Inclusion Complexation of Heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin with Tiaprofenic Acid: Pharmacokinetic Consequences of a pH-Dependent Release and Stereoselective Dissolution

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Abstract □ β-Cyclodextrin was ethylated at the 2- and 6-hydroxyl positions. Diethyl sulfate was employed as an alkylating reagent. NMR spectra data indicate that heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin (DCD) is the principal component of the product obtained. In addition, the FAB mass spectra obtained in nitrobenzyl alcohol and glycerin matrices gave pseudo-molecular ions with *m/z* ratios of 1630.75 and 1711.90 corresponding to $C_{70}H_{126}O_{35}$[2Na·NaCl] and $C_{70}H_{126}O_{35}$[$2C_3H_8O_3$], respectively. The dissolution of tiaprofenic acid (TA) enantiomers, from TA powder (10 mg) and inclusion complex and/or coprecipitate (IC) (TA:DCD 1:1 molar ratio, equivalent to 10 mg of free TA), were examined using the dispersion method at pH values of 1.5, 3.0, and 7.4. Complex formation with the hydrophobic DCD resulted in a significant reduction in the release rate of both *R*- and *S*-TA, as compared to that observed with the powder. At pH 1.5, tiaprofenic acid enantiomers were not released from IC, compared to 20.52 ± 1.47% of *R*-TA and 20.47 ± 1.64% of *S*-TA dissolved from the powder. The greatest stereoselectivity in release profiles was found at pH 3.0 from IC [*S*:*R* 24 h cumulative percent release (ΣR24) ratio of 0.88 ± 0.04]. Elevation of the pH to 7.4, which resulted in a faster dissolution and greater ΣR24 of enantiomers from both powder and IC, was accompanied by a parallel reduction in the stereoselectivity. Following single 20 mg/kg oral doses of racemic TA as both powder or IC to Sprague–Dawley rats, significant stereoselectivity was observed in the plasma concentration profiles of the enantiomers (*S*:*R* $AUC_{(0-\infty)}$ = 1.5). Despite significant reduction in the rate and extent of absorption, there was not a significant difference in the observed *in vivo* stereoselectivity between the two formulations. Therefore, the *in vivo* importance of the observed stereoselectivity in release at pH 3.0 is ruled out. Nevertheless, consideration must be given to the possibility of stereoselective release when chiral excipients are used in the formulation of racemic drugs.

Introduction

Tiaprofenic acid [(±)-5-benzoyl-α-methyl-2-thiophenacetic acid, TA] is a member of the 2-arylpropionic acid class of nonsteroidal antiinflammatory drugs (NSAIDs). It possesses a chiral center and exists as a racemate.[1]

Cyclodextrins are cyclic oligosaccharides that consist of six, seven, or eight glucose units for α-, β-, or γ-cyclodextrins, respectively, linked with α-1,4-glycosidic bonds.[2] Cyclodextrins, and their more water soluble derivatives in particular, have recently been widely utilized in the pharmaceutical formulation of various drugs.[3] Specifically, the unique structure of cyclodextrin enables it to form a host–guest complex by accommodating a wide variety of drug molecules inside its hydrophobic cavity.[4] β-Cyclodextrin has been used to improve the dissolution characteristics of sparingly soluble drugs such as NSAIDs.[5,6]

Cyclodextrins have a truncated cone shape with the wider side formed by the secondary hydroxyl groups and the narrower side by primary hydroxyls. The cyclodextrin provides a microheterogeneous environment since the exterior of the molecule is hydrophilic while the cavity is hydrophobic due to the relatively high electron density.[2] β-Cyclodextrin and its derivatives are also optically active and therefore have been utilized as chromatographic stationary phases to resolve the enantiomers of racemic therapeutic agents.[7,8] In the chiral recognition process, the formation of hydrogen bonds between the hydroxyl groups, located at the entrance of the β-cyclodextrin cavity, and the drug enantiomers is essential. Chiral recognition is dependent on additional factors such as tightness of fit between guest and host molecules, relative distance of the chiral center of the guest molecule from the entrance hydroxyl groups, and the structure and size of the guest molecule. The presence of at least one aromatic ring in the racemic guest molecule structure greatly increases the possibility of stereoselective interaction between the enantiomers and the β-cyclodextrin.[7] Since 2-arylpropionic acids, including TA, possess this structural feature, they are potential candidates for enantioselective interactions. A stereoselective interaction of flurbiprofen enantiomers with trimethyl-β-cyclodextrin has been reported.[9,10]

If cyclodextrins are used in the formulation of racemic orally administered pharmaceuticals, stereoselective release and subsequent absorption of the enantiomers may result. In light of the fact that the pharmacological activity is mostly attributable to the *S* enantiomers of the 2-arylpropionic acid subgroup of NSAIDs, a change in the relative absorption of the enantiomers may influence the pharmacodynamic properties of the drug.

β-Cyclodextrin and its water soluble derivatives such as trimethyl-β-cyclodextrin usually improve the dissolution characteristics of slightly soluble drugs like NSAIDs and thus enhance the rate of dissolution.[5,6,9,10] A rapid rate of dissolution may render detection of stereoselectivity (if it exists) difficult. We have ethylated the β-cyclodextrin to obtain a more hydrophobic derivative. In contrast to trimethyl-β-cyclodextrin, which has amphiphilic characteristics and high aqueous solubility,[9,10] the ethylated β-cyclodextrin exhibits very low water solubility. A reduced rate of release and subsequent dissolution for a water soluble drug, diltiazem hydrochloride, from diethyl-β-cyclodextrin (DCD) has been previously demonstrated.[11] A reduction in the rate of release of TA enantiomers, by inclusion complexation with hydrophobic DCD, may provide suitable conditions for stereoselective intermolecular interactions and/or release. Stereoselective release has indeed been reported for TA from a commercially available sustained release formulation (Surgam SR, Roussel)[12] and propranolol hydrochloride from hydroxypropylmethylcellulose matrices.[13] Furthermore, we have investigated the pharmacokinetics of TA enantiomers in rats and evaluated the possibility of such stereoselective release and its consequence on the absorption.

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Experimental Section

Materials—Racemic tiaprofenic acid and internal standard (IS) (±)-ketorolac tromethamine were obtained from Roussel Canada Inc. (Montreal, Canada) and Syntex Research (Palo Alto, CA), respectively. β-Cyclodextrin was purchased from Sigma Chemical Co. (St. Louis, MO) and used as supplied. All the reagents and solvents employed were of analytical grade.

Synthesis of Diethyl-β-cyclodextrin—Heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin was prepared by modification of a previously described method.[14] β-Cyclodextrin was dissolved in a 1:1 mixture of DMSO–DMF; $Ba(OH)_2{\cdot}8H_2O$ and BaO were then added in portions with continuous stirring over a period of 20 min. After cooling of the mixture to 0 °C, diethyl sulfate was slowly added with vigorous stirring over a period of 2 h. The temperature of the reaction mixture was kept below 10 °C and stirring was continued for an additional 72 h. After reaction of excess diethyl sulfate with ammonium hydroxide solution, the mixture was extracted with ethyl acetate and the product crystallized on standing.

Structure Confirmation—Proton and carbon-13 NMR spectra of the DCD in DMSO-d_6, with tetramethylsilane as internal reference were performed on a Bruker AM-300 FT NMR spectrometer at 22 and 75 °C. The FAB mass spectrum were recorded in nitrobenzyl alcohol as well as glycerin on an MS9 A.E.I (Manchester, England) spectrometer, (operating conditions: mass range, 132–1750; sampling rate, 256; signal level threshold, 1; minimum peak width, 5; scan rate (s/dec), 10.0; total scans in run, 7).

Preparation of Inclusion Complex and/or Coprecipitate—The inclusion complex and/or coprecipitate (IC) of TA and DCD was prepared by the kneading method.[11] Tiaprofenic acid (150 mg) and DCD (900 mg) (1:1 molar ratio) were triturated with 2–5 mL of water. The slurry was thoroughly kneaded for an additional 40 min and the resulting material was then freeze-dried (Freeze Dryer 4.5, Labconco Corp., Kansas City, MI). Formation of IC was evaluated and confirmed by differential scanning calorimetry (2910 TA Instruments Inc., Newcastle, DE) at a heating rate of 10 °C/min under nitrogen.

Solubility Studies—The solubility of TA was determined in the presence and absence of DCD. An excess amount of racemic TA was added to 1 mL of buffer solution at pH 3 (Sorensen glycine-HCl buffer, $\mu = 0.2$) and 7.4 (Sorensen phosphate buffer, $\mu = 0.27$). The same experiment was repeated in the presence of 100 mg of DCD (equivalent to the amount of DCD used in dissolution experiments). Samples (0.01 mL) were taken after 48 and 72 h from the supernatants of the previously centrifuged solubility medium. All experiments were performed in triplicate at 37 °C.

***In Vitro* Dissolution Studies**—The dissolution of TA as a powder and as its IC (<100 mesh) were studied at three different pH values: pH 1.5 (KCl-HCl buffer, ionic strength, $\mu = 0.4$), 3 (Sorensen glycine-HCl buffer, $\mu = 0.2$), and 7.4 (Sorensen phosphate buffer, $\mu = 0.27$). On separate occasions, 10 mg of rac-TA powder and IC (containing the equivalent of 10 mg TA powder) were dispersed in 900 mL of dissolution medium previously equilibrated to 37 ± 0.5 °C.[11] The dissolution medium was stirred at 50 rpm, and samples were withdrawn from the dissolution medium, using a syringe attached to a filter (pore size, 0.22 μm; Millipore Corp., Bedford, MA), just prior to addition of the products, and at 0.08, 0.17, 0.25, 0.33, 1.0, 2.0, 3.0, 6.0, 8.0, 12.0, 24.0 h after the addition of the products. The samples were stored at −20 °C until analysis. Six release studies were performed at pH 3 for IC while the rest of the dissolution experiments were repeated in triplicate.

***In Vivo* Study**—A total of 20 male Sprague–Dawley rats with body weights of 300–350 g were used in this study. All rats were catheterized by insertion of Silastic tubing (0.025 in. i.d. × 0.047 in. o.d.; Dow-Corning, Midland, MI) into the right jugular vein. Rats received 20 mg/kg single oral dose of tiaprofenic acid either as powder ($n = 10$) or IC ($n = 10$). Both formulations were administered as solid particles (>100 mesh) via gastric intubation followed by 500 μL of water. All animals were fasted overnight with free access to water and food was allowed 2 h postdose. Blood samples were withdrawn from the jugular vein cannula at 0, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 h postdose and immediately centrifuged, and the plasma was collected and kept at −20 °C until analysis.

Assay—The concentration of TA enantiomers in the samples obtained from the dissolution medium were determined using a previously reported stereospecific HPLC method.[12,15] Briefly, the method involves acidification of the samples containing IS followed by extraction with isopropyl alcohol–isooctane (5:95). After evaporation of the organic layer the residue was derivatized with trichloroethyl chloroformate and L-leucinamide. The formed diastereomers were extracted into chloroform, and the solvent evaporated. The residue was dissolved in 0.2 mL of mobile phase [0.06 M monopotassium phosphate–acetonitrile–triethylamine (70:30:0.02)] and an aliquot of 0.01–0.05 mL was injected into the HPLC. The HPLC system was equipped with an autoinjector, a multiple wavelength UV detector set at 310 nm, and a stainless steel 10 cm reverse phase column packed with 5 μm octadecyl-bonded silica.

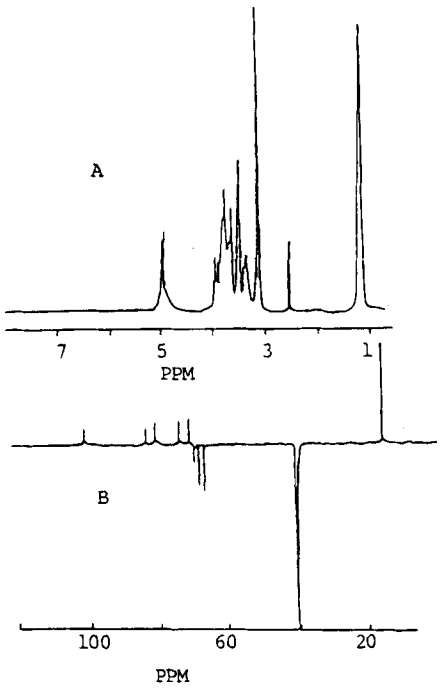


Figure 1—(A) ^1H NMR spectrum of diethyl-β-cyclodextrin obtained in DMSO-d_6 at 75 °C and (B) ^{13}C NMR spectrum of diethyl-β-cyclodextrin obtained in DMSO-d_6 at 75 °C.

Data Analysis—The cumulative amount released (%) was plotted *vs* time. Using the Student's *t*-test for paired data ($\alpha = 0.05$), we assessed the significance of the differences between the enantiomers released in each and every dissolution experiment. We also tested the significance of the differences observed between the enantiomers by pooling data points from all dissolution studies. The release rate constants were estimated using the Hixson–Crowell cube root equation: $M_0^{1/3} - M_t^{1/3} = \kappa t$, where M_0 and M_t are the original mass of the drug particles at time 0 and t, respectively, and κ is the cube root of dissolution rate constant (K).

Following oral administration of the both formulations, plasma concentrations were plotted *vs* time. The $t_{1/2}$ of the *R*- and *S*-TA were obtained by linear regression of the terminal portion of log plasma concentrations *vs* time curves. The area under the plasma concentration–time curves from time 0 to 24 h (AUC_{0-24}) were estimated by the linear trapezoidal rule. The extrapolated area under plasma concentration time curves from 24 h to infinity ($AUC_{0-\infty}$) were estimated from C_{24}/β, where C_{24} and β are the concentration at 24 h and the terminal elimination rate constant, respectively. Peak plasma concentrations (C_{max}) and the time of their attainment (T_{max}) were obtained from our experimental data points. Differences between the concentration time profiles of the enantiomers and their respective pharmacokinetic parameters within each treatment group were assessed by the Student's *t*-test for paired data ($\alpha = 0.05$). Observed difference in the pharmacokinetic indices between treatment groups were examined using independent Student's *t*-test ($\alpha = 0.05$). All results are expressed as the mean ± SD.

Results

NMR spectral data of the DCD are depicted in Figure 1 and corresponding assignments given in Tables 1 and 2. The

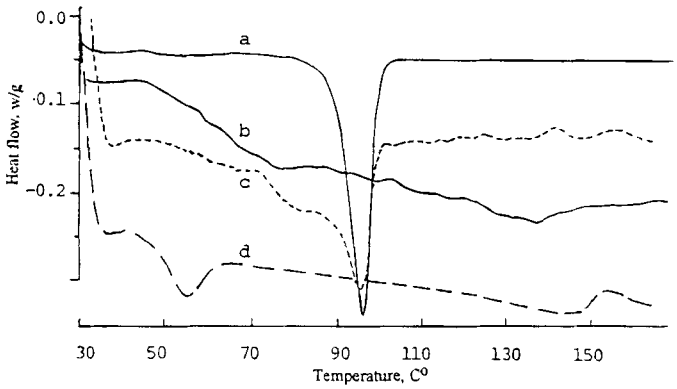
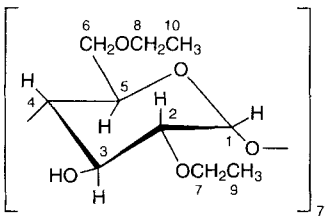


Figure 2—(a) DSC curves of freeze-dried tiaprofenic acid, (b) DSC curves of freeze-dried diethyl-β-cyclodextrin, (c) DSC curves of freeze-dried physical mixture, and (d) DSC curves of freeze-dried inclusion complex and/or co-precipitate (IC).

Table 1—300 MHz Proton NMR of Diethyl-β-cyclodextrin in DMSO-d_6



Chemical Shift (ppm from TMS)	Relative Number of Protons	Assignment
4.92	1	C3 OH
4.88	1	C1 H
3.9	1	C7 H
3.8	1	C3 H
3.75	1	C5 H
3.7	1	C7 H
3.68	1	C6 H
3.61	1	C6 H
3.5	2	C8 H
3.8	1	C4 H
3.31	1	C2 H
1.0–1.25	6	CH_3

Table 2—300 MHz ^{13}C NMR Data of Diethyl-β-cyclodextrin in DMSO-d_6

Chemical Shift (ppm from TSM)	Assignment	Chemical Shift (ppm from TSM)	Assignment
100.7	C1	68.7	C6
82.9	C4	67.2	C7
79.9	C2	65.2	C8
72.8	C3	14.97	C9, C10
69.9	C5		

results indicate that the β-cyclodextrin starting material was ethylated at the 2- and 6-hydroxyl positions. The proton and carbon spectra were comparable to the 400 MHz spectra kindly provided and more recently published[15] by Dr. Kenato Uekama (Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862, Japan).

The FAB mass spectrum obtained in the nitrobenzyl alcohol matrix gave a pseudo-molecular ion with a *m/z* ratio of 1630.75, corresponding to a molecular formula of $C_{70}H_{126}O_{35}$·[2Na·NaCl]. When glycerin was employed as a matrix, a pseudo-molecular ion with *m/z* ratio of 1711.90, corresponding to $C_{70}H_{126}O_{35}[2C_3H_8O_3]$, was observed.

Figure 2 represents the DSC curves of freeze-dried *rac*-TA, DCD, a 1:1 molar ratio of TA:DCD mixture prepared by simple physical mixing, and the product obtained by the kneading method (IC). The disappearance of the endothermic peak at approximately 92 °C in the DSC pattern of IC may indicate inclusion complexation of *rac*-TA and DCD. However, we were not able to obtain the exact stoichiometry of the interaction between enantiomers of TA and DCD due to the lack of finite solubility of IC.

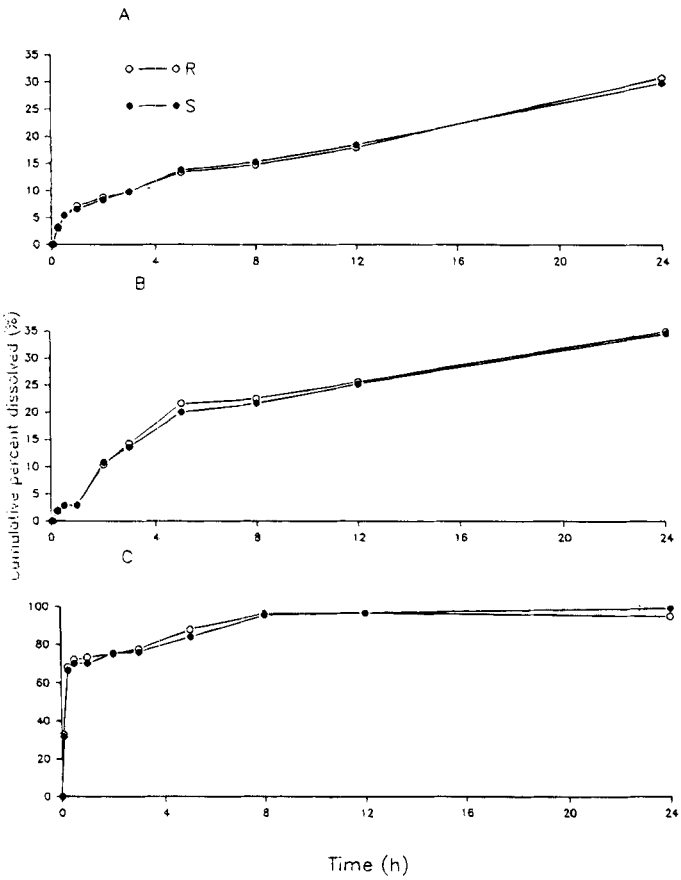


Figure 3—Representative plots of cumulative percentage of dissolved (*R*)- and (*S*)-tiaprofenic acid powder *vs* time at pH 1.5 (A), 3.0 (B), and 7.4 (C).

Solubility Experiments—Equilibrium was established within 72 h, since the concentrations obtained at 72 h were not different than those observed at 48 h. Solubility of both enantiomers of TA was 0.0142 ± 0.0008 mM in the pH 3 buffer at 37 °C. The presence of DCD did not alter the solubility of the enantiomers.

Higher solubility values were obtained for TA enantiomers in pH 7.4 buffer at 37 °C (0.38 ± 0.05 mM) with no stereoselectivity. Addition of DCD into the buffer system did not alter the solubility of the enantiomers at pH 7.4.

***In Vitro* Dissolution Experiments**—A representative dissolution profile of the TA enantiomers, when TA was added to the pH 1.5 dissolution medium as a powder, is depicted in Figure 3A. A lag-time of 0.22 ± 0.03 h was observed for both enantiomers, and their dissolution profiles were superimposable. Within 24 h of the experiment, 20.52 ± 3.01% of *R*-TA and 20.47 ± 2.83% of *S*-TA were dissolved (*S*:*R* ΣR24 ratio of 0.99 ± 0.07). Significant stereoselectivity in the dissolution of the enantiomers was not observed.

The concentrations of enantiomers released from the IC at pH 1.5 could not be measured, as they were below the sensitivity limit of our assay.

Dissolution profiles for TA enantiomers from TA powder at pH 3.0 are illustrated in Figure 3B; dissolution was not stereoselective. Compared to pH 1.5, the ΣR24 values were higher and there was no statistically significant difference

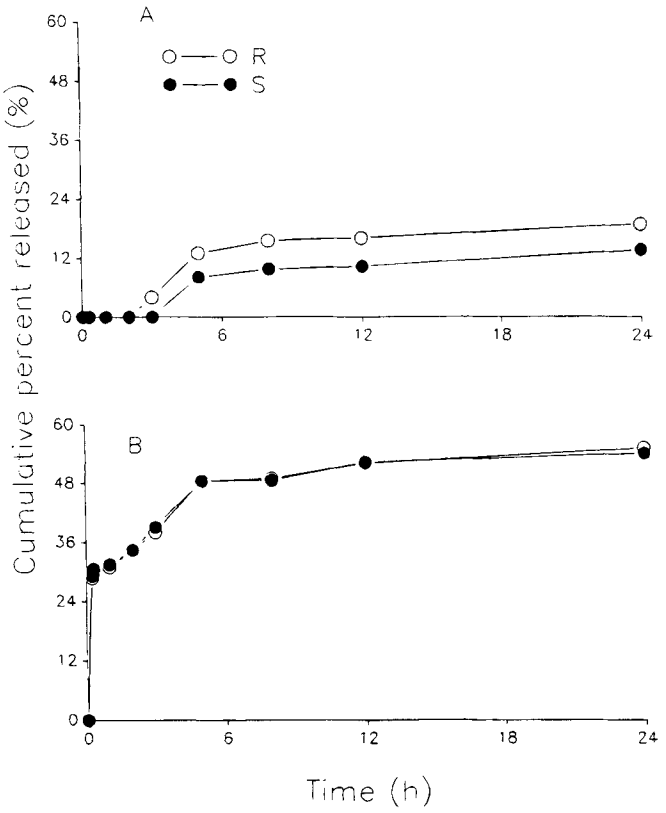


Figure 4—Representative plots of cumulative percentage of dissolved (*R*)- and (*S*)-tiaprofenic acid IC *vs* time at pH 3.0 (a) and 7.4 (b).

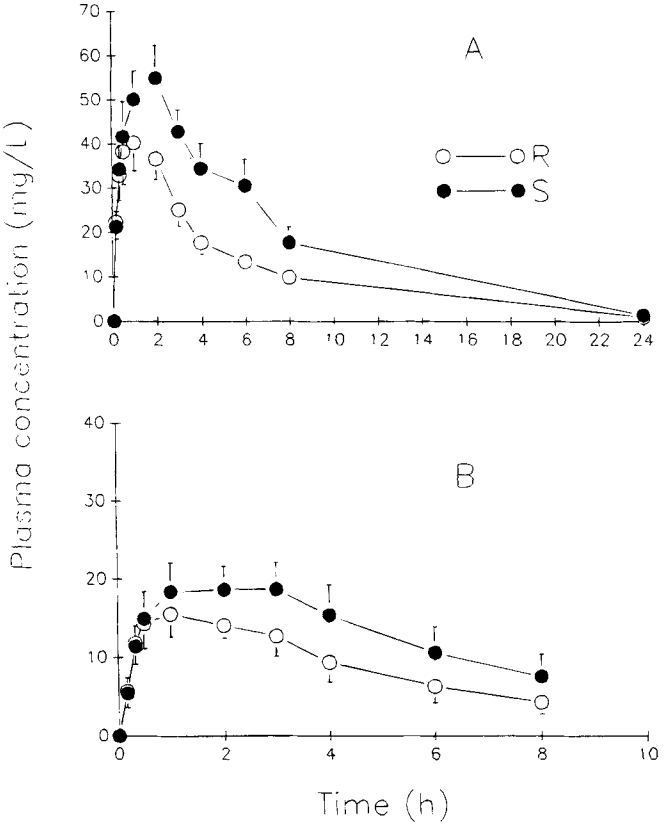


Figure 5—Mean plasma concentration *vs* time plot of TA enantiomer following a single oral dose of 20 mg/kg racemic TA as powder (A) and as IC (B).

between *R* and *S* enantiomer concentrations. The slope of the dissolution rate plots were not different between enantiomers (the release rate constant, $K_R = 0.56 \pm 0.0479$ h^{-1} and 0.55 ± 0.0471 h^{-1} for *R*-TA and *S*-TA, respectively). The ΣR24 values of 54.40 ± 14.01% and 55.9 ± 11.76% were observed for *R*- and *S*-TA, respectively, with an *S*:*R* ΣR24 ratio of 1.04 ± 0.05. Furthermore, dissolution of the enantiomers from the powder did not exhibit any lag-time.

At pH 3.0, the release of TA enantiomers from the IC and their subsequent dissolution were significantly slower than observed with the powder (Figure 4A) ($K_R = 0.169 \pm 0.046$ h^{-1} for *R*-TA and 0.174 ± 0.046 h^{-1} for *S*-TA). A significant stereoselectivity was found between the release profiles of *R*- and *S*-TA in every experiment performed at this pH. Moreover, when data points from all experiments were pooled, the difference was also found to be significant. In general, the release of *R*-TA was faster than that of *S*-TA. There was a trend toward a shorter lag-time (0.50 ± 0.77 h for *R*-TA *vs* 0.92 ± 1.28 h for the antipode) and a greater ΣR24 for the *R* enantiomer [12.80 ± 8.83% and 11.11 ± 7.60% for *R*- and *S*-TA, respectively (*S*:*R* ratio of 0.88 ± 0.04)].

The dissolution of the enantiomers at pH 7.4 was rapid ($K_R = 3.28 \pm 0.24$ h^{-1} and 3.28 ± 0.22 h^{-1} for *R*- and *S*-TA, respectively) with ΣR24 values of 92.87 ± 4.38 and 94.16 ± 5.85% for *R*- and *S*-TA, respectively (*S*:*R* ratio of 1.04 ± 0.02). There was no stereoselectivity in the dissolution patterns of the TA enantiomers, as indicated by the superimposable dissolution profiles (Figure 3C).

At pH 7.4, the release of *R*- and *S*-TA from the IC was considerably faster compared to that observed at pH 1.5 and 3.0 ($K_R = 2.49 \pm 0.25$ h^{-1} for *R*-TA and 2.50 ± 0.25 h^{-1} for *S*-TA). Both enantiomers were immediately released without any lag-time. As shown in Figure 4B, the release profiles of the enantiomers were similar and there was an appreciable reduction in stereoselectivity. At this pH only one of three release experiments gave evidence of significant stereoselectivity. After 24 h, 49.28 ± 4.46% of *R*-TA and 47.98 ± 5.86% of *S*-TA were released (*S*:*R* ratio 1.01 ± 0.03). This difference between ΣR24 values was not statistically significant.

***In Vivo* Studies**—Significant stereoselectivity in the plasma concentration *vs* time profiles of the enantiomers were observed, following a single oral dose of 20 mg/kg of racemic TA as a powder (Figure 5A). The concentration of *S*-TA was consistently higher than that of *R*-TA. The T_{max} was longer and C_{max} greater for the *S* enantiomer. No significant stereoselectivity was observed with respect to the mean terminal $t_{1/2}$ (Table 3).

The absorption rate of the both enantiomers of TA was significantly reduced after a single oral dose of 20 mg/kg of racemic TA given as IC reflected by longer T_{max} for both enantiomers as compared with the powder (Table 3). Similar to the powder, there was a significant stereoselectivity in the plasma concentration–time profiles of the enantiomers in favor of *S*-TA (Figure 5B). The $t_{1/2}$ values for both enantiomers of TA after IC were not significantly different than those observed for the powder. The relative bioavailability values of TA enantiomers from the IC were approximately 47% and 42% for *R*- and *S*-TA, respectively.

The *S*:*R* concentration ratio over time for both formulations increased from approximately 1 to 1.5 at 4 h and remained constant thereafter (Figure 6). No apparent differences were observed in the *S*:*R* concentration ratio between the IC and powder. Similarly *S*:*R* ratios for $AUC_{0-\infty}$ was not significantly different when the two formulation were compared to one another (Table 3).

Discussion

The preparation of partially substituted β-cyclodextrins such as heptakis(2,6-di-*O*-methyl)-β-cyclodextrin and hep-

Table 3—Bioavailability Data Obtained after a 20 mg/kg Dose of Racemic TA

	T_{max} (h)		C_{max} (mg/L)		$AUC_{0-\infty}$ (mg h/L)			$t_{1/2}$ (h)	
	R-TA	*S*-TA	*R*-TA	*S*-TA	*R*-TA	*S*-TA	AUC Ratio *S*:*R*	*R*-TA	*S*-TA
Powder									
Mean	0.83	2.05[a]	45.43	57.33[a]	221.6	353.9[a]	1.60	6.01	5.18
SD	0.16	0.26	6.77	6.99	33.35	55.31	0.07	1.43	0.91
Inclusion complex									
Mean	1.85[b]	2.45[a,b]	21.36	26.64[a]	103.9	148.4[a]	1.44	5.05	6.47
SD	0.41	0.52	2.81	4.25	22.66	33.91	0.07	1.63	2.47

[a] Significantly different than the corresponding value for the *R* enantiomer. [b] Significantly different than the corresponding value for the inclusion complex.

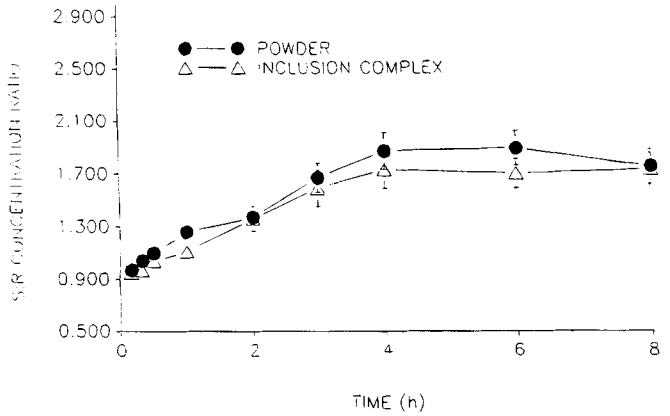


Figure 6—Mean plasma *S*:*R* concentration ratio *vs* time after administration of a single oral dose of 20 mg/kg racemic TA.

takis(2,3,6-tri-*O*-methyl)-β-cyclodextrin has been previously reported.[14] These methylated β-cyclodextrin derivatives are extremely water soluble and highly surface active.[9] The rapid dissolution of 2-arylpropionic acid after complexation with β-cyclodextrin and its hydrophilic derivatives masks stereoselectivity as the entire amount of both enantiomers is very rapidly released. However, when a more hydrophobic substituted β-cyclodextrin such as heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin (DCD) is used for the preparation of the inclusion complex, a significant reduction in the solubility and/or release rate of enantiomers of a racemic compound may become apparent. This reduction may provide a means for a proper assessment of any differences in the release characteristics of enantiomers. Therefore, we synthesized DCD with a modification of the method employed for methylating anhydrous β-cyclodextrin.[14] The ethylation of β-cyclodextrin was accomplished in the presence of BaO and $Ba(OH)_2 \cdot 8H_2O$ with diethyl sulfate as an alkylating reagent. The yield of the reaction was 74%. Although it is possible that the final product is a mixture of mono, di, and/or triethyl-β-cyclodextrins, the proton and carbon NMR spectra indicate that heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin is the main component. Furthermore, comparison of our NMR spectra with those of authentic heptakis(2,6-di-*O*-ethyl)-β-cyclodextrin[16] confirms the above conclusion. As a consequence, further attempts were not made to isolate DCD from the other substituted β-cyclodextrins, since the objective of this work was reduction of the release rate of the enantiomers from IC and evaluation of the difference in the solubility and release rate of *R*- and *S*-TA from the DCD inclusion complex and/or coprecipitate.

It has been demonstrated that (*S*)-flurbiprofen is preferentially included in the cavity of trimethyl-*O*-cyclodextrin.[9,10] This interaction has been attributed to changes in the capacity for intramolecular hydrogen bonding due to the replacement of the hydroxyl by methoxy groups.[9,10] In a recently published study, the difference in the binding enthalpy, *H*, was determined for the individual enantiomers of fenoprofen, flurbiprofen, 1-phenylethanol, and mandelic acid. An enhanced enantioselectivity in favor of the *S* enantiomer of the aforementioned drugs (high difference in binding enthalpy values) was observed when the alkylated cyclodextrins were employed as host molecules. This difference in enantiodiscriminating ability (i.e. derivatized *vs* native cyclodextrins) was attributed to significant intermolecular contact with the exterior of the derivatized cyclodextrins whereas there is only interior contact with the native cyclodextrins. Therefore, it seems that chiral discrimination may take place on the exterior of cyclodextrins.[18] We have similarly observed the preferential inclusion of *S*-TA into DCD. A slower rate of release and longer lag-time for *S*-TA was evident, suggesting preferential interactions and/or release of this enantiomer with DCD. Stereoselectivity seems to be pH dependent with the largest differences between the enantiomers observed at pH 3.0 (*S*:*R* ∑R24 ratio of 0.88 ± 0.04). Moreover, elevation of the pH to 7.4 significantly reduced stereoselectivity. This may be explained by the faster dissolution of TA as a result of increased ionization at higher pH. Indeed, the influence of pH on the release rate of various NSAIDs from the cyclodextrin has been reported.[5,6] Hence we did not find any significant change in the solubility of the enantiomers at pH values of 3 and 7 in the presence or absence of DCD; the observed stereoselectivity at pH 3 may be attributed to a differential release of TA enantiomers from IC. It appears that an elevation in the pH of the dissolution medium results in a decrease in the apparent stability constants of NSAIDs–CD inclusion complexes.[6] We similarly observed a pH-dependent rate of release of TA enantiomers from both the powder and IC. Complexation resulted in a significant reduction in the rate of dissolution and 24 h cumulative recovery of the enantiomers at pH values of 1.5 and 3.0. The release at pH 7.4, on the other hand, was rapid from the inclusion complex. The slow release and dissolution of diltiazem hydrochloride, a water soluble drug, from a DCD inclusion complex, on the other hand, does not appear to be affected by pH, agitation, or low surfactant concentrations.[11]

Both the extent and rate of release from DCD appear to increase with elevation of pH with rapid and complete release at pH 7.4 (Figure 4). This suggests that the prolonged T_{max} observed after oral administration of IC (Figure 5) is due to slow or no release in the stomach and duodenum where low pH ranges are expected. Once in the distal intestine, the drug appears to be released from the formulation and absorbed immediately and completely. It is not surprising, therefore, to observe no significant *in vivo* stereoselectivity (Figure 6) as the major site of release after IC was the distal intestine where pH is alkaline and we detected no stereoselectivity in release at pH 7.4. Moreover, any stereoselectivity in the release and subsequent absorption of the enantiomer may be masked by *in vivo* variability. It is also plausible that stereoselectivity in the release of TA enantiomers may not be readily evident due to the presence of presystemic chiral inversion of *R*-TA to its antipode.[17] This is, however, unlikely as the majority of the included drug is released and subse-

quently absorbed in the distal intestine, which has a pH range of 7–8, at which no stereoselectivity in release was observed.

The possibility of stereoselective release of TA enantiomers from a commercially available sustained release formulation and its affect on the pharmacokinetics of the enantiomers in humans have been previously reported.[12] Similar to the present observation, the stereoselectivity in the release of the enantiomers from the sustained release dosage form at lower pHs disappeared once the pH of the medium increased to a point at which a complete and rapid release of the enantiomers was achieved. Hence, a single oral dose of the above mentioned sustained release products to humans produced the same *S*:*R* concentration ratio as that of a regular release tablets (AUC *S*:*R* of 1),[12,17] indicating that the observed stereoselectivity in the *in vitro* dissolution of the sustained release product did not alter the disposition of the TA enantiomers.[12]

After a single oral dose of TA, plasma concentrations of the *S* enantiomer were predominant. This is contrary to the observation in humans, where superimposable plasma concentrations were noticed for the enantiomers.[12,17] We did not investigate the mechanism of the observed stereoselectivity of TA pharmacokinetics in rats; nevertheless, it may be due to stereoselectivity in the distribution and/or elimination processes. Furthermore, similar to other 2-arylpropionic acids, TA may a undergo unidirectional chiral *R* to *S* inversion.[1]

In conclusion, we have observed that the rate and stereoselectivity of release of TA from DCD are pH-dependent. Although in this case, the stereoselectivity of release had no apparent consequence on the pharmacokinetics indices, in the formulation of a racemic drug, the possibility of stereoselective release should be considered when optically active excipients are included in the dosage form.

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