

In vitro and *in vivo* evaluation of mefenamic acid and its complexes with β -Cyclodextrin and HP- β -Cyclodextrin

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The objective of this research was to improve solubility, dissolution, and, consequently, bioavailability of mefenamic acid, a poorly water-soluble nonsteroidal anti-inflammatory drug, by complexation with β -Cyclodextrin and HP- β -Cyclodextrin. The complexes of mefenamic acid with β -Cyclodextrin and HP- β -Cyclodextrin were prepared by kneading method and were characterized and evaluated to study the effect of complexation on dissolution profiles and *in vivo* advantage. The complexes were characterized by Fourier transform infrared spectroscopy, X-ray diffraction, and differential scanning calorimetry studies. Phase solubility studies indicated complex formation with possible stoichiometry of 1:1 and a stability constant of 176 M^{-1} for β -Cyclodextrin and 103.8 M^{-1} for HP- β -Cyclodextrin. The characterization studies confirmed the inclusion of mefenamic acid within the nonpolar cavity of β -Cyclodextrin and of HP- β -Cyclodextrin. Remarkable improvement was observed in the *in vitro* drug release profiles in 0.1-N HCl and pH-6.8 phosphate buffer with all complexes. Mefenamic acid also showed improvement in the *in vivo* activity when administered orally to rats as compared with mefenamic acid *per se*.

Key words: β -Cyclodextrin, complexation, HP- β -Cyclodextrin, mefenamic acid

INTRODUCTION

There has been great interest in cyclodextrin inclusion compounds as a means of increasing the solubility and dissolution rate of poorly soluble drugs. β -Cyclodextrin and its derivatives have been used in pharmaceutical formulations to enhance the solubility, dissolution rate, membrane permeability, stability, and bioavailability of slightly soluble drugs. This is due to their ability to molecularly encapsulate a wide variety of drugs into their hydrophobic cavity, which imparts changes in physicochemical properties, resulting in the enhancement of water solubility and drug-dissolution rate. Poorly water-soluble drugs usually show low bioavailability as their absorption is dissolution-rate limited and is consequently low. Cyclodextrins are considered to be good candidates for dissolution-rate enhancement of drugs having poor water solubility.^[1-4]

Cyclodextrins are cyclic malto oligosaccharides in which the glucose units are linked by α -1, 4 glycosidic bonds. The particular arrangement of the glucose units imparts a conelike structure to the molecule, making

the exterior of the cone hydrophilic; and the interior, hydrophobic in nature. The characteristic shape of the polymer leads to the encapsulation of the drug in its cavity, resulting in the improvement in solubility and drug release.^[5-7]

Mefenamic acid (MA) is a potent nonsteroidal anti-inflammatory drug (NSAID) of the enolic acid class, which shows preferential inhibition of cyclooxygenase-2 (COX-2) and inhibits the prostaglandin synthesis. It is highly prescribed in the treatment of rheumatoid arthritis, osteoarthritis, and other joint diseases. However, its oral bioavailability is very low, probably due to poor solubility in water and insufficient dissolution rate. MA also acts as an irritant to the gastrointestinal mucosa when administered orally. Thus there is a need to find an approach to increase its oral bioavailability by enhancing its dissolubility using a pharmaceutical carrier. It has been well established that the ulcerogenic potential of several acidic NSAIDs, including phenylbutazone, salicylic acid, indomethacin, ketoprofen, etc., could be reduced by β -Cyclodextrin complexation.^[2-3,8-9] However, there is no investigation reported on complexation of β -Cyclodextrin with mefenamic acid.

The main objective of the present study was to evaluate the effect of β -Cyclodextrin (β -CD) and Hydroxy Propyl

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β -Cyclodextrin (HP- β -CD) on the solubility, dissolution rate, and, consequently, bioavailability of mefenamic acid. MA complexes with β -CD and HP- β -CD were prepared by kneading method and were characterized by differential scanning calorimetry (DSC) and x-ray diffractometry (XRD). The phase solubility, dissolution rates, and absorption studies in rats were carried out and compared.

MATERIALS AND METHODS

MA was obtained as gift sample from Blue Cross Lab Ltd., Nashik, India. β -CD and HP- β -CD of commercial purity grade were obtained from Cerestar, U.S.A. Inc., Hammond, Indiana. Double-distilled water was used throughout the study. All other solvents and reagents were of analytical grade.

Animals

Healthy male Sprague-Dawley rats (250-300 g) were purchased from Hindustan Antibiotics, Pune (India). Prior to the experiments, the rats were housed in a temperature- and humidity-controlled room (23°C, 55% air humidity) with free access to water and standard rat chow. The rats were acclimated for at least 5 days and fasted overnight but supplied with water *ad libitum* before the experiments. The permission for the use of animals was taken from the Ethical Review Committee.

Phase solubility studies

The phase solubility studies were carried out according to the method reported by Higuchi and Connors.^[10-11] Excess amounts of MA were added to aqueous solutions of β -CD and HP- β -CD at various concentrations ($1-8 \times 10^{-8}$ M) and were shaken for 24 h at room temperature on a rotary flask shaker. After equilibration, the solutions were carefully filtered through 0.45- μ m Whatman filter paper, adequately diluted, and analyzed for drug content using a UV-Visible Spectrophotometer (Shimadzu UV-250-1-PC spectrophotometer) at 284 nm. The experiments were carried out in triplicate.

Preparation of mefenamic acid complexes

The inclusion complexes of MA with β -CD (molar ratio, 1:1), and MA with HP- β -CD (molar ratio, 1:1) were prepared by kneading method.^[12-13] MA was dissolved in acetone at 25°C, to which the required quantity of β -CD or HP- β -CD was added in a mortar so as to obtain a homogeneous paste. The thick slurries were kneaded for 1 h. The pastes were dried under vacuum at room temperature and pulverized and sieved through mesh no. 100.

The physical mixtures were prepared by mixing the calculated and exactly weighed amounts of drug with β -CD and HP- β -CD and pulverized in a ceramic mortar and carefully mixed.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of samples were taken on the FTIR

spectrophotometer (Perkin Elmer dispersive type) using the KBr disk.

X-ray diffractometry (XRD)

Powder x-ray diffractometry was carried out using a Philips PW 1050 scanner with filter Ni, CuK_α radiation over the interval $5-40^\circ/2\theta$. The operation data were as follows: voltage 40 kV, current 20 mA, filter Ni, and scanning speed $1^\circ/\text{min}$.

Differential scanning calorimetry (DSC)

The samples were analyzed by DSC using a Mettler Toledo S R System. The samples (6 mg each) were placed into a pierced aluminum sample container. The studies were performed under static air atmosphere in the temperature range of 50°C to 280°C, at a heating rate of 12°C/min. The peak temperatures were determined after calibration with a standard.

Dissolution studies

The *in vitro* dissolution studies of different samples were performed using USP XXIII Apparatus-II. An amount of each powdered sample equivalent to 250 mg MA was placed into a hard gelatin capsule. Phosphate buffer (pH, 7.2) and 0.1-N HCl, each 900 mL, were employed as the dissolution media at a temperature of $37^\circ\text{C} \pm 0.5^\circ\text{C}$. The stirring speed was 50 rpm. The aliquots were withdrawn at various time intervals and analyzed spectrophotometrically.^[14-16]

Anti-inflammatory activity

The carrageenan-induced paw edema method was used. Male albino rats weighing 200 to 250 g were housed in groups of six in standard laboratory housing conditions. One group acted as a control and another group received the test compounds. The animals were fasted for 24 h before drug treatment. They were deprived of food and water during the experiment. Into the subplantar region of the left hind paw, 0.1 mL of 1% lambda carrageenan solution in saline was injected. The paw volume was measured at 0, 1, and 3 h after the injection of carrageenan, with plethysmograph. The edema volume was determined and expressed as percentage swelling, compared with the initial hind paw volume of each rat.^[15,17-20]

Statistical analysis

The values are expressed as mean \pm SD. A statistically significant difference between the control and the treated groups was calculated using a one-way analysis of variance (ANOVA), followed by a Mann-Whitney U test (two tailed); $P < 0.01$ was considered to be significant for interpretation of the results using the software PRISM (Graphpad, San Diego, CA).^[21]

RESULTS AND DISCUSSION

Phase solubility studies

The solubility method is useful for studying inclusion

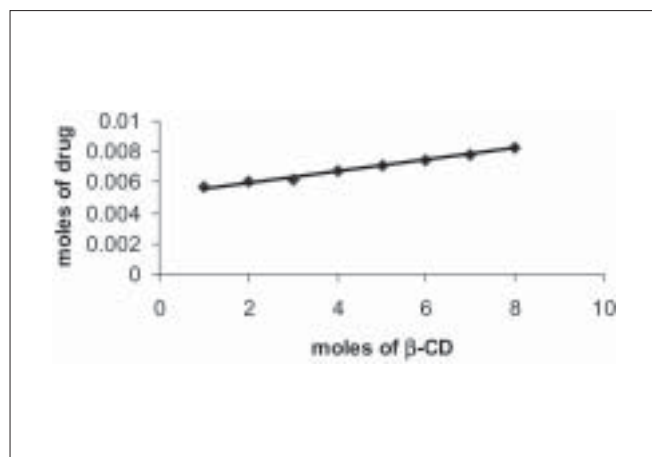


Figure 1: Phase solubility diagram of mefenamic acid and β -CD

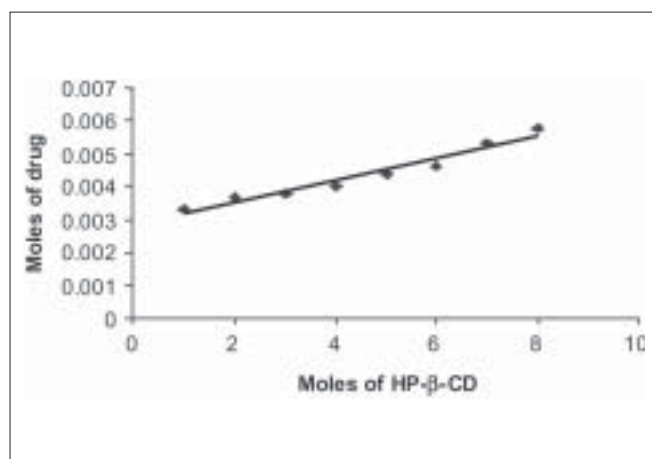


Figure 2: Phase solubility diagram of mefenamic acid and HP- β -CD

compounds of poorly soluble drugs with CDs in water because it gives not only the solubilizing ability of CDs but also the stability constant (K_s) of the complexes by analyzing the solubility curves.^[10]

The phase solubility profile for MA with β -CD and HP- β -CD systems in water at 25°C is presented in Figures 1 and 2 respectively. The solubility of MA increases linearly as a function of concentration of β -CD and HP- β -CD, showing a typical A_L -type phase solubility curve. This curve may be ascribed to the formation of a stoichiometric 1:1 complex of MA with β -CD and HP- β -CD. The apparent 1:1 stability constant K_c was calculated from the straight line of the phase solubility diagram by means of the following equation: $K_c = \text{slope}/\text{intercept} (1 - \text{slope})$. The value of the constant was found to be 176 M^{-1} and 103.8 M^{-1} with β -CD and HP- β -CD respectively.

Fourier transform infrared spectroscopy

Fourier transform infrared spectra of MA with β -CD and HP- β -CD inclusion complexes are shown in Figure 3. The spectra of pure drug showed peaks at 3300 cm^{-1} (N-H stretch),

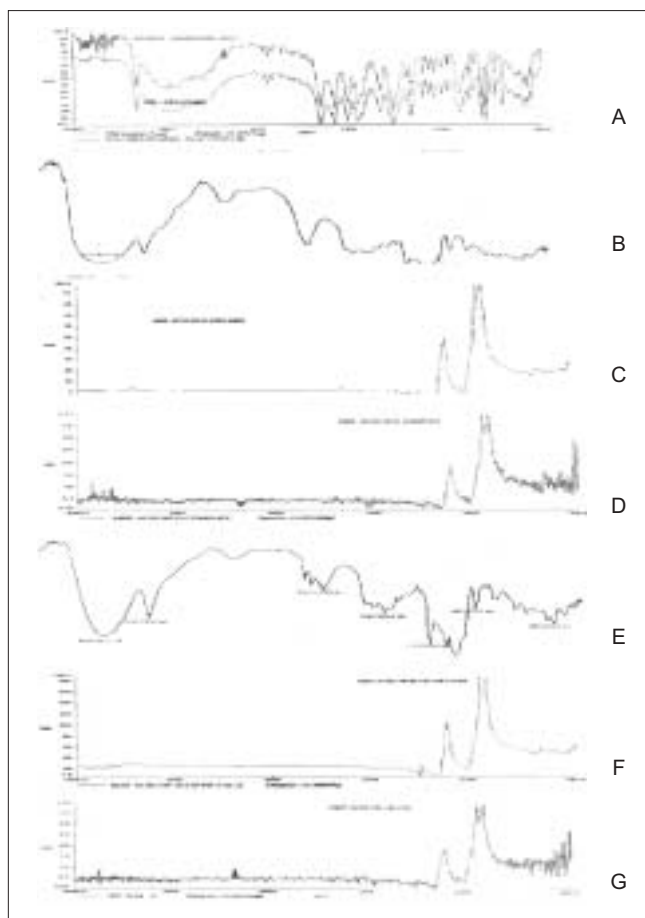


Figure 3: FT-IR curves of mefenamic acid (A), β -CD (B), physical mixture of β -CD and drug (C), complex formation of β -CD and drug (D), HP- β -CD (E), physical mixture of HP- β -CD and drug (F), complex formation of HP- β -CD and drug (G)

1647 cm^{-1} (C = O stretch), 1572 cm^{-1} (N-H bending), 1504 cm^{-1} (C = C stretch), 757 cm^{-1} (aromatic stretch). Inclusion complexes showed the absence of the above-noted peaks, which indicates entrapment of drug into the cavity and confirms the complex formation.

X-ray diffractometry

The X-RD patterns of the MA, β -CD, and HP- β -CD inclusion complexes are shown in Figure 4. It has been observed that the diffraction patterns of the inclusion complexes is somewhat diffused compared to diffraction patterns of MA. It also indicates that the crystallinity of the inclusion complex is less than that of the MA, β -CD, and HP- β -CD.

Differential scanning calorimetry (DSC)

The DSC patterns for MA with β -CD and HP- β -CD and inclusion complexes are shown in Figure 5. DSC thermogram of MA exhibits endothermic peaks at 235.6°C corresponding to its melting point and is confirmed by literature data.^[2,22] β -CD showed broad endothermic peak at 119°C, which may be attributed to a dehydration process. The shift of the endothermic peak to 234.4°C indicates a weak interaction in

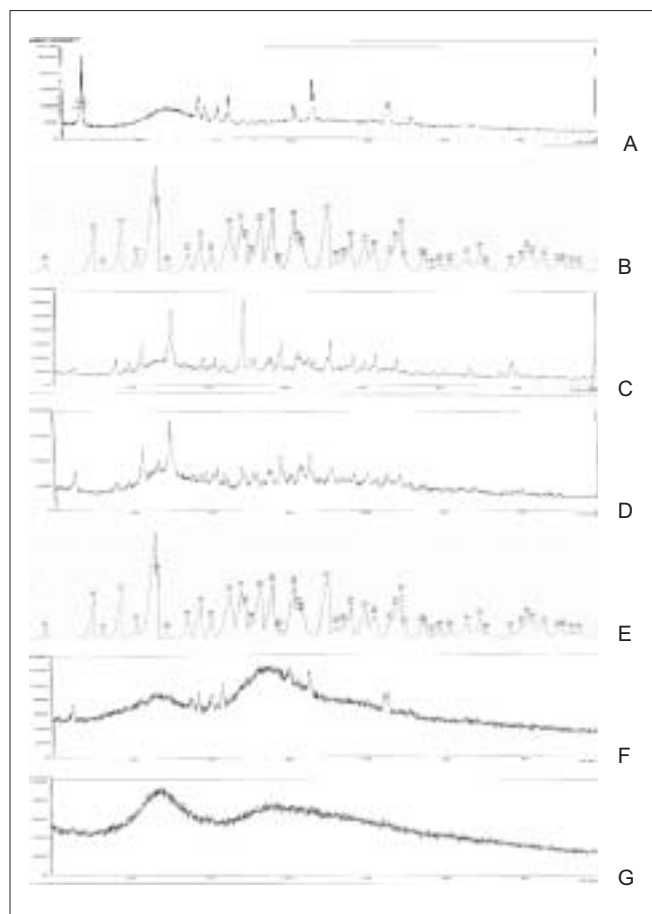


Figure 4: X-ray diffraction patterns of mefenamic acid (A), β -CD (B), physical mixture of β -CD and drug (C), complex formation of β -CD and drug (D), HP- β -CD (E), physical mixture of HP- β -CD and drug (F), complex formation of HP- β -CD and drug (G)

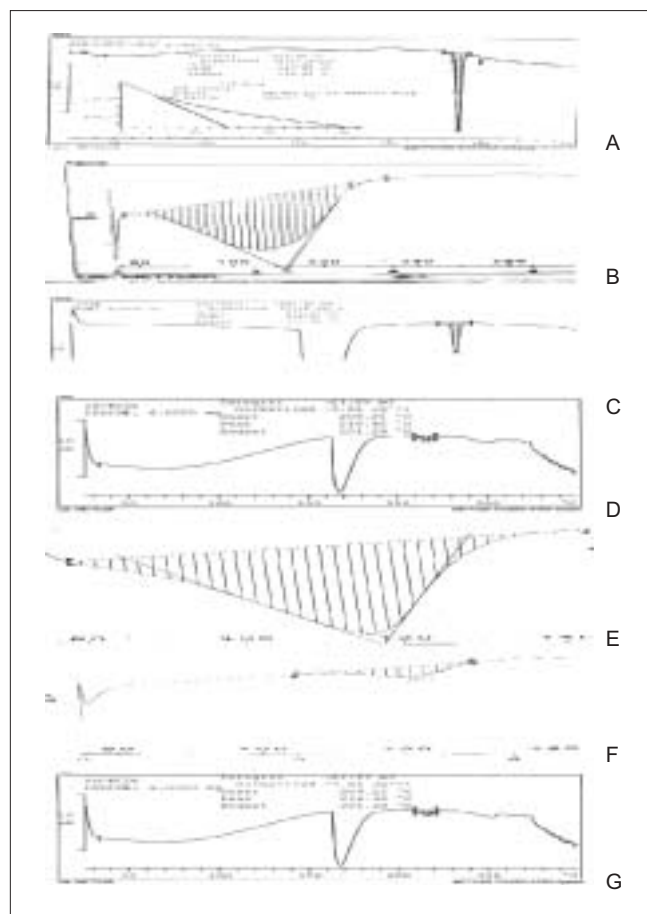


Figure 5: DSC patterns of mefenamic acid (A), β -CD (B), physical mixture of β -CD and drug (C), complex formation of β -CD and drug (D), HP- β -CD (E), physical mixture of HP- β -CD and drug (F), complex formation of HP- β -CD and drug (G)

the physical mixture in the case of β -CD. In contrast, the DSC curve of inclusion complexes showed a weak endothermic peak corresponding to the drug melting peak in the case of a 1:1 β -CD or a 1:1 HP- β -CD complex and thus it confirmed the complex formation.

Dissolution studies

The rate constants of the dissolution profiles of MA and inclusion complexes are shown in Table 1. The highest dissolutions of 93.12% and 93.91% were obtained from 1:1 and 1:1 complexes of MA with β -CD and HP- β -CD respectively in phosphate buffer. In the case of 0.1-N HCl, the highest dissolutions of 94.56% and 95.2% were obtained from the complexes of MA with β -CD and HP- β -CD respectively. The rate constants were found to be higher for the complexes when compared to the pure drug and physical mixtures.^[23]

Anti-inflammatory activity

The anti-inflammatory effect of MA with and without β -CD and HP- β -CD on carrageenan-induced edema is shown in Table 2. The MA complex showed significant improvement [$P < 0.01$ in comparison to vehicle (ANOVA) followed by

Table 1: Release rate constants of mefenamic acid

Sample	$K_1 \times 10^3 \text{ min}^{-1}$ in phosphate buffer	$K_1 \times 10^3 \text{ min}^{-1}$ in 0.1N HCl
Mefenamic acid (MA)	0.102	0.112
MA: β -CD (phy)1:1	0.365	0.412
MA: β -CD (phy)1:2	0.256	0.395
MA: β -CD (com)1:1	0.443	0.439
MA: β -CD (com)1:2	0.416	0.428
MA: HP- β -CD (phy)1:1	0.344	0.357
MA: HP- β -CD (phy)1:2	0.326	0.336
MA: HP- β -CD (com)1:1	0.414	0.552
MA: HP- β -CD (com)1:2	0.477	0.360

phy: physical mixture; com: complex; HCl: hydrochloric acid

Mann-Whitney U Test] in the *in vivo* activity when administered orally to the rats as compared with MA *per se* and also reduced side effects.^[24-26]

In conclusion, the dissolution rate of the MA with β -CD and HP- β -CD complexes was much higher than that of MA alone as a consequence of increased solubility and decrease in crystallinity due to complexation and also reduced side effects. As a result of this study, it may be concluded that

Table 2: The effect of mefenamic acid with and without β -CD and HP- β -CD on carrageenan-induced edema

Treatment	No of rats	Dose (mg/kg)	Mean edema at 3 hrs \pm S.E.	% Inhibition
Vehicle	6	-	171.2 \pm 2.43	-
Mefenamic acid (MA)	6	12	59.46 \pm 2.30*	65.30
MA- β -CD (com)1:1	6	12	50.06 \pm 2.21*	70.79
MA- β -CD (phy)1:1	6	12	60.96 \pm 2.52*	64.39
MA-HP- β -CD (com)1:1	6	12	52.07 \pm 2.40*	69.62
MA-HP- β -CD (phy)1:1	6	12	70.20 \pm 2.61*	58.99

* $P < 0.01$ in comparison to vehicle (ANOVA) followed by Mann-Whitney U Test; com: complex inclusion; phy: physical mixture

the complexes may be useful to improve solubility, dissolution rate, and, subsequently, bioavailability of marginally soluble drugs.

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