RESEARCH PAPER

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Influence of hydroxypropyl β- cyclodextrin on dissolution of piroxicam and on irritation to stomach of rats upon oral administration

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Solid dispersions of hydroxypropyl β- cyclodextrin (HPB), a highly water soluble derivative of β-cyclodextrin and piroxicam (PRX) were prepared by kneading, co-evaporation and freeze-drying. X-ray diffraction, differential scanning calorimetry, IR-spectral studies and thin layer chromatography were used to characterise the solid dispersions and also to study the possibility of complexation of the drug with HPB. A marked difference in characteristics of dispersions was observed due to their methods of preparation. The dissolution of PRX from the solid dispersion was studied by the dispersed powder technique and also as per USP 1990 which was found to have improved considerably over that of the pure drug alone. Coevaporated and freeze dried dispersions caused significant reduction in irritation to stomach mucosa of rats upon oral administration.

CYCLODEXTRINS have been extensively used to increase solubility[1,2] and to improve bioavailability of poorly water soluble drugs.[1,2,3] Hydroxypropyl β- cyclodextrin (HPB) a chemically modified β- cyclodextrin, is highly water soluble and does not have limitations such as renal toxicity[1] associated with β-cyclodextrin or other chemically modified cyclodextrins.

Piroxicam (PRX) a potent NSAI drug, is widely used in the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and acute gout. It is practically insoluble in water and its prolonged use is associated with side effects like gastric bleeding and mucosal damage[4]. Therefore it was thought worthwhile to improve solubility of PRX by preparing solid dispersions using Hydroxypropyl β-cyclodextrin.

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Materials and Methods

Materials

PRX and HPB were generously donated of Pfizer (India) Ltd., and Amaizo (American Maize Products Company, USA). Double distilled water (DDW) was used throughout the study.

Solubility studies

Solubility studies were carried out according to the method of Higuchi and Connors[5] Solution of HPB (molecular weight = 1371.6, ds = 4.9) of different concentrations (5,10,15,20 and 25 mM/Lit) were added to excess amounts of PRX and shaken at room temperature for 24 hours. After equilibrium, the solutions were filtered. The concentration of PRX in the filtrate was determined spectrophotometrically at 360 nm with reference to a suitably constructed standard curve.

Preparation of solid dispersion

Solid dispersions of PRX and HPB were prepared in the molar ratio of 1:1. Physical mixture (PM) of PRX with HPB was prepared by simply mixing the powders together with a spatula. The kneaded dispersion (KN) was prepared by wetting the physical mixture with minimum volume of a 1:1 (by volume) mixture of water and ethanol to obtain a dough like mass which was triturated in the mortar and was dried in vacuum oven at 45° for one hour. The coevaporated dispersions were obtained by evaporating solvent from a solution of HPB and PRX in (1) ethanol - water (CE) and (2) 25% ammonia - water (CEA). In both the cases aqueous solution of HPB was added to the PRX. The resulting mixtures were stirred mangentically at room temperature for one hour and evaporated at a temperature of 45 - 50° till nearly dry and then stored in a dessicator over anhydrous $CaCl_2$ up to constant weight. The coevaporated dispersions were sieved through 85 mesh B.S. The freeze - dried dispersion (FD) was prepared by freeze-drying a 25% aqueous solution of ammonia containing HPB and PRX in an Edward's Modulyo 4K Freeze-drier. The solid obtained was sieved through 85 mesh B.S. An attempt was made to freeze dry a 2% solution of PRX in 25% ammonia solution.

Methods for characterization and evaluation of solid dispersions

Solid dispersions were characterized and evaluated by differential scanning calorimetry, X-ray diffraction, IR-spectral studies and thin layer chromatography.

Differential Scanning Calorimetry (DSC)

DSC studies were performed using the scanning rate of 10°/min on a Shimadzu DT-40 Thermal Analyzer. Samples were heated from 30 to 350° and alumina was used as a reference material.

X-ray diffraction

Powder X-ray diffraction patterns were recorded using a Phillips X-ray diffractometer with a copper target, Voltage 40 KV, current 20 mA at a scanning speed of 2 deg/min.

IR-Spectral studies

IR-spectral studies were carried out on a Jasco FTIR spectrometer using KBr disc method.

Thin Layer Chromatography (TLC)

All the solid dispersions were studied by TLC (the pure drug being spotted simultaneously with the dispersions) to determine the homogeneity and absence of drug degradation in the dispersions. Silica gel G was used as adsorbent and toluene : acetic acid (95:5) was the solvent system used.

Dissolution of PRX from solid dispersions

Solid dispersions were evaluated for dissolution of PRX according to the dispersed powder method[3]. Twenty five ml of double distilled water was used as the dissolution medium. A stirring rate of 100 rpm and a temperature of 37±0.5° were used. Samples of solid dispersions were stored for 3 months at room temperature and at 45° and evaluated again for reliability of dissolution profiles.

Filling of Capsules with solid dispersions and evaluation of Capsules for dissolution of PRX:

Hard gelatin capsules of size 3 were filled manually with drug and solid dispersions so as to contain 20 mg of PRX/capsule. These capsules were evaluated for the dissolution profiles of PRX in quadruplicate. 900 ml of stimulated gastric fluid T.S. prepared without pepsin was used as dissolution medium in a USP type 1 (basket) apparatus at 37 ± 0.5° and the basket was rotated at 50 rpm.

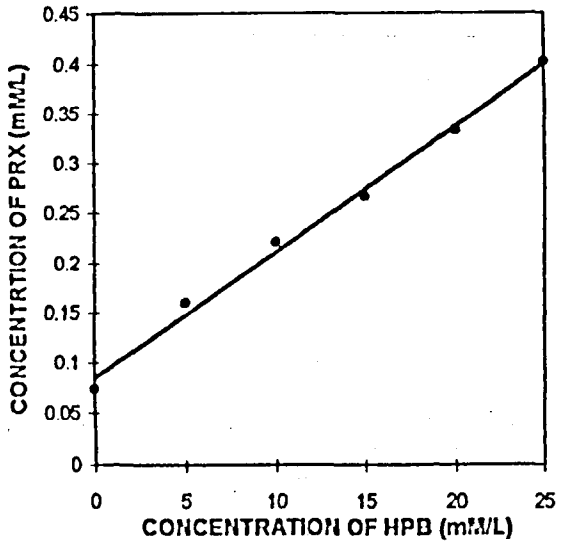

Fig. 1: Solubility Diagram

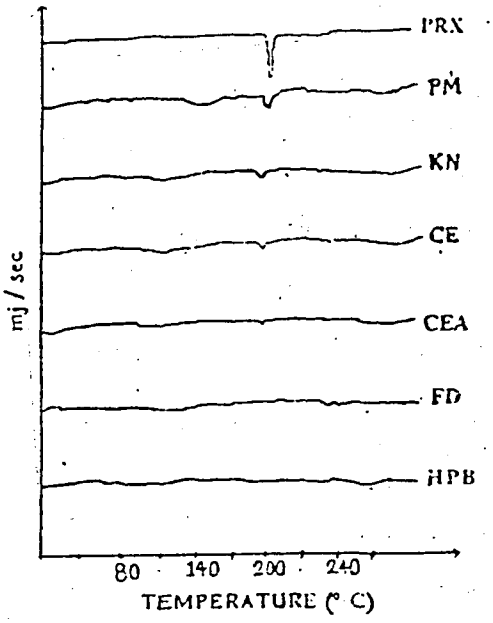

Fig. 2: DSC Thermograms of PRX, HPB and Solid Dispersions

Influence on the ulcerogenic effect

The influence of the formulations on the ulcerogenic effect of piroxicam was studied in Wistar rats using the method previously reported[6]. The drug was used as a positive control and HPB as negative control. A preliminary study was performed to determine the dose of PRX to be administered with a view to be able to observe difference in the effect among the samples. The rats were divided among 4 groups each consisting of 5 healthy rats in the weight range of 150-200 g. The rats were fasted overnight before administration and PRX (200 mg/kg), HPB, the coevaporated dispersion and the freeze dried dispersion were administered to one group each. PRX and the coevaporated dispersion were administered as suspensions while HPB and the freeze dried dispersion were administered as solutions. The solid dispersions administered contained the same amount of PRX and HPB as when administered alone. Seven hours after administration the rats were sacrificed by exposure to chloroform and the abdomen was opened. The stomach was removed and incised along the greater curvature and gently washed under the tap. The degree of injury was observed under a dissection microscope in 20 magnification, sketched and evaluated by the following scores.

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

0.5 - Latent injury or widespread bleeding.

1.0 - Slight injury (2 or 3 dotted injury).

2.0 - Severe injury (continuous lined injury or 5-6 dotted injuries).

3.0 - Very severe injury (several continuous lined injuries).

4.0 - Widespread lined injury or widened injury.

RESULTS AND DISCUSSION

The phase solubility diagram (Fig. 1) can be classified as type AL according to Higuchi and Connors[5]. Since the slope of the straight line was less than unity, it was assumed that the increase in the solubility observed was due to the formation of 1:1 complex. The apparent stability constant was

Table 1
Powder x-ray diffraction of PRX/HPB solid system expressed as 2θ° and "d" and relative diffraction intensity

"d" calculated according to Bragg's equation

	2θ°	d(Å) Interplanar distances	(I/I_o) Intensity
PIROXICAM			
	8.7	10.152	74.8
	11.7	7.549	46.88
	12.3	7.187	39.77
	14.6	6.059	68.06
	17.7	5.005	100.06
HPB			
	7.6 - 14.6	11.61 - 6.059	--
	15.2-22.4	5.822 - 3.964	--
PHYSICAL MIXTURE			
	8.7	10.152	32.46
	11.7	7.549	21.35
	12.3	7.187	20.31
	14.6	6.101	36.64
	17.7	5.005	39.06
KNEADED DISPERSION			
	8.7	10.152	32.46
	11.7	7.549	21.35
	14.6	6.059	40.625
	17.7	5.005	56.77
CO-EVAPORATED DISPERSION FROM ETHANOL			
	12.3	7.187	39.06
	17.4 - 20.6	5.09-4.31	--
CO-EVAPORATED DISPERSION FROM AMMONIA			
	7.8 - 12.6	11.321 - 7.02	--
	16.0 - 21.2	5.533 - 4.186	--
FREEZE DRIED DISPERSION			
	7.4 - 14.0	11.932 - 6.32	--
	15.0 - 22.2	5.899 - 3.999	--

calculated according to equation (1) and was found to be 179.69M^{-1}.

$$K_{1:1} = \frac{Slope}{So\,(1 - slope)} \rightarrow (1)$$

Where So is the solubility of PRX in the absence of HPB.

Supporting evidence for complex formation was also obtained from DSC studies (Fig. 2). A sharp endotherm at 200.2° due to melting of PRX, almost

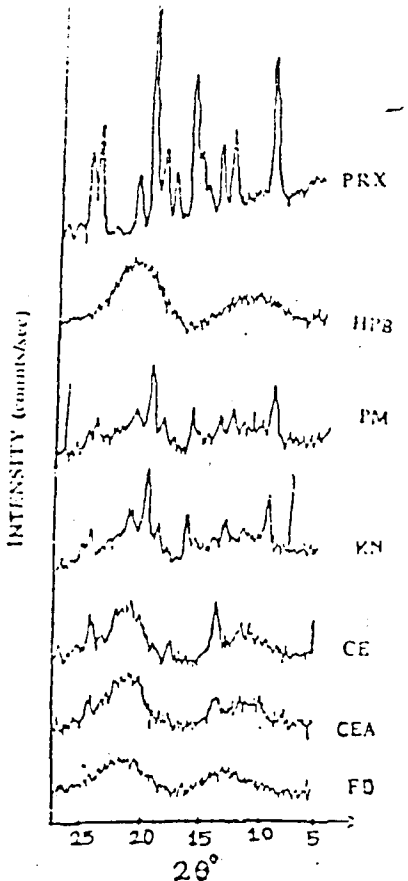

Fig. 3:X-ray Diffraction patterns of PRX, HPB and Solid dispersion

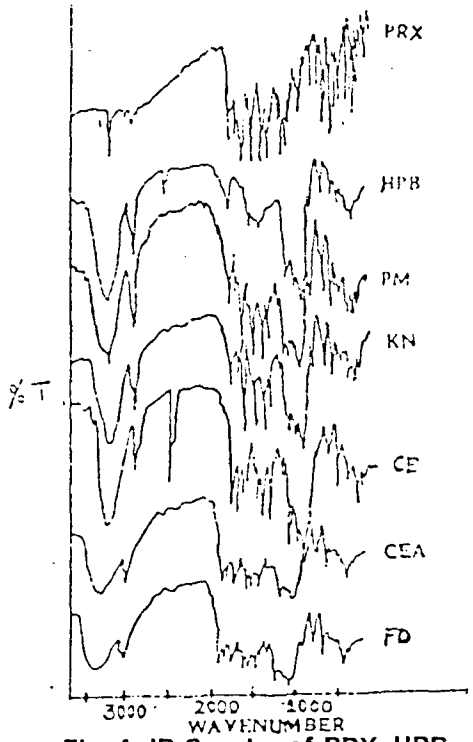

Fig. 4: IR-Spectra of PRX, HPB and Solid dispersions

disappeared in case of the freeze-dried dispersion and the dispersion coevaporated from ammonia. The thermogram of physical mixture appeared to be the combination of the thermograms of PRX and HPB. However, the thermogram corresponding to PRX was slightly broader due to some interaction of PRX and HPB on heating. The endotherm of kneaded dispersion and the dispersion coevaporated from ethanol were similar to physical mixture though slightly broader.

X-ray diffraction patterns (Fig. 3) of the physical mixture showed all the peaks for PRX with reduced intensities. In case of kneaded dispersion also, most of the peaks were seen indicating existence of crystalline nature. Dispersion coevaporated from ethanol showed only one significant peak indicating amorphization of PRX. While dispersion coevaporated from ammonia and the freeze-dried dispersion showed broad diffused spectra indicating an amorphous nature. Table 1 lists the interplanar distances for the different samples. The interplanar distance of the freeze dried and the co-evaporated dispersion from ammonia were closer to that for HPB indicating that the inclusion complex was formed.

The IR-spectrum of PRX (Fig. 4) showed a distinct peak at 3330 cm-1 which is characteristic of the cubic polymorph of PRX[7]. HPB showed a broad peak at 3375 cm-1. The physical mixture and the kneaded dispersion showed the peaks corresponding to both the PRX and HPB. IR-spectra of co-evaporated dispersion and the freeze-dried dispersion showed disappearance of the characteristic peak of PRX and number of peaks in the fingerprint region were significantly reduced indicating possibility of (1) transformation of PRX to amorphous state during co-evaporation and freeze-drying process and (2) interaction between PRX and HPB.

TLC-studies confirmed that there was no degradation of PRX in all solid dispersions and homogeneity of all the dispersions was indicated by the round spots obtained for all of them.

Fig. 5 shows the dissolution profiles of the different dispersion samples in double distilled water.

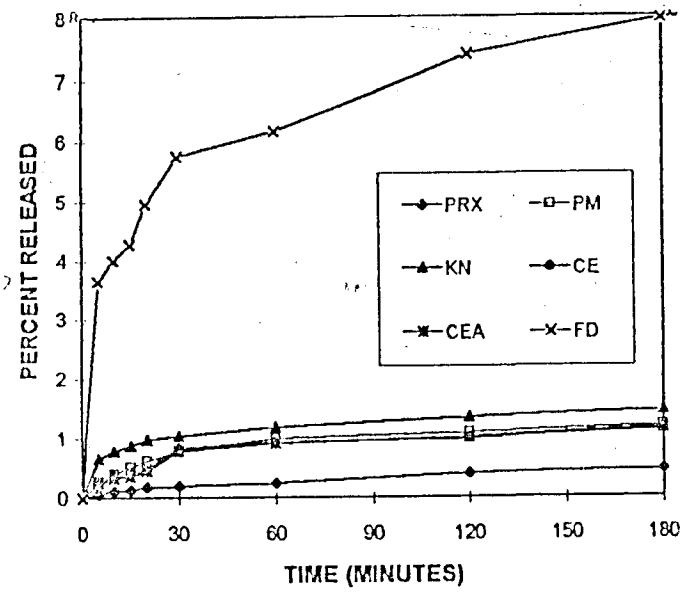

Fig. 5: Dissolution of PRX from solid dispersion (by dispersed powder method)

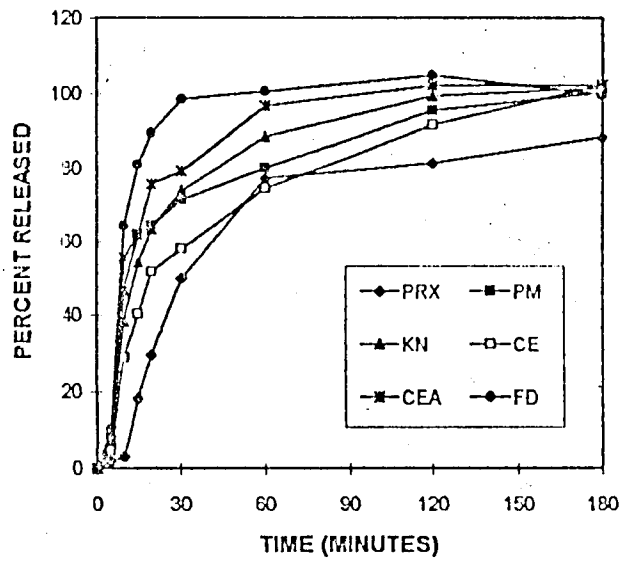

Fig. 6: Percent PRX dissolved from capsules filled with various solid dispersions v/s time

The freeze-drying process improved the dissolution of PRX to the maximum extent which is 17 times as that of PRX alone at the end of 3 hours. Physical mixture, kneaded dispersion and the dispersion co-evaporated from both ammonia and ethanol showed 2.5 to 3 fold increase in solubility of PRX during the same period of time.

Samples stored at room temperature and at 45° showed no changes in the dissolution pattern at the end of three months.

Fig. 6 shows the dissolution profiles of PRX from capsules filled with the dispersion. Values of t 50% as well as t 75% were the lowest for the freeze-dried dispersion; 7.0 min, 13.9 min respectively, followed by the dispersion coevaporated from ammonia; 9.0 min and 19.8 min respectively. Capsules filled with the physical mixture, kneaded dispersion, coevaporated and the freeze-dried dispersion exhibited complete dissolution of PRX at the end of 3 hours. Capsules filled with the drug alone showed dissolution of PRX to the extent of 89% in the same time period.

In the ulcerogenic studies, at the dose level of 200 mg/kg of PRX, both the coevaporated dispersion and the freeze dried dispersion gave the lower scores; 0.8 ± 0.46 and 0.7 ± 0.61 respectively as compared to PRX; 2.4 ± 0.46. This reduction in ulcer inducing effect of the dispersion was conclusively significant at 5% level by t-test. It is reported[8] that the crystals of non- steroidal antiinflammatory agents 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 faster and show an accelerated absorption. Moreover, it will not come in direct contact with the stomach wall in crystalline form, since until it is dissolved it remains encapsulated within the cylodextrin matrix.

In conclusion, solid dispersions with HPB improved solubility of PRX. Studies on solubility, DSC, X-ray diffraction, IR, TLC, and powder dissolution indicated complex formation. Interaction between PRX and HPB was greater after co-evaporation and

Table 2
Degree of injury to the stomach of Wistar rats

Sample	Amount administered (mg/kg)	Degree of injury in case 1	2	3	4	5	Average ± SE[a]
PRX	200	2	1	4	3	2	2.4 ± 0.46
HPB	828	0	0	0	0	0	0.0 ± 0.00
CEA	1028	0.5	1	1	0.5	1	0.8 ± 0.46[a]
FD	1028	0	0.5	1	1	0.5	0.7 ± 0.61[a]

a - Significant by t - test at 5% level

freeze- drying processes than kneading. Solvent used in preparation of coevaporated dispersion influenced the solubility of the dispersion. Solubility of PRX increased considerably due to complexation and amorphization. The freeze-dried dispersion exhibited a very good *in vitro* dissolution profile. Coevaporated and freeze dried dispersions caused significant reduction in irritation to stomach mucosa of rats upon oral administration.

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