilibrium, the samples were filtered and absorbance read at 268 nm (Shimadzu 1601 UV/Vis spectrophotometer, Shimadzu Corp, Kyoto, Japan).

The apparent stability constant was calculated from the initial straight portion of the phase solubility diagram using the following equation:

$$K_{1:1} = \frac{Slope}{Intercept(1 - slope)}$$

Preparation of Inclusion Complexes

Rofecoxib and HPβ-CD complexes were prepared in a molar ratio of 1:1 by the physical mixture and spray-drying methods.

Physical Mixtures (PMs)

The ground components were mixed in a mortar and sieved through a 100-μm mesh British Standard (BS) sieve.

Spray-dried Products (SDs)

Rofecoxib and HPβ-CD were dissolved in dichloromethane and ethanol (1:1). The resultant solution was stirred for 24 hours and spray dried under the atomization pressure of 1.8 kg/cm^2 and feed pump efficiency of 15% to 17% at a rotation rate of 20 000 rpm. The inlet temperature was kept at 69°C and outlet temperature at 58°C. The samples obtained were collected and passed through a 100-μm mesh BS sieve. Dichloromethane was taken as the solvent, as both the drug and cyclodextrin were completely soluble in it.

Characterization of Solid Complexes

The complexes were characterized and evaluated by the following:

- Differential scanning calorimetry (DSC): Thermal behavior of rofecoxib, HPβ-CD, and each inclusion complex was examined by using a DuPont (Wilmington, DE) model 910 thermal analyzer. Argon was used as the carrier gas and the DSC analysis was performed at a heating rate of 10°C/min and an argon flow rate of 35 cc/min. The sample size was 5 mg and examinations were made in the temperature intervals between 50 and 500°C.
- Powder x-ray diffraction studies (XRD): XRD of the samples was performed using a high-power x-ray diffractometer RU-200B from M/s Riguao, (Tokyo, Japan). The scanning rate was 4°/min. The voltage/current used was 40 kV/50 mA and the target/filter (monochromator) was copper.
- Fourier transform infrared spectroscopy (FT-IR): FT-IR spectral studies were carried on FT-IR Magma IR 750 by nicolet series II instrument using KBr disc method (Nicolet Analytical Instruments, Madison, WI). Scanning was done from 4000 to 500 cm^{-1}.
- Scanning electron microscopy (SEM): SEM of samples was performed using a Jeol scanning microscope JSM-840 (Tokyo, Japan) with a 10-kV accelerating voltage. The surface of the samples for SEM were previously made electrically conductive in a sputtering apparatus (Jeol Fine Coat, ion sputter, JFC-1100, Tokyo, Japan) by evaporation of gold. A magnification of ×1500 was used.

Aqueous Solubility Studies

The aqueous solubility of compounds, ie, pure drug powder and rofecoxib HPβ-CD inclusion complex, were determined at 37°C ± 0.5°C in pH 1.2 and pH 7.4. Solubility was measured by shaking a well-powdered or well-dispersed solute in excess (40 mg) with water (100 mL) until equilibrium was attained. Solute and solvent were placed in stoppered conical flasks immersed in a thermostated water bath and agitated continuously for 24 hours. The temperature during agitation was kept at 37°C ± 0.5°C. After 24 hours, the solution was filtered through a Millipore filter (0.22 μm) (Millipore, MA). It was diluted sufficiently with water and absorbance was recorded at 268 nm. By using the calibration curve, aqueous solubility was determined.

In Vitro Dissolution Rate Studies

Dissolution studies were carried for pure rofecoxib and for inclusion complexes using USP paddle type dissolution apparatus at 37°C ± 1°C at 100 rpm. The dissolution medium used was 900 mL of simulated gastric fluid (SGF) pH 1.2 without pepsin and phosphate buffer pH 7.4. The drug and the inclusion complex were filled in a hard gelatin capsule shell so as to contain 12.5 mg rofecoxib/capsule. The amount of PM and SD complexes filled in the capsule so as to contain 12.5 mg of rofecoxib was 80 mg. The dissolution rate studies of rofecoxib from marketed preparation (Rofebax 12.5 mg conventional tablets, Ranbaxy) was also done. Sampling was performed after 5, 15, 30, 45, 60, 90, and 120 minutes. The rofecoxib content was determined spectrophotometrically at 268 nm (Spectronic 21 UV/Vis spectrometer). All studies were performed in triplicate.

Pharmacodynamic Studies

Anti-inflammatory Studies

Anti-inflammatory studies were performed in a carrageenan-induced rat hind paw edema model.[11] Wistar male rats, weighing between 150 and 210 g were fasted prior to the experiment, but water was allowed ad libitum. The animals were divided into 4 groups of 4 animals each. Group 1 received 1.2 mg/kg of pure rofecoxib suspended in sodium carboxymethylcellulose (CMC). Group 2 received pure HPβ-CD solution prepared in water and Groups 3 and 4 received rofecoxib HPβ-CD physical mixture and spray-dried complex (1:1), respectively, at a dose of 1.2 mg/kg equivalent to rofecoxib. Immediately after drug administration, 0.1 mL of 1.0% carrageenan in sodium CMC was injected into the plantar surface of the hind paw and the paw volume was measured with the help of a plethysmometer (Ugo Basile biological research apparatus, Comerio VA, Italy) at 1, 2, 3, 4, 5, and 6 hours after administration of carrageenan.

Ulcerogenic Studies

The potential of the prepared inclusion complexes in producing gastric ulceration was studied in the fasted rat

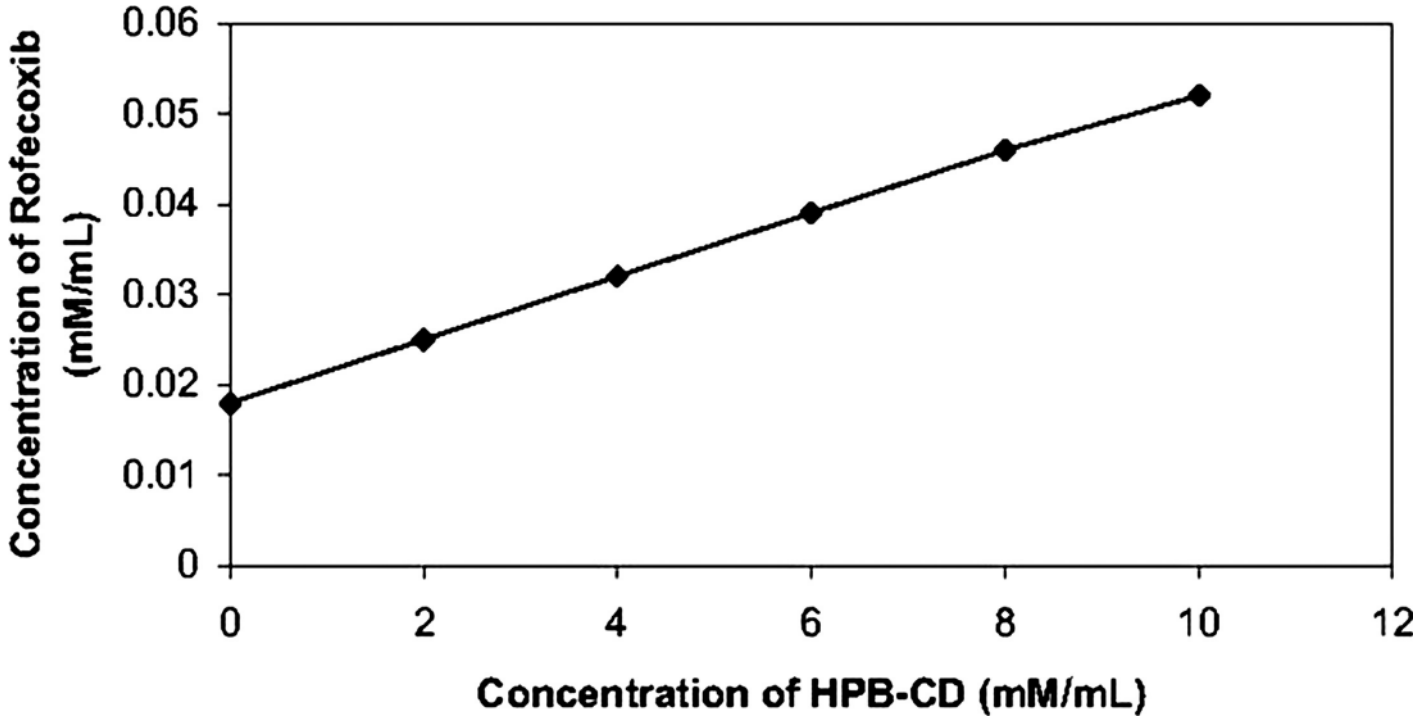

Figure 1. Phase solubility diagram.

model.[12] Wistar male rats weighing between 150 and 210 g were fasted for 24 hours prior to experimentation and water was allowed ad libitum. The animals were divided into 4 groups of 4 animals each. Group 1 received pure rofecoxib suspended in sodium CMC at a dose of 200 mg/kg and served as control. Group 2 received pure HPβ-CD solution. Group 3 received rofecoxib HPβ-CD inclusion complex prepared by the PM method. Group 4 received rofecoxib HPβ-CD inclusion complex prepared by the spray-drying method. 200 mg/mL/kg body weight, volume were administered orally with a mouth feeder to the animals for a total period of 4 hours. The rats were killed, under ether anesthesia, after 4 hours. Isolated stomach was opened up along the greater curvature and its contents carefully washed under tap water. The hemorrhagic lesions, produced in the glandular portion, were observed under a dissection microscope (Olympus, New Delhi, India), at ×20 magnification and evaluated using the following scores:

0.0 – Normal (no injury, bleeding, and latent injury)

0.5 – Latent injury or widespread bleeding

1.0 – Slight injury (2 to 3 dotted lines)

2.0 – Severe injury (continuous lined injury or 5 to 6 dotted injuries)

3.0 – Very severe injury (several continuous lined injuries)

4.0 – Widespread lined injury or widened injury

RESULTS AND DISCUSSION

Phase Solubility Studies

The phase solubility diagram for rofecoxib-HPβ-CD system in water is shown in Figure 1. A linear increase in solubility of rofecoxib was observed with increasing concentration of HPβ-CD. Since the slope of the diagram was less than 1 (0.0035), the complex stoichiometry was assumed to be 1:1. The value of the stability constant was found to be 195.11 M^{-1}.

Differential Scanning Calorimetry

The DSC graph of pure rofecoxib drug powder showed a sharp endotherm near 210°C, which is indicative of its melting temperature. In the thermogram of HPβ-CD, 2 endothermic peaks were observed. In the temperature range between 160°C and 190°C, loss of water occurs, and near 350°C, the endothermic peak corresponding to HPβ-CD, fusion is observed.

The DSC pattern of rofecoxib-HPβ-CD inclusion complexes (1:1) prepared by PM showed the presence of peaks of both pure compounds except with the difference that the drug-melting endotherm had slightly shifted from its original position of 210°C to 200°C and the endotherm did not appear as a sharp peak.

The thermogram of rofecoxib HPβ-CD complexes prepared by the spray-drying method (1:1) showed complete

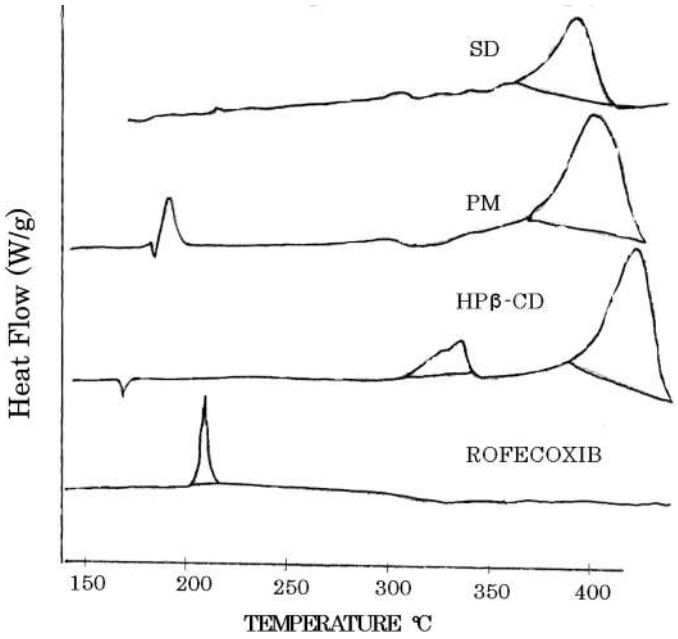

Figure 2. DSC thermograms of rofecoxib and HPβ-CD and their inclusion compounds.

disappearance of the endothermic peaks characteristic of rofecoxib, thus suggesting maximal/complete complex formation (Figure 2).

Powder X-Ray Diffraction

The XRD pattern of rofecoxib showed peaks that were intense and sharp, indicating its crystalline nature. Inclusion complexes of rofecoxib with HPβ-CD PM (1:1) showed undefined, broad, diffused peaks with low intensities. Though this signifies an amorphous nature, a few sharp peaks having less intensities were observed with a 1:1 ratio.

The inclusion complexes of rofecoxib with HPβ-CD prepared by the spray-drying method (1:1) showed peaks of diminished intensity suggesting almost complete amorphization of the drug (Figure 3).

Fourier Tranform Infrared Studies

Since FTIR is a highly sensitive method of analysis, all spectra of complexes show some or other changes from parent spectra, ie, pure drug and cyclodextrins. Some complex formation could thus be assigned to every method and every ratio. The IR spectra of rofecoxib showed the presence of the following peaks: 1737 cm^{-1} (5-membered lactone ring); 1640 cm^{-1} (unsaturation); and 1500 cm^{-1}, 970cm^{-1}, and 820 cm^{-1} (aromatic nucleus). The IR spectra of HPβ-CD showed prominent absorption bands at 3414 cm^{-1} (for O-H stretching vibrations); 2933 cm^{-1} (for C-H stretching vibrations); and 1164 cm^{-1}, 1083 cm^{-1}, and 1083 cm^{-1} (C-H, C-O stretching vibration). In the IR spectrum of rofecoxib-HPβ-CD physical mixture (1:1), the secondary amide band appeared at 3290 cm^{-1} along with the hydroxyl group band at 3414 cm^{-1} suggesting partial or little interaction of the drug with the HPβ-CD molecule. The IR spectrum of meloxicam-HPβ-CD PM in a molar ratio of 1:2 displayed absorption bands at 3404 cm^{-1} (-OH), 3290 (-CONH), 1620 (-CONH), 1550, 1530, 1346, 1161, and 1082 cm^{-1} indicating a mixture containing drug along with the complexing agent, ie, HPβ-CD. Slight shifting of -OH stretching vibration from 3414 cm^{-1} in the complexing agent, ie, HPβ-CD, to 3404 cm^{-1} in the PM (1:2) inclusion complex suggested interaction of the drug, ie, meloxicam, with the hydroxyl groups of HPβ-CD due to formation of weak hydrogen bondings.

However, in meloxicam-HPβ-CD freeze-dried complex in a molar ratio of 1:1, the amide N-H stretching vibration at 3290 cm^{-1} could not be detected indicating strong interaction between meloxicam and the hydroxyl groups of HPβ-CD.

In the IR spectrum of meloxicam-HPβ-CD freeze-dried complex (1:2), the amide N-H stretching vibration at

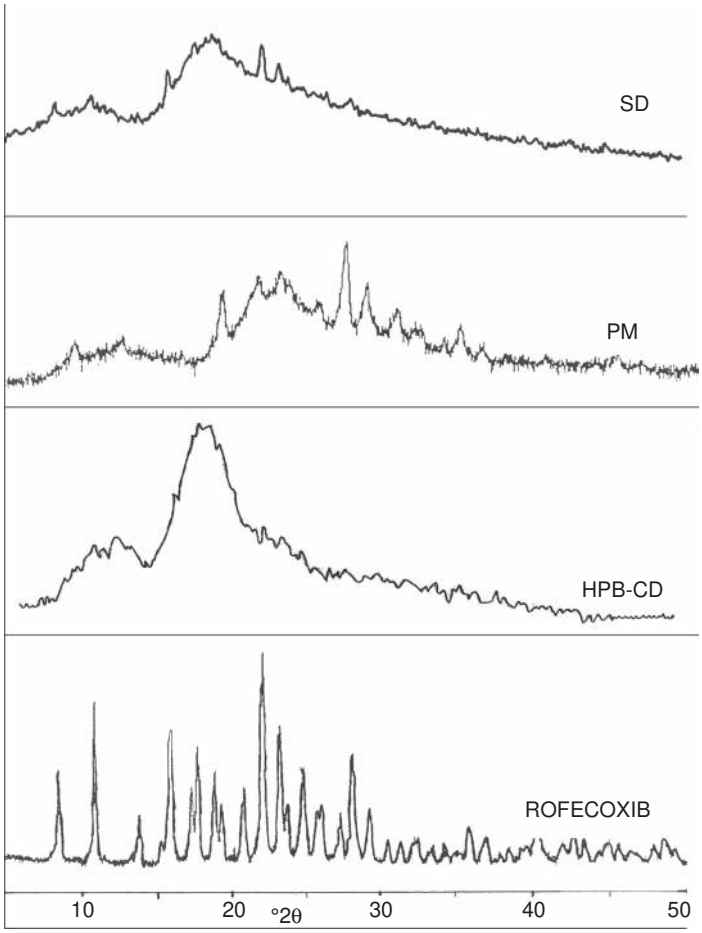

Figure 3. XRD patterns of rofecoxib and HPβ-CD and their inclusion compounds.

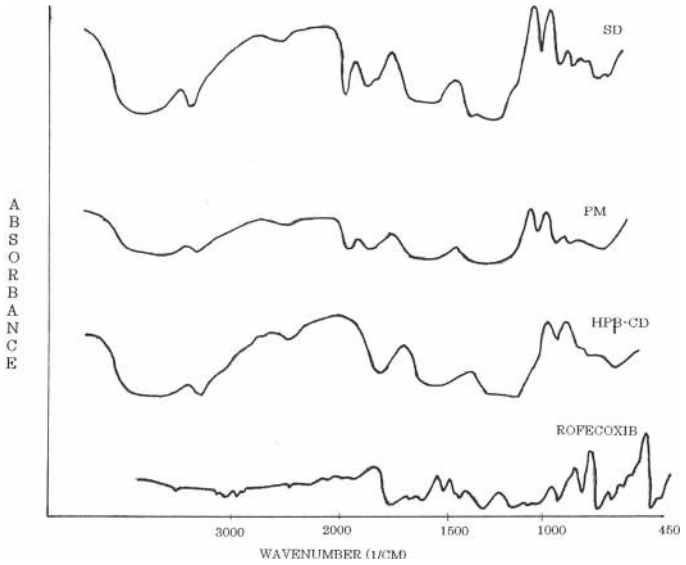

Figure 4. FT-IR spectra of rofecoxib and HPβ-CD and their inclusion compounds.

3291 cm^{-1} could not be detected, which might be due to co-occurrence of the N-H band with the O-H intensified band at 3400 cm^{-1}. This indicated a strong interaction and complete complex formation of meloxicam with HPβ-CD in a molar ratio of 1:2. The intensities of the bands appearing at 1623 cm^{-1}, 1523 cm^{-1}, 1460 cm^{-1}, 1349 cm^{-1}, and 1323 cm^{-1} were also affected due to such type of interaction (Figure 4).

Scanning Electron Microscopy

SEM is used to assess the microscopic aspects of the drug, the complexing agent, and the complexes formed. Although this method is not a very conclusive method to confirm complex formation, nevertheless it helps to assess the existence of a single component in the preparations obtained. Pure rofecoxib is characterized by the presence of a crystalline particle of regular size. Pure HPβ-CD also appears as crystalline particles without a definite shape. The inclusion complexes of rofecoxib/HPβ-CD PM showed the crystalline structure of both rofecoxib and HPβ-CD. Crystals of rofecoxib mixed with CD crystals were seen adhering to their surfaces.

The photomicrographs of spray-dried samples showed the typical morphology of preparations generally obtained by this method. That is, small-size particles tending to aggregation, suggesting the existence of an amorphous product with the presence of a single component in the complex, thus suggesting maximum or complete complex formation (Figure 5).

Aqueous Solubility Studies

The aqueous solubility of the optimized inclusion complexes, ie, rofecoxib/HPβ-CD, was more than the pure drug, ie, rofecoxib both in pH 1.2 and pH 7.4 (Table 1), indicating that a considerable portion of rofecoxib will be present in the nonionized form in the acidic gastric juice of the stomach. These nonionized molecules can easily penetrate

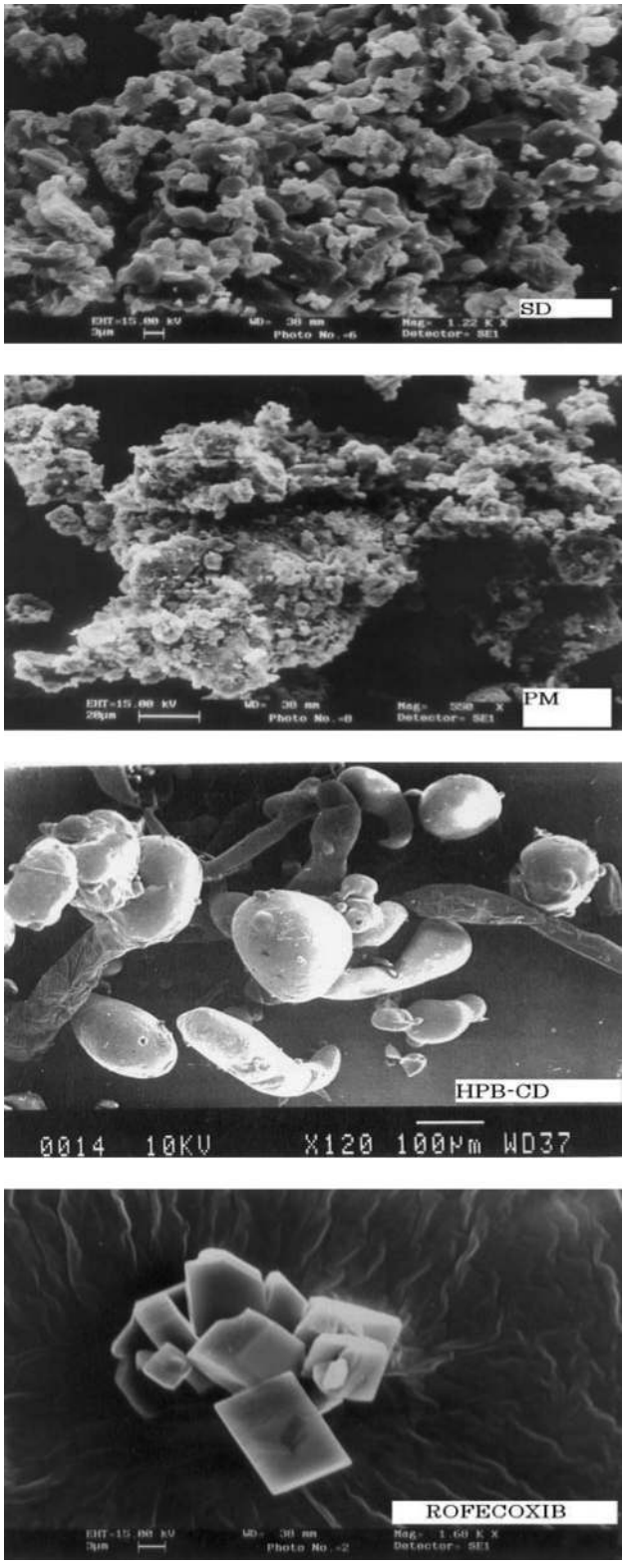

Figure 5. SEM photomicrographs of rofecoxib and HPβ-CD and their inclusion compounds.

Table 1. Aqueous Solubility Studies*

	Aqueous Solubility (μg/mL) n = 3	
Compound	**pH 1.2**	**pH 7.4**
Pure rofecoxib	5.6 ± 3.47	540 ± 4.45
Rofecoxib-HPβ-CD (PM) complex (1:1)	120 ± 3.77	850 ± 1.28
Rofecoxib-HPβ-CD (SD) complex (1:1)	568 ± 2.88	1480 ± 2.43

*HPβ-CD indicates hydroxypropyl beta cyclodextrin; PM, physical mixture; SD, spray-dried products.

through lipid membranes into the mucosal cells of the stomach wall, where the higher intracellular pH leads to ionization, which results in direct local damage to the gastric mucosa.

In Vitro Dissolution Rate Studies

It is assumed that the complexes show a higher release as compared with the pure drugs. Rapid dissolution is the characterstic behavior of inclusion complexes. The results in terms of percent of active ingredient dissolved at 5 and 15 minutes (DP_5, DP_{15}) are presented in Table 2 and the dissolution profiles are shown in Figures 6 and 7. One-way analysis of variance was used to test the statistical significance of differences between pure and treated samples. The DP_5 and DP_{15} values of SDs are significantly higher ($P < .05$) when compared with pure rofecoxib and marketed formulation.

The comparative release studies indicated that the release of active material was strongly affected by the method of formulation. The dissolution profiles of the complexes were studied in different dissolution media with the aim of differentiating between the dissolution behavior of the complexes. The dissolution characteristics of the complexes in SGF without pepsin pH 1.2 was studied to gain information about the dissolution of the drug in the acidic conditions of the stomach, which would have an influence on the ulcerogenic potential of the drug. It was observed that in SGF (pH 1.2) without pepsin after 5 minutes only 9.8% of pure rofecoxib was dissolved and after 2 hours only 54.3% of the drug went into solution, whereas in the case of rofecoxib-HPβ-CD inclusion complex prepared by the spray-drying method in a molar ratio of 1:1, 27.9% of drug was released after 5 minutes and after 2 hours 90.3% drug release was obtained. In phosphate buffer (pH 7.4), pure rofecoxib gave a maximum release of 55.6% at the end of 2 hours whereas rofecoxib-HPβ-CD inclusion complex (SD, 1:2) gave a complete release (99.6%) after 45 minutes. The dissolution studies revealed that all the formulations showed an increased rate and was more in alkaline medium, which may be due to the ionization of the drug as it is a weak acid. The improvement in the dissolution rate of the drug/cyclodextrin systems may be attributed to the degree of crystallinity of the active material, together with the increase in both the wettability and the solubility of the drug.

Anti-inflammatory Studies

The HPβ-CD complex prepared by the spray-drying method showed a faster onset of anti-inflammatory activity as compared with the pure drug rofecoxib spray-dried similarly (pH 5.5 ± 0.2), indicating maximum inhibition of edema. An 85% inflammation inhibition in the spray-dried group (pH 4.0 ± 0.1) was obtained after 20 minutes, whereas for rofecoxib, a 60% inflammation inhibition was observed after 1 hour (Figure 8). This indicates that in the complexed form the drug shows an improvement in the rate and extent of absorption. The difference was significant at the 5% level of significance indicating that in the complexed form the drug showed an improvement in the rate and extent of absorption ($P = .011$).

Table 2. $DP_{(5)}$, $DP_{(15)}$ and $DE_{(5)}$, $DE_{(15)}$ % Parameters of Pure Rofecoxib, Rofebax, and Inclusion Complex in pH 1.2 and pH 7.4*

		Dissolution Parameters			
		pH 1.2		pH 7.4	
Serial Number	**Product**	**$DP_{(5)}$**	**$DP_{(15)}$**	**$DP_{(5)}$**	**$DP_{(15)}$**
1.	Pure rofecoxib	9.8	22.2	8.5	14.5
2.	Rofebax (marketed preparation)	18.0	46.08	30.9	44.8
3.	Rofecoxib-HPβ-CD (PMs)	18.2	30.2	19.7	32.3
4.	Rofecoxib-HPβ-CD (SDs)	27.9	60.9	34.9	54.9

*$DP_{(5)}$ indicates percentage of drug released at 5 minutes; $DP_{(15)}$, percentage of drug released at 15 minutes; HPβ-CD indicates hydroxypropyl beta cyclodextrin; PM, physical mixture; SD, spray-dried products.

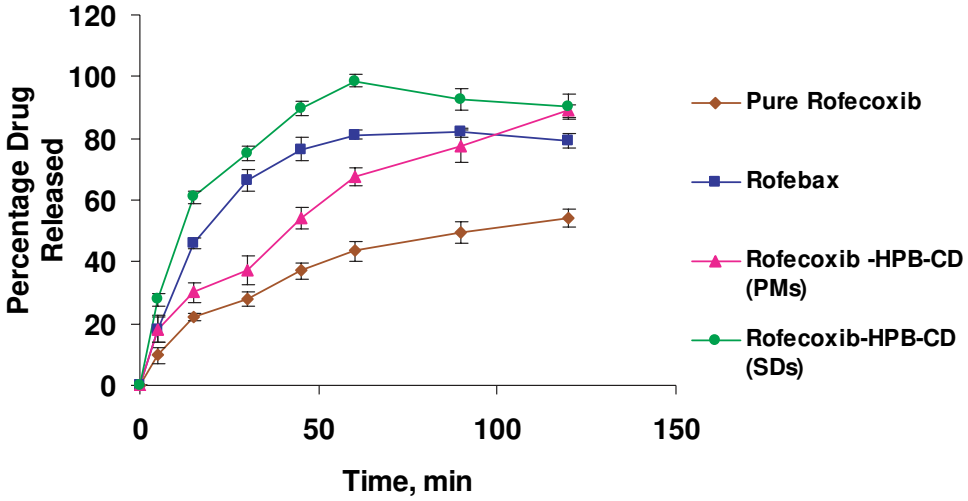

Figure 6. Dissolution profile in simulated gastric fluid pH 1.2.

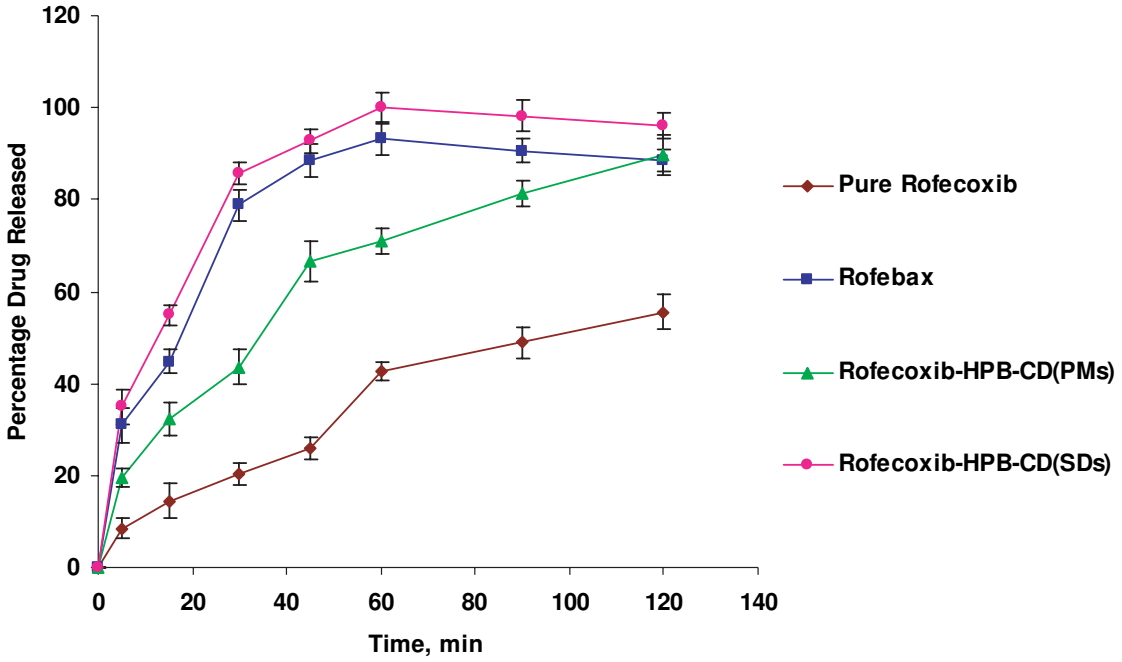

Figure 7. Dissolution profile in phosphate buffer pH 7.4.

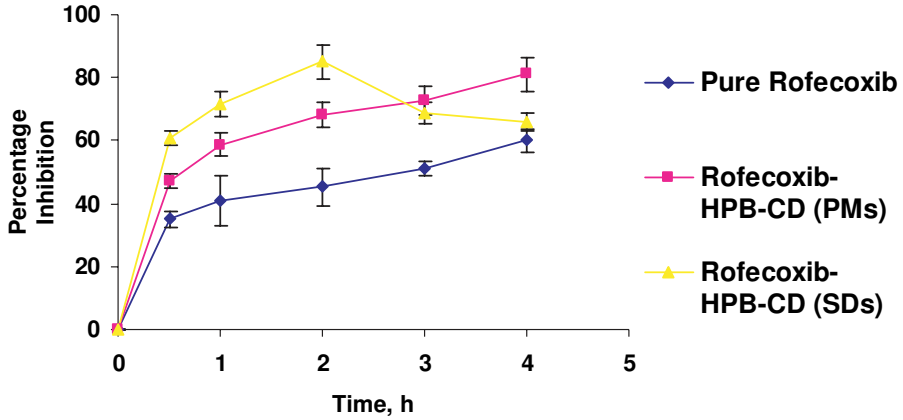

Figure 8. Anti-inflammatory studies of rofecoxib and rofecoxib HPβ-CD complex by rat hind paw edema method (mean ± SD; n = 4).

Table 3. Degree of Injury to the Stomach of Rats*

Sample	Degree of Injury
Pure rofecoxib	1.80 ± 0.63
Pure HPβ-CD	0.00 ± 0.00
Rofecoxib-HPβ-CD complex (PM)	1.75 ± 0.70†
Rofecoxib-HPβ-CD complex (SD)	0.75 ± 0.25†

*mean ± SD; n = 4; HPβ-CD indicates hydroxypropyl beta cyclodextrin; PM, physical mixture; SD, spray-dried products.
†$P < .05$

Ulcerogenic Studies

In the ulcerogenic studies, at the dose level of 200 mg/kg of rofecoxib, the spray-dried complex prepared with HPβ-CD gave the lowest score, 0.75 ± 0.25, as compared with rofecoxib, 1.8 ± 0.63 (Table 3). Complex prepared by physical mixing showed an intermediate effect (score: 1.75 ± 0.70). The difference was statistically significant at the 5% level of significance ($P = .04$). It is reported that crystals of NSAIDs being poorly soluble in gastric acid remain in contact with the stomach wall for a longer period of time, resulting in a dangerously high local concentration. This leads to local irritation of the stomach wall and to ulceration. It is expected that in the complexed form, the drug will dissolve fast and show an accelerated absorption. Moreover, it will not come in direct contact with the stomach wall in the crystalline state since until it is dissolved it remains encapsulated within the cyclodextrin matrix.[12]

CONCLUSION

An inclusion complex of rofecoxib and HPβ-CD was prepared successfully by the spray-drying method in a molar ratio of 1:1. The inclusion complex was found to have improved in vitro drug release compared with the pure drug. The solubility profile of complexes of rofecoxib prepared using HPβ-CD as the complexing agent in a molar ratio of 1:1 by the spray-drying method in pH 1.2 and pH 7.4 indicated that the acid solubility of rofecoxib was enhanced considerably by formation of an inclusion complex with HPβ-CD. The above results also clearly demonstrated a significant decrease in the gastric ulcerogenic activity of rofecoxib through complexation with cyclodextrins. Even though the physical mixture of rofecoxib with cyclodextrins reduced ulcer formation, it was the spray-dried complex formation approach that minimized gastric ulceration. These findings are extremely important from a commercial point of view as the prepared complex removes a major drawback for rofecoxib in therapy.

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