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Enhancement of Oral Bioavailability of Rofecoxib Using β-Cyclodextrin

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Abstract

The purpose of the present work was to investigate the effect of complexation of rofecoxib with β-cyclodextrin on its dissolution characteristics and bioavailability. Inclusion complexes of rofecoxib with β-cyclodextrin were made by freeze-drying technique. Phase solubility studies were conducted as suggested by Higuchi and Connors. The samples were characterized by performing dissolution studies, X-ray Diffraction studies and Differential Scanning Calorimetry. The complexes were compressed into tablets and compared in-vitro with various marketed formulations. A single dose study on healthy human volunteers was performed in comparison with a marketed formulation of rofecoxib (without β-cyclodextrin) to investigate the relative bioavailability.

Phase solubility studies confirmed the formation of a 1 : 1 complex in solution of rofecoxib with β-cyclodextrin. Tablets of solid inclusion complexes of rofecoxib with β-cyclodextrin prepared by freeze drying technique showed enhanced dissolution rate in distilled water in comparison with all the marketed formulations analyzed. This is attributed to the increased solubility and wettability along with decreased crystallinity caused by complex formation, which is confirmed, by XRD and DSC studies. The bioequivalence studies performed showed statistically significant enhancement in bioavailability as compared to the marketed formulation. Apparently, tablets containing complexes of rofecoxib with β-cyclodextrin shows faster onset of action due to improved solubility, enhanced dissolution and faster absorption of the molecule. The results of this investigation with rofecoxib in β-cyclodextrin lend ample credence to its better oral bioavailability on complexation.

Introduction

Cyclodextrins show a remarkable ability to form inclusion complexes with various molecules that fit partially or entirely inside the cavity. This phenomenon modifies the physico-chemical properties such as solubility, dissolution rate and bioavailability of the guest molecules [1, 2]. Inclusion complexation of a number of drugs with β-cyclodextrin have been reported [3, 4]. Kneading, co precipitation, freeze-drying or sprays drying techniques are employed for the same.

Rofecoxib is a potent non-steroidal antiinflammatory drug (NSAID) strongly recommended for the treatment of arthritis. It is a selective COX-2 inhibitor with 1000 fold selectivity for COX-2 relative to COX-1. It shows high anti-inflammatory and analgesic activities in addition to low toxicity, moderate incidence of gastric side effects and a high therapeutic index [5–8].

However, it is practically insoluble in aqueous fluids; and as such its oral absorption is dissolution rate limited. Its aqueous solubility was found to be 0.01 mg/ml. The very poor aqueous solubility of the drug gives rise to difficulties in the formulation of dosage forms and may lead to variable dissolution rates and bioavailability. In the present work inclusion complexes of rofecoxib with β-cyclodextrin were tried to enhance the dissolution rate of rofecoxib.

Materials

Rofecoxib was supplied by Unichem Lab. Ltd. Mumbai, India. β-cyclodextrin (Cerestar) was generously donated by S.A. Chemicals, India. All other reagents and solvents used were of analytical grade.

Methods

Phase solubility studies

Solubility measurements were conducted in distilled water (pH 6.2 ± 0.1) according to Higuchi and Connors [9]. Excess drug was added to solutions of various increasing concentrations of β-cyclodextrin (β-CD) and suspensions equilibrated for 48 h on a mechanical shaker. Filtrates were analyzed after suitable dilutions using UV Spectrophotometer (CECW CE 2021 2000 SERIES) at 260 nm for rofecoxib content.

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Preparation of inclusion complexes [10, 11]

The freeze drying technique (LABCONCO, Freeze Dry System, Freezone 4.5) was used to prepare inclusion complexes of rofecoxib and β-cyclodextrin in two different molar ratios. Physical mixtures of plain rofecoxib and β-cyclodextrin were also prepared in 1 : 1 molar ratio by mixing the powders in geometric proportions.

Characterization of inclusion complexes

Differential scanning calorimetry (DSC) studies
The samples were subjected to DSC studies using Perkin Elmer DSC 7 model. Alumina was used as a reference material and samples were scanned at the rate of 10 °C/min.

Powder X-ray diffraction (XRD) studies
The XRD patterns were recorded using Philips X-ray generator (PW 1729) and automatic X-ray diffractometer model PW 1710 unit. The radiation used was Nickel filtered $CuK_{\alpha 1}$ radiation having a wavelength of 1.542A°, operating at 35 K watts and 20 mamps in the range (2θ) of 10° to 60°. Scanning rate used was 1.2°/min.

In-Vitro *dissolution studies*
The dissolution studies of samples were performed according to USP XXIII type II apparatus. Distilled water (pH 6.2 $\pm$ 0.1) was employed as dissolution medium at temperature of 37 $\pm$ 0.5 °C. The rotation speed was 100 rpm. The samples were withdrawn at various time intervals and analyzed spectrophotometrically (CECIL CE 2021 2000 SERIES) at 260 nm for rofecoxib content.

Tablet preparation
Tablets were made using rofecoxib (dose-25 mg), inclusion complexes and physical mixtures. Wet granulation was done using PVP K-30 (3%) as the binder in isopropyl alcohol. Final weights of the tablets were adjusted using Vivacel. Talc (1%) and magnesium stearate (0.5%) were used as anti-adherent and glidant respectively. The tablets were compressed using CADMACH single tablet press machine equipped with 10 mm flat beveled punches

Pharmacokinetic studies

Procedure
A single dose (25 mg) study was performed to compare the pharmacokinetic parameters and bioequivalence of Rofecoxib tablets formulated at our institute (test formulation) versus marketed tablets (standard formulation).

A total of twelve volunteers participated in the study. The volunteers were randomly assigned to the treatment groups. The volunteers were fasted overnight at least 10 hours prior to dosing. Blood samples (5 ml) were withdrawn at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 hours after administration of the dose using heparinised disposable syringes. Blood samples were collected in glass tubes. Plasma was separated by immediate centrifugation and stored in light protected containers at -20 °C until analysed.

Determination of rofecoxib from plasma
The plasma samples were analysed by Reversed Phase HPLC for the content of Rofecoxib and the pharmacokinetic parameters were computed and subjected to statistical analysis.

Extraction from plasma. To the plasma samples (1 ml), 2 ml of Acetonitrile was added and vortexed for 5 minutes and centrifuged for 15 mins at 4000 rpm. The supernatant was separated, filtered through 0.45-micron membrane filter and 100 μl was injected in the column.

Chromatographic conditions.

Instrument:	Jasco PU-980 HPLC pump with UV-975 detector.
Integrator:	Borwin Version 1.21 Chromatography software.
Column:	Waters Spherisorb S5 0DS2 [C-18 (250 mm $\times$ 40 mm, 5μ)].
Mobile phase:	Acetonitrile : Water [475 : 525 v/v].
Flow rate:	0.8 ml/min.
Injection volume:	100 μl.
Wavelength:	260 nm.
Retention time:	Rofecoxib- 6.64 mm.

Data analysis. A non-compartmental model was adopted for calculating the pharmacokinetic parameters. The parameters employed to evaluate bioequivalence of the formulations under investigation were $C_{\max}$, $T_{\max}$ and AUC values. $C_{\max}$ and $T_{\max}$ were read directly from the observed plasma concentration against time profile. AUC and other parameters such as K_{el} and $T_{1/2}$ were computed from observed plasma concentration against time profile using computer program in BASICA. The data obtained was statistically analyzed by ANOVA at 5% probability levels.

Results and discussion

Rofecoxib solubility in water was found to be 0.01 mg/ml. Phase solubility studies of rofecoxib in distilled water (pH 6.2 $\pm$ 0.1), confirmed the solubility enhancement capability of β-cyclodextrin, as shown in Figure 1. Solubility curve for β-cyclodextrin is classified as AL type, indicating the formation of a 1 : 1 stoichometry of the complex. The stability constant for rofecoxib-β-cyclodextrin was found to be 41.56 M^{-1}. The phase solubility studies indicated that the aqueous solubility of rofecoxib was greatly enhanced in the presence of β-cyclodextrin.

Freeze dried inclusion complexes of rofecoxib showed maximum dissolution compared to the drug alone or its physical mixture with β-cyclodextrin, as shown in Figure 2. The drug dissolution from tablets of 1 : 1.5 M complex showed better release rate as compared to the marketed formulation studied, as shown Figure 3. This may be due to the

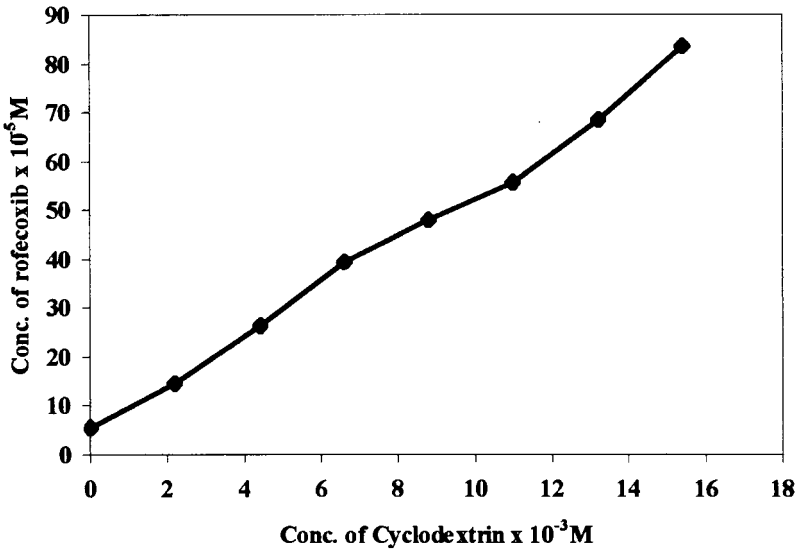

Figure 1. Phase solubility diagram of rofecoxib in distilled water.

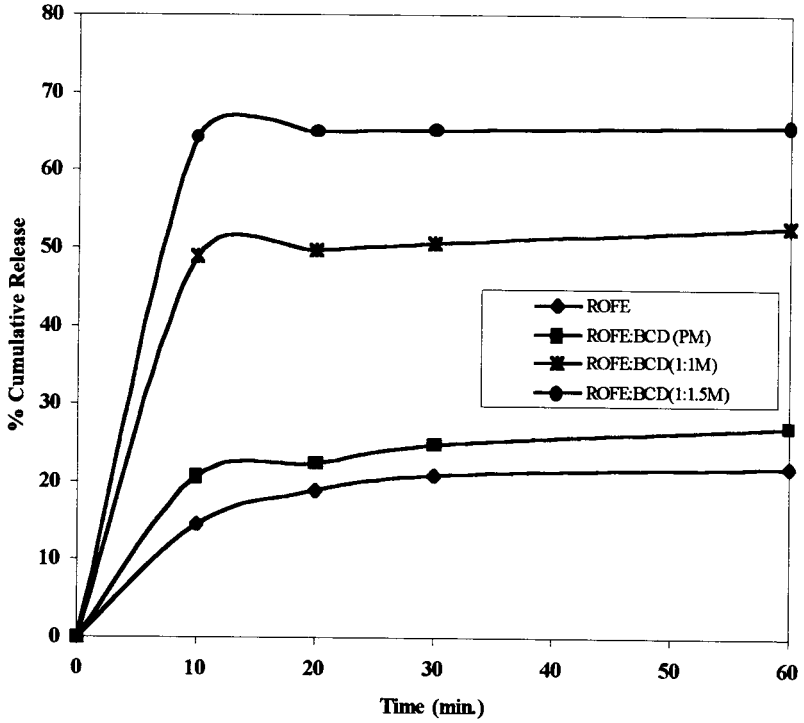

Figure 2. Dissolution profile of various rofecoxib-β-CD inclusion complexes in distilled water.

increased solubility and wettability along with the decrease in crystallinity caused by complex formation.

The DSC thermograms revealed the endothermic peak of rofecoxib at 268.5 °C. Thermograms of the rofecoxib-β-cyclodextrin complex showed absence of the characteristic endothermic peak of the drug, confirming formation of inclusion complex.

The XRD pattern of physical mixture was found to be a combination of drug and β-cyclodextrin. However, the XRD pattern of the rofecoxib-β-cyclodextrin complex was found to be diffuse and different, and the drug peak had disappeared. The drug peaks at 9.1, 12.7, 18.3, 22.5 and 26.4° observed in the physical mixture are diminished in the complex. Thus the XRD diltractograms showed the formation of a new solid phase for the inclusion complex.

The mean peak plasma levels and pharmacokinetic parameters of rofecoxib-β-cyclodextrin (containing 25 mg of rofecoxib – test formulation) and marketed tablet (containing 25 mg rofecoxib – standard formulation) are shown in Table 1. The mean peak plasma levels of the above formulations are shown in Figure 4.

There was a statistically significant difference in $C_{\max}$, $T_{\max}$ and AUC of the test formulation when compared with standard formulation of rofecoxib. The percent relative bioavailability of the test formulation as judged from AUC_{rm0-24} was 129.33 as compared to the standard formulation of rofecoxib. Thus, rofecoxib-β-cyclodextrin tablet

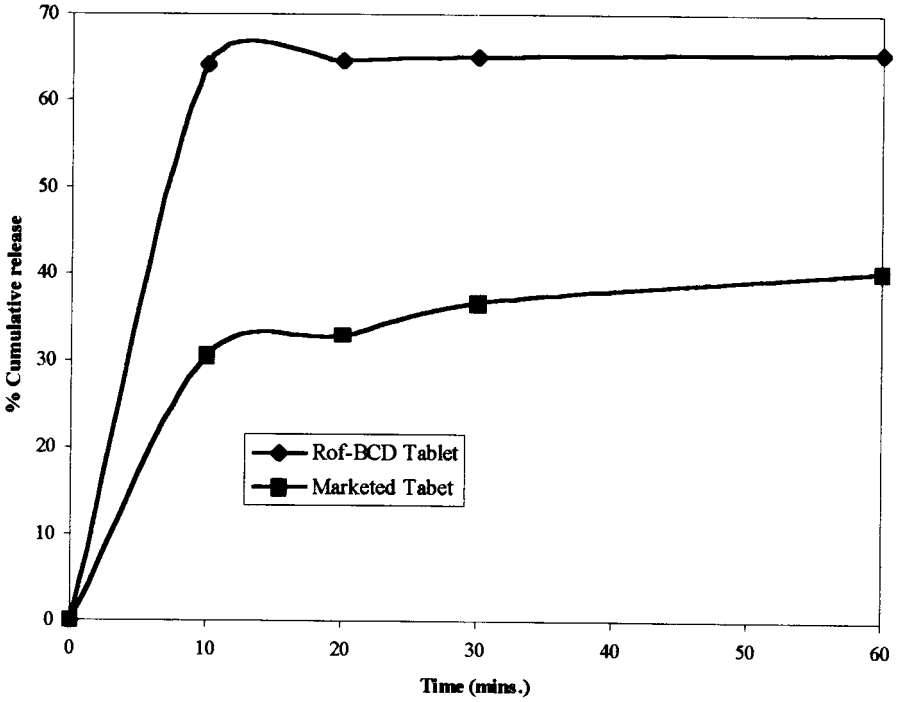

Figure 3. Dissolution data of tablet of rofecoxib-β-cyclodextrin freeze dried complex [1 : 1.5 M] as compared to the marketed formulation of rofecoxib [dose–25 mg].

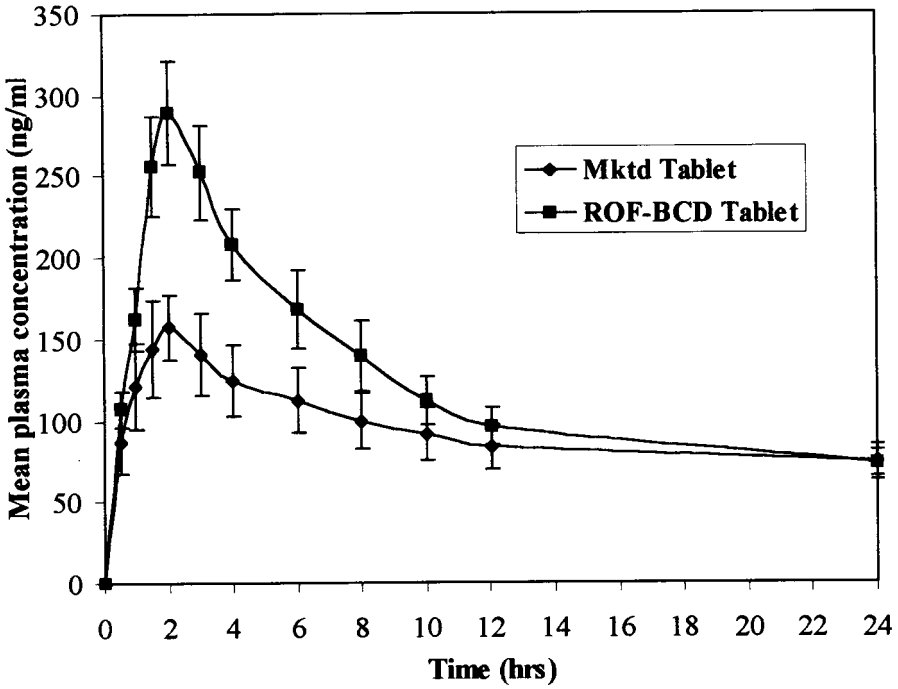

Figure 4. Mean plasma concentrations of rofecoxib in healthy human volunteers ($n = 12$) after single dose (24 mg) administration of Rof-BCD tablet in comparison to marketed tablet of rofecoxib.

formulation provides better oral bioavailability as compared to the marketed rofecoxib formulation.

Conclusion

The freeze-drying method could be successfully used for the formation of the inclusion complex of rofecoxib and β-cyclodextrin thus improving the solubility and dissolution rate of rofecoxib. Also, enhancement of drug bioavailability by complexation with β-cyclodextrin, as compared to the marketed rofecoxib formulation has been observed.

Table 1. Comparison of pharmacokinetic parameters after administration of Rof-BCD tablet and rofecoxib marketed tablet ($n = 12$)

Parameters	Rof-BCD tablet	Marketed tablet of rofecoxib	F ratio between the products
C_{max} (ng/ml)	300.82	162.30	6.18
	(± 21.40)	(± 22.63)	(S)
T_{max} (h)	1.83	1.83	0.00
	(± 0.26)	(± 0.37)	(NS)
K_{el}	0.0489	0.0419	8.92
	(± 0.0029)	(0.0028)	(S)
$T_{l/2}$ (h)	14.127	16.532	11.54
	(0.629)	(± 0.618)	(S)
AUC_{0--24} (ng h/ml)	4904.32	3791.92	9.83
	(± 382.9)	(± 324.7)	(S)
$AUC_{0-\infty}$ (ng h/ml)	5464.11	4016.78	7.35
	(± 4438)	(± 385.1)	(S)

F value from statistical table for ANOVA = 4.96.
S = Significant difference.
NS = Non-significant difference.

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