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Evaluation of Stereoselective Dissolution of Racemic Ketoprofen from Formulations Containing Chiral Excipients

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The dissolution characteristics of ketoprofen enantiomers from matrices containing chiral excipients sodium alginate, β-cyclodextrin and hydroxypropylmethylcellulose under three different pH values (pH 1.5, 4.6 and 7.4) in aqueous environment were determined. An achiral excipient, Eudragit RL 100, was used to generate a control dissolution profile. This study demonstrates that release of ketoprofen enantiomers from formulation containing hydroxypropylmethylcellulose matrix at pH 7.4 is stereoselective and statistically significant. It is observed that the release of S-ketoprofen (eutomer) was faster than the R-ketoprofen (distomer). Further, it indicates that the differential release is pH-dependent. The research implication is that by choosing the appropriate chiral excipient and other formulation conditions one can deliberately manipulate the release of a specific enantiomer from a racemic therapeutic. This intended stereospecific retardation/acceleration in the release of enantiomers could be exploited for the design of stereoselective drug delivery systems.

Within the pharmaceutical industry there is an inexorable move, driven by pressure from regulatory authorities, towards the provision of enantiopure drugs[1-5]. As a consequence, the fields of asymmetric synthesis and chromatographic chiral separations have mushroomed in recent years, as these methodologies are believed to offer the best routes to the provision of pure stereoisomers. However, there is another as yet largely overlooked potential means of achieving selective administration of the preferred stereoisomer, stereospecific retardation. This involves the formulation of a racemic therapeutic with a highly specific sorbent, which is capable of binding an excess of a particular enantiomer, leaving the other enantiomer less strongly bound and therefore more readily released from the formulation.

There have been few previous reports on stereoselective release of chiral drugs from pharmaceutical formulations including sustained release dosage forms[6] and formulations containing chiral excipients[7,8]. Most workers have apparently viewed the phenomenon of stereoselective dissolution as a potential problem than as an opportunity and there has also been some doubt expressed as to whether these claimed differences were of statistical validity.

Stereochemical interactions between chiral drugs and excipients are of interest due to potential dosage problems that may arise as a result of unequal rates of delivery of drug isomer. Intrinsic recognition processes such as transient hydrogen bonding can occur in the diffusion from chiral matrices and are possibly of significance in the release of chiral molecules. This is analogous to the mechanism of action of chiral stationary phases.

In the present study, the main objective was to under-

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stand the affect of chiral excipients on the release profile of the enantiomers of ketoprofen from compressed hydrophilic matrices, sodium alginate (NaAlg), β-cyclodextrin (CD) and hydroxypropylmethylcellulose (HPMC), in aqueous media.

MATERIALS AND METHODS

(R/S)-ketoprofen, probenecid (PB), L-leucinamide hydrochloride, Sodium alginate and β-cyclodextrin were obtained from Sigma Chemical Co., St. Louis, MO, USA. Ethyl chloroformate was procured from Fluka, Buchs, Switzerland. Acetonitrile used was HPLC grade while buffer salts and all other reagents employed were analytical grade supplied by SD Fine Chemicals (Mumbai, India). Acetonitrile was dried over a molecular sieve- 4 A° (Sigma Chemical Co.) before using in the preparation of the reagent solutions. Water HPLC-grade was generated using Milli-Q academic, Millipore, Bangalore. HPMC (K4M grade) and Eudragit RL were a gift from M/S Astra Zeneca Pvt. Ltd, Bangalore. The purity of ketoprofen was found to be 99.98% w/w by pharmacopoeial method[9].

Preparation of ketoprofen matrix tablets:

Two types of matrix tablets were prepared: the first one contained physical mixture (PM) of racemic ketoprofen and the chiral excipient (NaAlg/CD/ HPMC) or achiral excipient, eudragit RL 100 (Eudra). The second type is inclusion complex (IC) formed by lyophilisation of an equimolar mixture of ketoprofen and β-cyclodextrin[10]. The composition of each formulation is listed in Table 1.

TABLE 1: COMPOSITION OF KETOPROFEN FORMULATIONS.

	Formulation No.			
	KT1 (mg)	KT2 (mg)	KT3 (mg)	KT4 (mg)
(±)-Ketoprofen	150	150	109.8	150
Eudragit	450			
Sodium alginate		450		
β-Cyclodextrin			490.2	
Hydroxypropyl-methylcellulose				450
	600	600	600	600

Matrix tablet formulation of ketoprofen with eudragit (KT1), sodium alginate (KT2), β-cyclodextrin (KT3) and hydroxypropylmethylcellulose (KT4).

Preparation of ketoprofen-excipient physical mixture:

Ingredients of each formulation were mixed in a mortar and formed into tablets by direct compression with a pellet-maker (Pye Unicam, England) with a dye of 13 mm (compression force: 2500 kg). A high content (75%) of all the excipients was used as this was expected to provide enantioselectivity[11].

Preparation of ketoprofen-β-cyclodextrin inclusion complex:

Stoichiometric quantities of ketoprofen and β-cyclodextrin were weighed (KT:CD::254.3 mg:1135 mg) and dissolved in Milli-Q water. The drug is acidic and very slightly soluble in water. Hence a little (0.5 ml/l) ammonium hydroxide 25% was added to help ketoprofen to dissolve. The solution, when transparent, was stirred for 24 hours. It was then placed in a freezer at -12° until completely frozen (4-5 h). The frozen solution was then lyophilised using a lyophilizer (Chirst, LOC1, α 142 model, Germany). No residual ammonia was detected in the colyophilised product by the qualitative analysis using Nesslers reagent. The lyophilised product (600 mg) is tabletted by direct compression.

Differential scanning calorimetry (DSC):

A Shimadzu Model DSC-60 equipped with a data station (Thermal Analysis system, TA-60WS) was used to record the DSC curves. The DSC curve for all the samples was recorded in the solid state (PM/IC). Drug-excipient sample (ratio as used in the formulation; Table 1) was weighed (Precisa® 290SCS series balance, Precisa Instruments AG, Switzerland) into standard aluminum sample open pan. Covered with an aluminium lid and sealed it with a SSC-30 crimper (201-52000-90). A heating rate of 10°/min with nitrogen purge (60 ml/min.) was employed throughout the study.

Fourier transform infrared spectroscopy (FT-IR):

FT-IR spectra were recorded for all the formulations in the solid state (PM/IC) using a Nicolet, FT-IR absorption spectrometer (Avtar®, 360 FT-IR ESP, Nicolet Instruments Corporation, USA) according to KBr disk method.

Release studies:

The dissolution experiments (6 replicates) for all the formulations were carried out using the rotating basket (USP 23 Apparatus I; Electrolab®, Tablet dissolution tester TDT-06P, Mumbai) at 50±1 rpm. Matrix tablet in the basket were placed in 900 ml of dissolution medium equilibrated to 37±0.5°. Samples (5 ml) were withdrawn from the dissolu-

tion vessel and replaced with fresh dissolution medium using auto-sampler at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 min. The samples were filtered and immediately stored at -10° until quantification of the enantiomers of ketoprofen by the optimised indirect chiral HPLC analysis. The dissolution studies were carried out at three different pH values: pH 1.5 (KCl-HCl buffer), 4.6 (acetate buffer) and 7.4 (phosphate buffer).

Analytical methods:

The amount of ketoprofen in the matrix tablets was determined using a reported HPLC method[12] and expressed in percentage based on the theoretical content of ketoprofen in the respective formulation. The concentration of ketoprofen enantiomers in the samples obtained from the dissolution medium was determined using indirect chiral HPLC analysis[13]. Stock solutions of (±)-ketoprofen (1mg/ml) and internal standard, probenecid (120 μg/ml) was prepared in acetonitrile and stored at -10° until use. Fifty microlitres of internal standard at 120 μg/ml concentration was used for the assay.

Work-up and derivatisation of dissolution samples:

To 950 μl sample of the aqueous dissolution medium containing ketoprofen was added 50 μl of internal standard solution. To acidify, 500 μl of 0.6 M sulfuric acid was added. The sample was extracted with 5 ml of a mixture of isooctane-isopropanol (95:5) after vertex mixing for 30 s and centrifuging on a laboratory centrifuge (Remi®, R4C, Remi Equipment, Mumbai, India) for 5 min at 4000 g. The organic layer (4 ml) was transferred to clean tubes and evaporated to dryness under gentle stream of nitrogen at ambient temperature. The residue was reconstituted in 100 μl of 50 mM triethylamine in acetonitrile. To this mixture was added, at 30s intervals, 50 μl of 60 mM ethyl chloroformate in acetonitrile and 50 μl of a mixture of 1M L-leucinamide hydrochloride and 1M triethylamine in methanol. After 2 min 50 μl of water was added followed by 750 μl of mobile phase. 20 μl of the solution was injected into the HPLC system.

Three separate calibration curves each were generated for R- and S-ketoprofen with standard solutions of racemic ketoprofen for the three different pH values of the dissolution studies. These standards were also taken through the sample preparation and derivatisation methods described above. The samples were suitably diluted, whenever necessary, so that the concentration is in the range of the calibration curve.

Data analysis:

A standard calibration curve was constructed separately for the R- and S-KT enantiomers, at three different pH values as used for dissolution studies, using the racemic KT covering the appropriate ranges and used to quantify the release of both the enantiomers of KT in the dissolution medium. The cumulative (%) release of enantiomers (ΣR) of ketoprofen was presented as mean±standard deviation (SD). The dissolution profiles for all the formulations are depicted graphically as plots of cumulative (%) release vs. time. The S/R KT ratio is taken as the measure of chiral discrimination. If this ratio is very close to unity there is no difference in the release of the enantiomers.

Statistical analysis:

Prior to each dissolution experiment, the racemate and not individual enantiomers, was incorporated into the formulations. Therefore, the two-sample *t* test is inappropriate to examine the significance of the difference between the enantiomers released. This is because the assumption of independent samples is not valid in the present study since the concentrations have been measured in pairs[14]. Hence, the difference between the two enantiomers released was evaluated for statistical significance using the paired *t* test (α=0.05). The paired *t* test was deemed more appropriate than the two-sample *t* test because only the paired *t* test compares the concentration of enantiomers in a given experiment and is not influenced by variation between experiments[14]. A summary of the results of paired *t* test is presented in Table 2.

RESULTS AND DISCUSSION

The ketoprofen content in the matrix tablet formulations KT1, KT2, KT3 and KT4 were found to be 99.9, 100.1, 96.6 and 100.8%, respectively. The thermal curves of KT1, KT2 and KT4 showed the melting peak of ketoprofen at 96.08°, 96.06° and 96° respectively indicating the absence of any interaction in the solid state between KT and the excipients Eudra, NaAlg and HPMC. The endothermic melting point peak of standard racemic KT appeared at 96.13°. In the KT3 the freeze dried product the melting peak of KT and the peak in the β-cyclodextrin DSC curve at about 99.35° corresponding to the evaporation of water from the cyclodextrin cavity completely disappeared (fig. 1). These observations confirm the formation of an inclusion complex between KT and β-cyclodextrin by the freeze-drying technique. The DSC curve of the separately lyophilised KT, β-CD was very similar to that of the untreated samples, indicating that the lyophilisation process did not substantially affect the solid-

state properties.

In the normal FT-IR spectra the C=O, the acid and ketonic carbonyl bands, stretching of ketoprofen appeared at 1698 and 1656 cm^{-1}. In the IR spectrum of the freeze-dried product (KT3) a reduction in the intensity of the characteristic acid carbonyl-stretching band of the pure ketoprofen is observed. The reduction in the intensity might be due in part to the vibrational restrictions imposed on the guest molecule in the cyclodextrin cavity. Lin and Kao[15], found that spray-dried inclusion complexes prepared using several drugs and β-cyclodextrin also behaved like this. The FT-IR spectra corresponding to KT and freeze dried KT show no differences between bands for one or the other product which suggests the differences is a consequence of the inclusion of ketoprofen within the cyclodextrin cavity. The infrared spectra corresponding to KT2, and KT4 showed no band displacement further reveal the absence of any interaction between KT and the respective excipients in the solid state (PM).

The rotating speed of the basket in the study was maintained at a low rpm of 50, as vigorous dissolution procedure (e.g.,100 rpm speeds) may contribute to the practically equivalent release rates of ketoprofen enantiomers by promoting drug release through erosion mechanism. Erosion of enantiomers through the hydrated matrix is non-stereoselective and diffusion of enantiomers through hydrated matrix is presumably an enantioselective process[16].

It is observed that the dissolution curve for the release of enantiomers of ketoprofen from formulation KT1 is practically superimposable for all the cases. This as expected since an achiral excipient is employed in the matrix tablet KT1. A representative dissolution profile is depicted (fig. 2a). At pH 7.4, the cumulative release profile within 480 min of the experiment, (ΣR 480), 50.2±0.18 % of S-KT and 48.2±0.17 % of R-KT, respectively were dissolved. At pH 4.6, ΣR 480

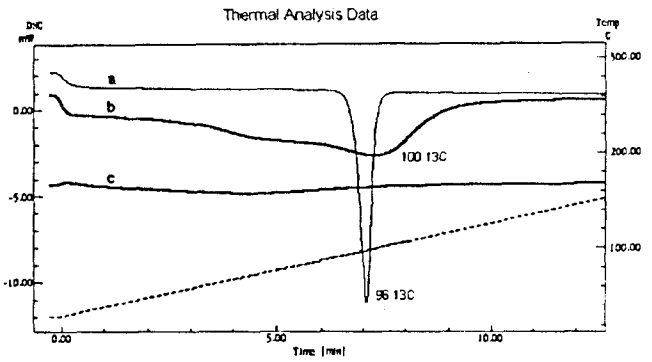

Fig. 1: DSC patterns.

DSC curve of ketoprofen (a), β-cyclodextrin (b) and ketoprofen-β-cyclodextrin inclusion complex (c).

TABLE 2: SUMMARY OF PAIRED *t* TEST.

Formulation	pH	Degrees of freedom	t_{stat}	$t_{critical}$ ($t_{0.05}$)	Remarks
KT1	1.5	10	0.232	1.812	Not significant
	4.6	10	0.595	1.812	Not significant
	7.4	10	0.501	1.812	Not significant
KT2	1.5	10	0.631	1.812	Not significant
	4.6	10	0.807	1.812	Not significant
	7.4	10	0.367	1.812	Not significant
KT3	1.5	4	0.281	2.131	Not significant
	4.6	5	0.638	2.015	Not significant
	7.4	5	0.153	2.015	Not significant
KT4	1.5	10	0.668	1.812	Not significant
	4.6	10	1.299	1.812	Not significant
	7.4	10	4.889	1.812	Significant

Matrix tablet formulation of ketoprofen with eudragit (KT1), sodium alginate (KT2), β-cyclodextrin (KT3) and hydroxypropylmethylcellulose (KT4).

showed values of 24.2±0.28% and 23.2±0.30% of S- and R-KT, respectively and at pH 1.5, ΣR 480 showed values of 6.4±0.25% and 5.9±0.37% of S- and R-KT, respectively were dissolved. The S/R ratio of ketoprofen enantiomers released is close to unity (0.92-1.02). No enantioselectivity in release was observed under the tested conditions. This release pattern is used as a benchmark for comparing the release profile of enantiomers of ketoprofen from other matrices.

The enantioselective experiments on KT2 (alginate matrix) after 480 min, the dissolution of enantiomers at pH 7.4 was found rapid with ΣR 480 values of 50.2±0.18% of S-KT and 48.2±0.17% of R-KT, At pH 4.6 the dissolution registered values of 44.6±0.24% and 41.2±0.22% of S- and R-KT, respectively and at pH 1.5, showed values of 3.28±0.19% and 3.15±0.18% of S- and R-KT, respectively. The dissolution curve for the release of enantiomers of ketoprofen was practically superimposable for all the cases. A typical dissolution profile is shown in fig. 2b. It is noted that the S/R ratio of KT released is close to unity (0.92-1.03). No statistically significant enantioselectivity was observed in release under the tested conditions.

The dissolution pattern of the enantiomers of ketoprofen from KT3 formulation (KT-β-cyclodextrin matrix), under the experimental conditions, was non-enantioselective as indicated by the superimposable dissolution profile. The absence of stereoselective release profile is further supported by the value of S/R ratio that is very close to unity (0.96-1.04). The β-cyclodextrin inclusion complex of racemic ketoprofen was found to be in amorphous state (being lyophilised product). The ketoprofen molecules are almost completely included in the cyclodextrin cavity and the solubility of the complex is influenced more by the interaction between water molecules and β-cyclodextrin and hence shows high aqueous solubility, in all the three-pH values, as evidenced by the high dissolution rates. At pH 7.4, the KT enantiomers were released with ΣR120 values of 43.2±0.28% and 44.9±0.30% of S-KT and R-KT, respectively. It is observed that the cumulative release profile attained a constant value at 120 min at pH 7.4. At pH 4.6, the KT enantiomers were released with ΣR120 values of 46.5±0.34% and 44.6±0.33% of S-KT and R-KT, respectively. At pH 1.5 the KT enantiomers were released with ΣR120 values of 43.7±0.39% and 42.8±0.38% of S-KT and R-KT, respectively. The ΣR attained constant values at 90 min. both at pH 4.6 and 1.5. A representative dissolution profile is depicted in fig. 2c.

Similarly, the stereoselective dissolution of KT4 formulation (HPMC matrix) was also evaluated at three-pH values (1.5, 4.6 and 7.4) and was significantly slower than that observed for KT1, KT2 and KT3 formulation. The mean dissolution profile of enantiomers of ketoprofen from HPMC ma-

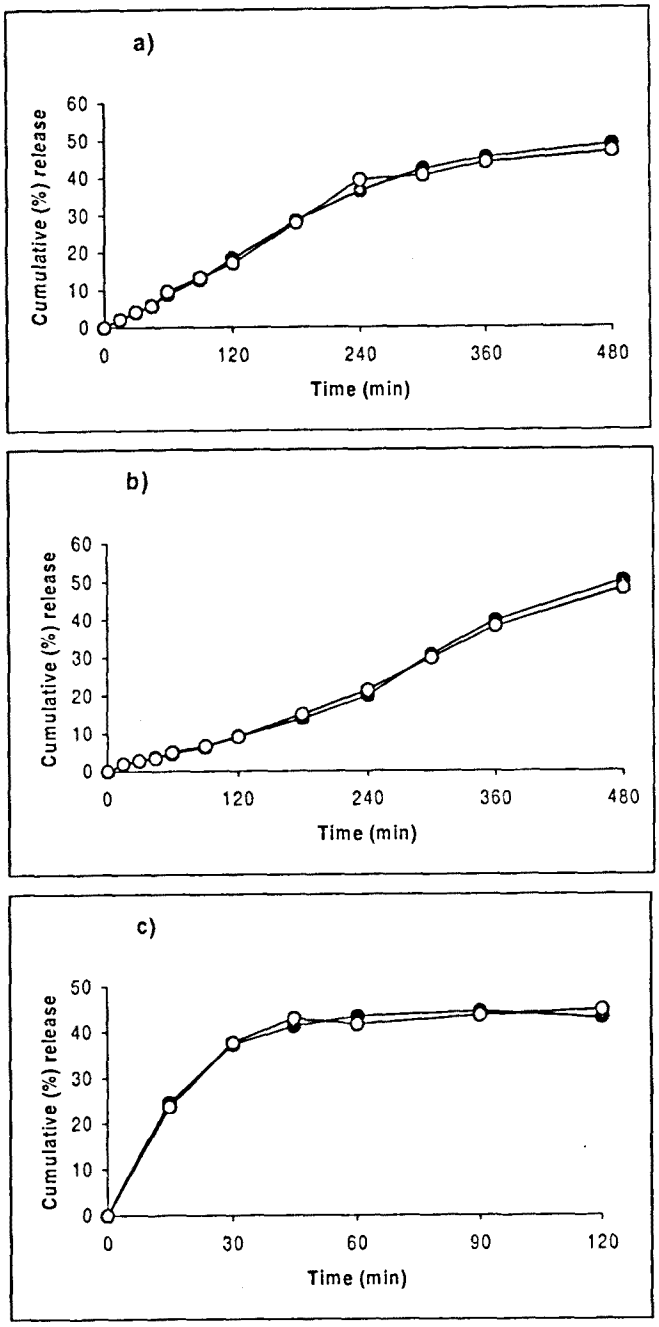

Fig.2: Dissolution profiles of Ketoprofen matrix tablets. Representative plots of cumulative (%) release of ketoprofen enantiomers from KT1 matrix tablets vs time at pH 7.4 (a), KT2 matrix tablets at pH 7.4 (b) and KT3 matrix tablets at pH 7.4 (c); (-●-) represents S-KT and (-○-) represents R-KT.

trix is graphically depicted in fig.3 (a-c). The release of enantiomers of ketoprofen from KT4 at pH 7.4 revealed that there is enantio-discrimination. Within 480 min of the experiment, 15.2±0.27% of S-KT and 9.5±0.17% R-KT were dissolved. A significant stereoselectivity was found between the release of profiles of R- and S-KT in every experiment performed at pH 7.4. The S/R was in the range 0.91 to 1.99. It is noticed that the S/R ratio during the first 30 min was close to unity (0.92-0.96) and later gradually increased to a factor of 1.99 at the end of dissolution experiment, 480 min. It is noticed that the release of S-KT was faster than that of R-KT. Further, at pH 4.6 and 1.5 the dissolution profile of the enantiomers of ketoprofen from HPMC matrix showed no significant stereoselectivity.

The mechanism of stereoselective release of ketoprofen enantiomers evident from the results of KT2 (alginate) and KT4 (HPMC) matrices warrants some discussion. The tablets were prepared by direct compression of a dry mixed powder. Therefore stereoselective interaction prior to the dissolution experiment would be improbable. As the dissolution medium penetrates the tablet the drug would begin to dissolve, and only then could individual drug molecules interact with the chiral matrix and establish differential binding/interaction, before eventually diffusing from the matrix into the bulk dissolution medium. Drugs closer to the tablet surface will have lesser time to interact with the chiral excipient leading to lower or no stereoselectivity. This is evident from the S/R ratio close to unity during the first 30 min in the release studies made on KT4 (HPMC matrix tablets). It follows that a drug molecule deeper in the matrix will experience greater time to interact and be more subject to the enantioselectivity of the excipient. Evidence of increased stereoselectivity with time was indeed found in the KT4 release studies (S/R ratio ranging from 1.4 to 1.99).

In the case of HPMC matrices, KT4, in aqueous media HPMC gels and preserves the matrix integrity. This may provide suitable conditions for stereoselective diffusion of the enantiomers through the chiral environment of the hydrated matrix and/or stereoselective complexation or hydrogen-bonding interaction of the enantiomers, in the ionised form (pH 7.4), with the hydrated chiral polymer, which makes it possible to modulate the release rate of enantiomers. At pH 4.6, possibly the ketoprofen enantiomers is not sufficiently ionised to stereoselectively form complex or interact through hydrogen bonding with the hydrated chiral polymer. Obviously at pH 1.5 ketoprofen in the un-ionised form may not enter into stereoselective interaction with the hydrated chiral polymer. In the case of KT2 (alginate matrix) the matrix disintegrated and gel forming of the polymer is not observed. Besides, the release of ketoprofen from the matrix also is quite rapid at pH 7.4 and 4.6. There could probably be an erosion mechanism that operates in the case of release of

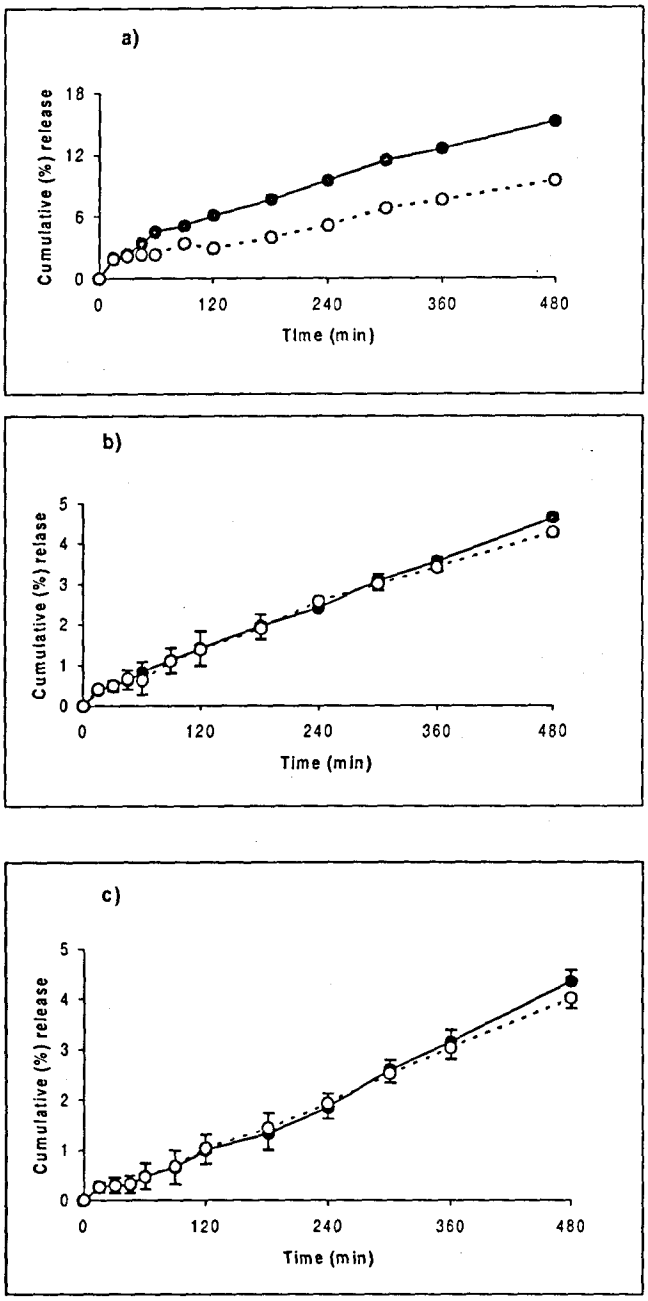

Fig. 3: Dissolution profiles of KT4 matrix tablets.

Plots of cumulative (%) release of ketoprofen enantiomers from KT4 matrix tablets vs time at pH 7.4 (a), pH 4.6 (b) and pH 1.5 (c); (-●-) represents S-KT and (-○-) represents R-KT.

KT enantiomers from alginate matrix and hence lack of chiral discrimination. At pH 1.5, the KT is in the un-ionised form and not able to stereoselectively interact with the hydrated polymer.

Ketoprofen enantiomers have been successfully separated on a β-cyclodextrin based chiral stationary phase[17]. But on the contrary to the expectations the results of the release study, on the matrix tablets prepared from KT-β-cyclodextrin inclusion complex, KT3, revealed lack of chiral discrimination in the drug release. This observation is in line with that of a previous work[7], where release studies on propranolol from β-CD inclusion matrices demonstrated lack of chiral discrimination. There again, propranolol was resolved on a β-CD based chiral stationary phase[17]. Lack of chiral discrimination in the release profile of enantiomers of ketoprofen from CD matrix could be explained as below:

Possibly there existed a difference in the stability of ketoprofen-β-cyclodextrin inclusion complex. The stability difference though minor appears sufficient to elicit enantiodiscrimination by interacting, when chromatographed, with the chiral stationary phase as ketoprofen traverse the chiral environment of the stationary phase. But in the release experiments this difference in stability of the complex probably is not large enough to demonstrate chiral discrimination, as there is interaction between ketoprofen and β-cyclodextrin molecules on 1:1 basis. May be that ketoprofen molecules are almost completely included in the cyclodextrin cavity and the solubility of the complex is influenced more by the interaction between water molecules and β-cyclodextrin than by their interaction with the individual enantiomers. Further, as the matrix disintegrated so fast that the drug has less time to interact with the excipient to demonstrate enantioselectivity.

The results suggest that stereoselective release, retardation, could occur as a result of the presence of a chiral excipient in the tablet matrix in an aqueous environment and also that the process of chiral discrimination is pH-dependent. Further, it is observed that some chiral excipients are less efficient than others in achieving this, which reflect differences between the compounds in terms of their ability to interact differentially with the enantiomers of ketoprofen.

It may be concluded that chiral excipients have the potential to interact stereospecifically with racemic therapeutics and modulate the release profile of component enantiomers. Consequently stereoselective studies of chiral drugs are important in formulation and development in order to overcome potential problems of compatibility, dissolution and subsequent bioavailability. Further, by choosing the appropriate chiral excipient and formulation conditions one can deliberately manipulate the release of a specific enantiomer from a chiral drug. This deliberate stereospecific retardation in the release of enantiomers could be exploited for the design of stereoselective solid dosage forms.

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