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Chiral separations of underivatized arylpropionic acids by capillary zone electrophoresis with various cyclodextrins Acidity and inclusion constant determinations

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Abstract

Chiral separations of non-steroidal anti-inflammatory drugs of the family of arylpropionics acids (carprofen, flurbiprofen, indoprofen, ketoprofen, naproxen, pranoprofen and suprofen) were studied by capillary electrophoresis in different pH buffers (pH 4, 6, 8 and 10) containing various neutral cyclodextrins (CDs) (β-CD, hydroxypropyl-β-CD, dimethyl-β-CD, trimethyl-β-CD and hydroxypropyl-γ-CD). Baseline resolution of all the racemates was only achieved with trimethyl-β-CD at pH 4.0. The solute acidity constants and apparent formation constants of their inclusion complexes were also derived from these experiments as an aid for understanding chiral recognition and selectivity optimization. It is clearly shown that no conclusion with regard to the stereoselectivity of a CD can be drawn from the strength of the fit between the CD and the enantiomers.

Keywords: Capillary electrophoresis; Buffer composition; Enantiomer separation; Acidity constant; Inclusion complex formation constants; Arylpropionic acids; Anti-inflammatory drugs, non-steroidal; Cyclodextrin

1. Introduction

2-Arylpropionic acids (APAs) represent an important group of non-steroidal anti-inflammatory drugs (NSAIDs). All are chiral and, with the exception of naproxen, are marketed as racemates. Their activity resides mainly in the enantiomers with the *S*-configuration. The difference in activity for most APAs is compensated by metabolic inversion of the (*R*)-(−)- to the (*S*)-(+)-enantiomer [1,2]. The extent of this reaction appears to depend both on the structure of the acid and on the animal species under investigation [3]. Some, such as flurbiprofen, undergo no observable transformation [4,5]. For benaxoprofen, the decreased rate of metabolism and excretion of the inactive enantiomer in elderly patients lead to hepatotoxicity and, consequently, the drug has been withdrawn from the market [6,7].

Chiral separations are therefore needed to control the optical purity and the metabolic rate of arylpropionic acids. The first chiral liquid chromatographic separations were obtained after derivatization of the racemate with an appropriate chiral reagent to produce diastereoisomeric derivatives [8]. In general, the APA carboxylic group is transformed into an ester func-

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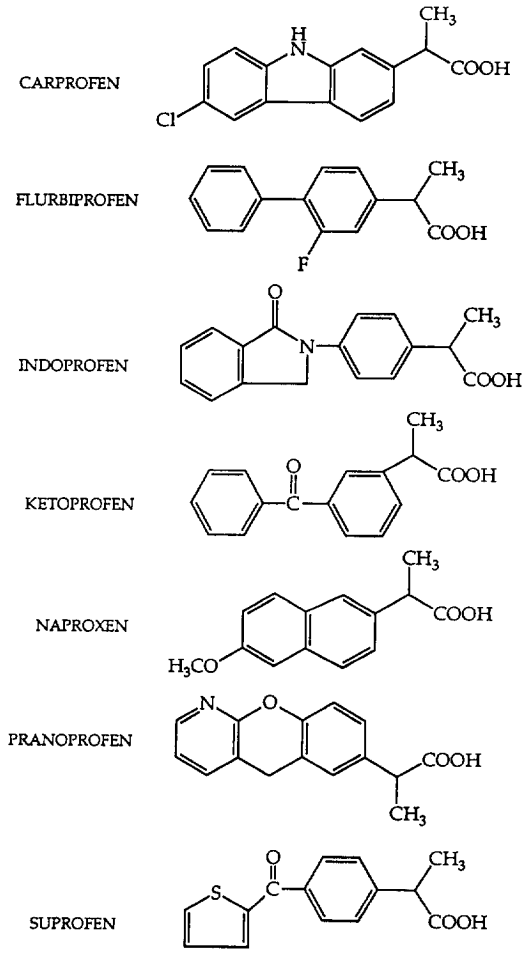

Fig. 1. Structures of the arylpropionic acids studied.

tionality with reagents such as L-leucine amide [9] and (*R*)-(+)-α-phenylethylamine [10]. This indirect method may introduce inaccuracies into the determination of enantiomeric ratios owing to the chiral impurities in the derivatizing agent and/or to racemization during the derivatization procedure [11]. Direct resolution of underivatized APAs was also obtained with chiral stationary phases of Pirkle and co-workers [12,13], ligand exchange [14], protein (bovine [15] and human [16] serum albumin, α-acid glycoprotein [17], ovomucoid [18], flavoprotein [19], and avidin [20]), cyclodextrin (CD) [21], and cellulose {tris(3,5-dimethylphenylcarbamate) of cellulose and amylose [22]} types. Chiral separation was also obtained by supercritical fluid chromatography with an immobilized polysiloxane-anchored permethyl β-CD stationary phase [23].

Recently, arylpropionic acids have been studied by capillary electrophoresis. The first separations (carprofen, fenoprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen and suprofen) were achieved using linear oligo- or polysaccharides as chiral agents [24–26]. The separation was highly dependent on the nature of the maltodextrins used. Oligomers with a degree of polymerization higher than 7 led to the highest enantioselectivity [26]. It was noted that no separation was obtained with cyclic polysaccharides such as β-CD, dimethyl-β-CD and hydroxypropyl-β-CD when using a pH 7.0 buffer [24]. Simultaneously with this work, two groups have shown that cyclodextrins can be suitable for the direct separation of APAs. Rawjee and coworkers [27–29] illustrated their model for the chiral separation of enantiomers of weak acids with the separation of ibuprofen and naproxen enantiomers with β-CD and hydroxypropyl-β-CD, respectively. Chiral resolution was obtained with a buffer having a pH close to the pK_a of the analyte (ca. 4.5), for CD concentrations higher than 10 m*M* in the presence of hydroxyethylcellulose to suppress electroosmosis [28]. Fanali and Aturki [30] studied the enantiomeric separation of fenoprofen, ibuprofen, flurbiprofen, suprofen, ketoprofen and indoprofen with three derivatized CDs (heptakis-2,6-di-O-methyl-β-CD, heptakis-2,3,6-tri-O-methyl-β-CD and 6^A-methylamino-β-CD). They demonstrated that tri-O-methyl-β-CD is the best chiral selector, allowing baseline separations at pH 5 with a 25 m*M* concentration. They also showed that methanol had a positive effect on the separation, mainly for flurbiprofen. Karger et al. [31] reported the slight separation of tiaprofen, flurbiprofen and carprofen by micellar electrokinetic chromatography in the presence of γ-CD. Another successful approach for flurbiprofen and ketoprofen was to use a protein, avidin, with a pH 6 buffer in presence of 10% of organic solvent (ethanol or 2-propanol) [32]. Recently, macrocyclic antibiotics, vancomycin [33] and ristocetin A [34], proved to be good chiral selectors for carprofen, ibuprofen, indoprofen,

fenoprofen, flurbiprofen, ketoprofen, naproxen and suprofen. Chiral separation of ibuprofen and flurbiprofen was also obtained by open-tubular capillary electrochromatography with an immobilized polysiloxane-anchored permethyl β-CD [35].

Concurrently with the work of Rawjee and Vigh [28] and Fanali and Aturki [30] and complementary to them, a systematic study of the influence of pH, ionic strength and nature of the CD (β-CD, hydroxypropyl-β-CD, dimethyl-β-CD, trimethyl-β-CD and hydroxypropyl-γ-CD) on the separation of some arylpropionic acids (carprofen, indoprofen, flurbiprofen, ketoprofen, naproxen, pranoprofen and suprofen) was undertaken. This allowed the determination of the acidity constants of these weakly soluble analytes directly in aqueous solution, some of them previously not available in the literature. In addition, the experiments enabled us to derive apparent formation constants of the inclusion complexes for each analyte–CD pair at different pH values. These values were used to understand better the inclusion of this series of APA with regard to ionization state, molecular shape and nature of the CD. They were also analysed to provide support for the understanding of the chiral recognition mechanism.

2. Experimental

Separations were performed with a Quanta 4000 apparatus (Waters, Marlborough, MA, USA). Data were collected with a NEC Powermate 386/25 computer using Maxima 825 acquisition software. Untreated 50 μm I.D. (363 μm O.D.) × 60 cm (52.5 cm to the detector) fused-silica capillaries (Waters) were used. Injection was performed hydrodynamically by raising the sample vial and the capillary inlet 10 cm above the capillary outlet for 10 s. Separations were performed at 30 kV. UV detection was monitored at 254 nm. The temperature in the capillary compartment was maintained at ca. 26–27°C with a fan. New capillaries were conditioned by performing the following flushes: 1 *M* NaOH for 10 min, water for 5 min, 6 m*M* NaOH solution containing 0.5 *M* NaCl for 10 min, water for 5 min and the buffer for 10 min. Prior to each run, the capillaries were rinsed with 10 m*M* NaOH for 1 min and buffer for 4 min. All the experiments were duplicated.

β-Cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPβCD) with a degree of substitution (DS) of 0.6, dimethyl-β-cyclodextrin (DMβCD) with a DS of 1.8 and hydroxypropyl-γ-cyclodextrin (HPγCD) with a DS of 0.6 were a kind gift from Wacker-Chemie (Munich, Germany, and Lyon, France). Heptakis (trimethyl-β-cyclodextrin) (TMβCD) was obtained from Sigma (St. Louis, MO, USA). Formic acid (99%), morpholinoethanesulfonic acid (MES), bicine and β-alanine were purchased from Aldrich (Milwaukee, WI, USA). Carprofen, indoprofen, flurbiprofen, ketoprofen, pure (*S*)-naproxen, pranoprofen and suprofen were a kind gift from Rhône-Poulenc Rorer (Vitry-Alfortville, France).

All buffers were prepared with water from a Milli-Q water-purification system (Millipore, Bedford, MA, USA) and were adjusted to the desired pH with 1 *M* NaOH (pH 4.0, formic acid; pH 6.0, MES; pH 8.0, bicine; pH 10.0, β-alanine). The ionic strength was maintained at 75 m*M* in all buffers, unless indicated otherwise. Buffers were filtered and thoroughly degassed prior to use. Analytes (0.5 m*M*) were dissolved in water–methanol (50:50, v/v).

3. Results and discussion

The main parameters affecting chiral separations with CD-based electrolytes are expected to be the nature and concentration of the CD and the buffer pH. Secondary parameters are ionic strength, presence of an organic solvent (nature and percentage), electroosmotic flow modifiers [static capillary coating, cellulose, poly(vinyl alcohol)] and other additives (e.g., urea). To simplify the first screening, the ionic strength was set to 75 m*M* and no solvent or other additives were used. High ionic strength buffers were used as they were shown to improve chiral separations [36]. To minimize the time for

method development, experiments were done under 30 kV. APAs are anionic at pH values higher than the pK_a of their carboxylic group ($pK_a \approx 4.5$). The study was conducted at four different pH values (4.0, 6.0, 8.0 and 10.0) with four CDs (βCD, DMβCD, HPβCD and HPγCD) at two concentration levels (1 and 10 mM). These CDs have turned out to be particularly favourable for chiral separations [37]. The optimum CD concentration for a 1:1 CD–guest molecule complex was shown to be inversely proportional to the square root of the product of the formation constants of the inclusion complex of each enantiomer with the CD [38]. The formation constants are generally in the range 100–1000 M^{-1}, which corresponds to an optimum concentration between 1 and 10 mM. For each analyte, experiments without CD were carried out to characterize their electrophoretic behaviour while they are free. The results of this first screening (see below) led us to study the stereoselectivity of TMβCD at 1, 4, 10 and 20 mM concentration levels in two buffers (pH 4.0 and 8.0) and for different ionic strengths (35, 60 and 75 mM).

3.1. Influence of pH on analyte ionization and determination of acidity constant

In the course of these experiments, it was first ascertained that the analyte mobilities were nearly constant in the absence of CD at pH values higher than 6.0, which corresponded to the expected range where the analytes were almost fully ionized. The data obtained were processed to derive the analyte acidity constants, some of them being unavailable in the literature. These constants can be easily determined from the equation [39,40]

$$pK_a = pH + \log\left(\frac{\mu}{\mu_{A^-} - \mu}\right) + \frac{0.5085 z^2 \sqrt{I}}{1 + 0.328 a \sqrt{I}} \quad (1)$$

where μ is the electrophoretic mobility at the pH of the buffer, μ_{A^-} the electrophoretic mobility of the fully ionized acid, z the valency of the ion, I the ionic strength of the buffer solution and a the ion size parameter, generally unknown but assumed to be 5 Å. The third term in Eq. 1 is equal to $-\log\gamma$, where γ is the activity coefficient of the ions in the solution. This term corresponds to 0.1 pH unit for a 75 mM ionic strength buffer. pK_a values were initially determined from Eq. 1 using the mobilities obtained at pH 4.0 and 10.0 (fully ionized acid). The use of the linear regression between the inverse of the mobility and the inverse of the hydrogen concentration gave identical results. The experimental values and the available literature values are shown in Table 1. Considering an

Table 1
Acidity constants (pK_a) of arylpropionic acids in water as determined in this work and reported in the literature

APA	This work (CE, 26–27°C)	Literature values						Hammett and Taft prediction 25°C)
		[41][a] (25°C)	[42][a,b]	[43][a,b]	[44] (DMS, 25°C)	[45] (A or S)[b]	[28] (CE, 35°C)	
Carprofen	4.29							
Flurbiprofen	4.13	4.13				4.9		4.09
Indoprofen	4.29				4.42			
Ketoprofen	4.03	4.00			4.45	3.7		4.06
Naproxen	4.26	4.15		4.2	4.29	4.1	4.68	4.30
Pranoprofen	4.35							
Suprofen	4.00		3.91					4.01

All values corrected for ionic strength. CE = capillary electrophoretic measurements; DMS = extrapolation obtained from pK_a value obtained in water–dimethyl sulfoxide (20:80, w/w) by potentiometric determination; A = absorbance measurements; S = solubility measurements.

[a] Method not defined. [b] Temperature not given.

accuracy of 0.02 in buffer pH measurements and $0.2 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} in electrophoretic mobility measurements, the accuracy of this determination is estimated to be less than 0.1.

The values obtained here in water at 26–27°C by capillary electrophoresis (CE) are in good agreement with those reported elsewhere [41–43]. It should be noted that in the papers cited the methods used were not specified. Fini et al. [44] determined pK_a values in water indirectly from those obtained in water–dimethyl sulfoxide (20:80, w/w) by a potentiometric method, which is expected to lead to poorer accuracy. However, the latter determinations are in good agreement with those obtained by CE, except for ketoprofen. Herzfeldt and Kummel [45] obtained values either by UV absorbance (determination of the absorbance maximum with respect to pH at a given wavelength) for flurbiprofen and naproxen or by solubility for ketoprofen. The value derived for flurbiprofen seems doubtful. Lastly, the naproxen pK_a value obtained by Rawjee and Vigh [28] by CE at 35°C in a 160 mM ionic strength aqueous buffer does not match the other determinations, even after correction for ionic strength. The temperature difference does not explain either this value, knowing that the pK_a temperature variation coefficient of acetic acid is $1 \cdot 10^{-3}$ °C^{-1} [46]. The APA pK_a values can also be estimated with an accuracy of ±0.2 using Hammett and Taft's method, considering each fragment of the molecule as an acidity entity (see Table 1) [46]. The pK_a prediction obtained with this method is in excellent agreement with our determination.

Capillary electrophoresis thus appeared to be an easy and suitable method of direct pK_a determination for species having a low water solubility and available in mixtures or in small amounts.

3.2. Determination of inclusion complex formation constant

A knowledge of the formation constant for inclusion complexation is of interest first to appreciate the extent of the inclusion of an analyte into the cavity of a CD and also to predict the optimum CD concentration leading to the best enantioselectivity [28,38,47,48].

Assuming the existence of a 1:1 inclusion complex, the apparent mobility, μ, of an enantiomer in presence of a CD at a given pH is

$$\mu = \frac{1}{1 + K[\mathrm{CD}]} \cdot \mu_f + \frac{K[\mathrm{CD}]}{1 + K[\mathrm{CD}]} \cdot \mu_c \qquad (2)$$

where μ_f is the electrophoretic mobility of the enantiomer in its free form, μ_c that of the enantiomer in its complexed form, K the formation constant of the inclusion complex and [CD] the CD concentration. The mobility μ_f is the value that is determined directly in the absence of CD. Therefore, for a given CD and at a given pH, it is possible to estimate rapidly the two unknown parameters, μ_c and K, from two experiments ($i = 1, 2$) at two different CD concentrations by resolving the set of two equations

$$\mu_c = \frac{1 + K[\mathrm{CD}]_i}{K[\mathrm{CD}]_i} \left(\mu_i - \frac{1}{1 + K[\mathrm{CD}]_i} \cdot \mu_f \right) \qquad (3)$$

($i = 1, 2$) and

$$K = \frac{([\mathrm{CD}]_2 - [\mathrm{CD}]_1)\mu_f - \mu_1[\mathrm{CD}]_2 + \mu_2[\mathrm{CD}]_1}{[\mathrm{CD}]_1[\mathrm{CD}]_2(\mu_1 - \mu_2)} \qquad (4)$$

From Eq. 4, it can be shown that the best precision of K determination is obtained when the difference between μ_1 and μ_2 and between μ_1 and μ_f is maximum, which means that μ_2 and μ_1 should be close to μ_c and $1/2(\mu_f + \mu_c)$, respectively. It is clear that the two levels of CD concentration that were systematically studied, 1 and 10 mM, fitted the best for a log K value of the order of 3. When log $K < 3$, higher CD concentrations are needed to obtain a more acceptable estimate. In the case of TMβCD, the log K value was recalculated using CD concentrations of 1 and 20 mM for log $K > 2.7$, 4 and 20 mM for $2 <$ log $K < 2.7$ and 10 and 20 mM for log $K < 2$. A correction of the measured effective mobility for electrolyte viscosity was also achieved at high CD concentration through the measurement of the ratio of the current obtained without CD to that obtained in the presence of CD [38,48]. The correction factors were 1.03, 1.06 and 1.12 at 4, 10 and 20 mM,

Table 2
Calculation of apparent inclusion constant (log K) of various APAs with various cyclodextrins at pH 4.0 and 8.0 from mobility measurements at two different CD concentration levels

APA	βCD		HPβCD		DMβCD		TMβCD		HPγCD	
	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8	pH 4	pH 8
Carprofen	2.8	2.6	3.5	3.4	3.8	3.4	3.0	2.8	2.9	2.3
Flurbiprofen	3.6	3.4	3.7	3.5	4.3	3.8	3.0	2.7	2.7	2.3
Indoprofen	2.3	1.9	2.7	2.3	3.1	2.4	2.1	1.5	2.2	2.0
Ketoprofen	2.8	2.5	3.1	2.6	3.1	2.7	1.9	1.6	2.3	1.9
Naproxen	3.1	2.4	3.3	2.6	3.5	2.9	2.3	1.7	2.4	2.0
Pranoprofen	2.6	2.3	2.9	2.4	3.1	2.3	2.0	c.a. 1.1	2.4	2.1
Suprofen	3.1	3.0	3.3	3.0	3.6	3.1	2.2	1.9	2.2	2.0

Electrolyte, 75 mM ionic strength; temperature, 26–27°C; for further explanations, see text.

respectively, with TMβCD and 1.03 at 10 mM with all the other CDs. Table 2 gives the apparent formation constants for inclusion complexation so determined for the various APAs and CD studied in 75 mM ionic strength aqueous buffers of pH 4.0 and 8.0. Using this approach, the confidence interval in log K determinations was calculated to be ±0.2. When a chiral separation was observed, it was possible to determine the inclusion constant for each enantiomer. However, the calculated log K difference was less than 0.1, which is less than the confidence interval. Hence only the formation constant of the more complexed enantiomer is given in Table 2. It should be noted that a correction of the mobility of 1.03 for viscosity at a CD concentration of 10 mM affected the result by less than 0.05, which is substantially within the error margin. In the case of pranoprofen with TMβCD at pH 8, the inclusion constant was too low to be determined directly within the range of CD concentration studied. The mobility of its fully complexed form was taken to be similar to that of carprofen, which has almost the same molecular mass. From these data and the experiment at a CD concentration of 20 mM, a value of 1.1 can be estimated for this constant.

Most of the calculated formation constants were between 2 and 3.5, which demonstrated good inclusion of the APAs inside the CD cavity. The aromatic rings of the APAs are likely to be included in the CD cavity, to minimize the exposure to the aqueous solvent, with the carboxylate group protruding from the upper rim.

Concerning the nature of the CDs, the following order of complexation was observed in most cases, irrespective of pH: DMβCD > HPβCD > βCD > HPγCD > TMβCD. DMβCD leads to more stable inclusion complexes than the other CDs. DMβCD is characterized by the presence of methyl and hydroxyl groups on the wider rim of its cavity. Crystallographic studies have shown that the methylation at the O(2) and O(6) hydroxyl groups on the ring extends the intramolecular cavity, whereas the round structure of the macrocyclic ring is still maintained by intramolecular O(2)–H–O(3) hydrogen bonds [49]. The extension of the cavity modifies the position of the APA carboxylate group with regard to the upper rim of the CD. The observation that DMβCD is a better complexing agent than βCD and HPβCD may be due to the fact that for DMβCD, the carboxylate group is closer to the secondary hydroxyl group and forms stronger hydrogen bonds. However, using absorbance measurements, βCD was found to be a better complexing agent than DMβCD for naproxen [50]. It was considered that DMβCD may have prevented the carboxylate groups from protruding as far into the solvent water. However, it was also found that ibuprofen was better complexed by DMβCD than βCD. HPγCD has a relatively weaker complexing ability than βCD and

HPβCD. The larger size of this CD allows the analytes to have a greater degree of freedom than that exists with βCDs. TMβCD leads to the least stable inclusion complexes. TMβCD is characterized by the presence only of methyl groups and the absence of secondary hydroxyl groups on its wider rim. It has been shown by crystallographic studies that the macrocyclic ring of TMβCD is markedly distorted compared with that of βCD because of the steric hindrance created by its methyl group and its inability to form intramolecular hydrogen bonds [51]. This distortion promotes flexibility and should lead to a better fit with analytes. However, βCD and DMβCD appeared to be better complexing agents than TMβCD. This indicates that secondary hydroxyl groups are involved in the complexation of APAs and that hydrogen bonding occurs.

With all the CDs studied, the APAs formed more stable inclusion complexes at pH 4.0 than at the other pH values. It was observed that the inclusion constants at pH 4.0 were about twice those obtained at higher pH and that pH no longer has an influence on the inclusion at pH ≥ 6. These results can be explained by the higher inclusion of APAs when they are in their molecular form. Denoting APAs by their capital first letter, the following order of complexation was established:

for βCD, F > S > N > C, K > P > I;
for HPβCD, F > C > S > N > K > P > I;
for DMβCD, F > C > S > N > K > I, P;
for TMβCD, C, F > N > S > I > P > K;
for HPγCD, C > F > P, N > K > I > S.

Flurbiprofen and carprofen formed the most stable inclusion complexes with all the CDs except β-CD, and ketoprofen, indoprofen and pranoprofen appeared to be the least included in all the βCDs. Fanali and Aturki [30] suggested that an aromatic ring bearing a substituent in the *para* position would fit better in the βCD cavity and therefore give higher inclusion constants. Our results showed that it is difficult to draw a conclusion concerning the degree of complexation just by considering the *meta* or *para* position of the substituent on the aromatic ring. For example, this approach does not explain the high degree of complexation of carprofen and the low degree of complexation of indoprofen, the latter having its propionic group in the *para* position.

The method developed here appears to be an easy and rapid way to determine a formation constant from a small number of experiments.

3.3. Influence of nature of CD and pH buffer on APA chiral separations

The nature of the CD and the electrolyte pH are the fundamental parameters regarding the resolution of enantiomers. The influence on the chiral resolution of racemic APA mixtures of four neutral CDs (βCD, HPβCD, DMβCD and HPγCD) at two different concentration levels (1 and 10 m*M*) and of pH values between 4.0 and 10.0 was systematically investigated. No separation was obtained with β-CD and HPβCD (DS = 0.6) whatever their concentration and pH. At pH 4.0, a slight resolution was obtained with DMβCD (DS = 1.8) for indoprofen, suprofen and carprofen, and baseline separation was achieved for pranoprofen. Only pranoprofen was slightly separated at higher pH. HPγCD (DS = 0.6), which has a lower complexing ability, resolved ketoprofen and pranoprofen enantio-

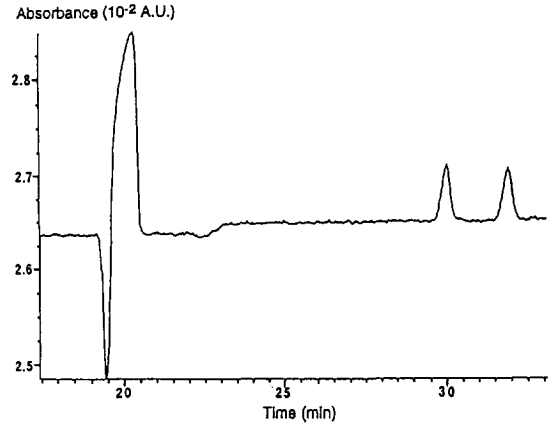

Fig. 2. Chiral separation of pranoprofen. Capillary, 50 μm I.D. × 60 cm (52.5 cm to detector); electrolyte, 58.6 m*M* formic acid adjusted to pH 4.0 with 1 *M* NaOH (ionic strength 35 m*M*), 10 m*M* TMβCD; *V* = 30 kV; UV detection at 254 nm; sample, 0.5 m*M* pranoprofen; hydrodynamic injection, 10 s at 10 cm.

mers at pH 4.0. This screening showed that the highest enantioselectivities were achieved with DMβCD at pH 4.0, i.e., under conditions where APAs are partially protonated. This CD has an apolar character and some of its secondary hydroxyl groups located on the wider rim of the cavity are substituted. This aspect seem to be crucial in the chiral recognition mechanism. This partial derivatization of hydroxyl groups on the rim may help to create distinctive stereoselective interactions (hydrogen bonds and/or hydrophobic interactions) with the enantiomers, which can be modulated by steric hindrance. HPβCD, which has DS < 1, is probably substituted on its more reactive primary hydroxyl group located on the small rim. This could account for its stereoselective behaviour, similar to that of βCD.

To confirm the trend shown by DMβCD, the chiral resolution of the preceding series of APAs was studied with TMβCD as an electrolyte additive at pH 4.0. This CD, when introduced at a concentration of 10 m*M*, allowed the baseline resolution of five out of the six studied APA racemates (carprofen, indoprofen, ketoprofen, pranoprofen and suprofen) and slight resolution was obtained for flurbiprofen. Only partial separations were observed with addition of 1 m*M* TMβCD. Resolution data are given in Table 3. Chiral separations of pranoprofen and carprofen are shown in Figs. 2 and 3. It should be noted that in the presence of an electroosmotic flow, the (*R*)-(−)-enantiomer is expected to migrate faster than its antipode, since Fanali and Aturki [30] have shown by spiking the racemic samples with the pure optical isomers that with TMβCD the (*R*)-(−)-isomer forms more stable inclusion complexes than their antipodes. Also shown in Table 3 is the effect of the ionic strength of the buffer on chiral resolution. As mentioned previously, chiral resolution is improved by an increase in ionic strength. The electroosmotic flow (EOF) values are also indicated in Table 3, the resolution being inversely proportional to the sum of the electroosmotic mobility and the average enantiomer electrophoretic mobility. The EOF mobility is expected to decrease with increase in ionic strength. However, in this work, an increase in the EOF with increase in ionic strength (35, 60 and 75 m*M*) was observed. This tendency may be due to a variation of the surface charge of the capillary wall. Minimizing the EOF with hydroxyethylcellulose [28] or with a coated capillary [30], similar APA separations

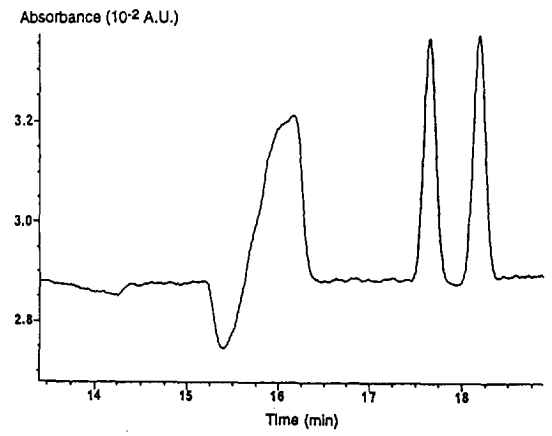

Fig. 3. Chiral separation of carprofen. Capillary, 50 μm I.D. × 60 cm (52.5 cm to detector); electrolyte, 117.2 m*M* formic acid adjusted to pH 4.0 with 1 *M* NaOH (ionic strength 75 m*M*), 10 m*M* TMβCD; *V* = 30 kV; UV detection at 254 nm; sample, 0.5 m*M* carprofen; hydrodynamic injection, 15 s at 10 cm.

Table 3
Enantiomeric resolutions of some APAs obtained with TMβCD as chiral selector and influence of the ionic strength of the electrolyte

APA	Ionic strength, *I* (m*M*)		
	35	60	75
Carprofen	1.5 (9.7)	2.0 (10.2)	2.2 (11.2)
Flurbiprofen	0 (9.1)	nd[a]	0.7 (11.2)
Indoprofen	2.4 (8.7)	nd	nd
Ketoprofen	1.5 (8.7)	nd	nd
Pranoprofen	3.8 (8.9)	2.1 (10.9)	nd
Suprofen	1.3 (8.7)	1.6 (12.0)	1.9 (11.3)

Resolution, R_s, is calculated from $R_s = 1.177(t_{M2} - t_{M1})/(\delta_1 + \delta_2)$, where δ is the width at half-height. Fused-silica capillary, 50 μm × 60 cm (52.5 cm to detector), pH 4.0 formate buffers of various concentrations, containing 10 m*M* TMβCD. Analyte concentration, 0.5 m*M*; hydrodynamic injection for 10 s, except for the last column (15 s). The actual electroosmotic mobility (in 10^{-5} cm^2 V^{-1} s^{-1}) is given in parentheses after each value of resolution.
[a] nd = Not determined.

were reported. Finally, TMβCD appeared to be the best CD for the chiral separation of APAs. The higher flexibility of the intramolecular cavity of this CD may be responsible for its greater stereoselectivity. On the other hand, this study confirms that there is no direct connection between the stability of the inclusion complex and the enantioselectivity of a chiral agent. For example, the formation constant of inclusion complexation for pranoprofen with TMβCD, which exhibited the highest chiral resolution, was one of the smallest with respect to the other APAs and the other CDs. This study also confirms that rapid screening of various CDs at various pH values can lead to the chiral separation of enantiomers. Following this screening, a further optimization with regard to efficiency, analysis time, CD concentration, additive use and a quantitative analysis can be carried out.

4. Conclusion

This study has shown that capillary electrophoresis appears to be a valuable method for the determination of physico-chemical constants such as acidity constant (pK_a) and apparent inclusion constants, especially with compounds that are only slightly soluble in water or are available only in small amounts or in mixtures. The pK_a values obtained by using this technique for a series of arylpropionic acids were in excellent agreement with previously determined values and theoretical expectations. As a result of the screening of various neutral CDs under various pH conditions, DMβCD, TMβCD and HPγCD have been demonstrated to be potential chiral agents for the resolution of APA enantiomers. Baseline separation of most arylpropionic acids was achieved at pH 4.0 with TMβCD. The knowledge of the inclusion constant is then advantageous for establishing the optimum CD concentration more rapidly and for clarifying the chiral recognition mechanism. The fact that trimethyl-β-cyclodextrin can resolve all the APA enantiomers studied, while showing a lower complexing ability than the other CDs tested, confirms that a strong fit between the CD and the enantiomers is not mandatory for their chiral separation.

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