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Enantioseparation of nonsteroidal anti-inflammatory drugs by capillary electrophoresis using mixtures of anionic and uncharged β-cyclodextrins as chiral additives

Nine nonsteroidal anti-inflammatory drugs (NSAIDs) were enantioseparated by capillary electrophoresis using an anionic cyclodextrin derivative (sulfobutyl ether β-cyclodextrin or carboxymethyl-β-cyclodextrin) in combination with a neutral cyclodextrin as chiral additives to a pH 3 phosphoric acid-triethanolamine buffer. In the presence of a negatively charged cyclodextrin, the analytes were given an appropriate mobility but relatively low enantioselectivities were generally obtained when such a cyclodextrin was the only selector added to the buffer. The addition of an uncharged cyclodextrin, such as the native β-cyclodextrin or one of its derivatives (dimethyl-, trimethyl- and hydroxypropyl-β-cyclodextrin), to this kind of buffer containing an anionic cyclodextrin, was found to give rise to considerable improvement in chiral resolution for all compounds studied. Resolution and analysis time were optimized by varying the nature and concentration of the two cyclodextrins. The best compromise was usually achieved by the simultaneous addition of sulfobutyl ether β-cyclodextrin and trimethyl-β-cyclodextrin. Under optimum conditions, the enantiomers of all NSAIDs examined could be completely separated (most often with resolution values higher than 5) in short analysis times (generally lower than 15 min).

1 Introduction

The increasing need for optically pure drug substances has led to the development of various methods for analytical and preparative chiral separations. The inherent ability of capillary electrophoresis (CE) to provide high separation efficiencies combined with its potential for rapid method development and minimal consumption of reagents makes it an ideal technique for rapid and high-resolution chiral analysis [1–7]. Over the past few years, a number of direct CE enantiomeric separations have been performed with cyclodextrins (CD) as chiral selectors. These additives proved to be effective not only in capillary zone electrophoresis (CZE) but also in electrokinetic chromatography (EKC), using different modes such as cyclodextrin-mediated micellar EKC (CD-MEKC) and EKC with charged cyclodextrins (CD-EKC) [8, 9]. Vigh and co-workers [10–12] recently developed a rational approach to predict the electrophoretic mobility differences between the enantiomers of weak acids and bases in CE using CDs as chiral selectors. They identified three types of CD-based chiral CE separations, differentiated by the fact that stereoselective interactions can occur either with the uncharged or charged forms of the enantiomers or with both forms. Therefore the buffer pH should be considered as a major factor influencing CE enantiomeric separations. However, both the type and concentration of CD also have a strong influence on enantiomeric resolution. Vigh *et al.* [10, 11] applied their model for the chiral separation of weak acids to the enantiomeric resolution of nonsteroidal anti-inflammatory drugs (NSAIDs), namely fenoprofen, ibuprofen, and naproxen, with native β-CD or its hydroxypropyl derivative. Enantioseparation was obtained with buffers having a pH close to the pK_as of the analytes (pH 4.89), the electroosmotic flow being suppressed by the addition of hydroxyethylcellulose. Fanali and co-workers [13] also separated arylpropionic acid enanatiomers using pH 5 buffers containing dimethyl-, trimethyl- or methylamino-β-CD. Karger *et al.* [14] reported the enantioseparation of NSAIDs using MEKC in the presence of γ-CD. Recently, linear oligo- or polysaccharides, proteins (avidin), and macrocyclic antibiotics (vancomycin and ristocetin A) also proved to be suitable chiral selectors for NSAIDs [15–18].

Dual CD systems, using a mixture of neutral and charged CDs, have been used by Sepaniak *et al.* [19] for the achiral separation of nonionizable solutes or by Lurie and co-workers [20] for the enantiomeric resolution of cationic drugs of forensic interest. Buffers of pH 9, containing the negatively charged carboxymethyl β-CD and a neutral CD, were used by Anigbogu *et al.* [21] for the enantioseparation of aminogluthetimide [21] while a mixture of the cationic mono (6-deoxy-6-amino) β-CD and the neutral trimethyl-β-CD was added to a pH 2.3 buffer by Lelièvre and Gareil [22] for the enantiomeric resolution of several acidic compounds. In a previous study, high chiral resolution was achieved for five

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Nonstandard abbreviations: CD, cyclodextrin; **β-CD,** β-cyclodextrin; **CMCD,** carboxymethyl-β-cyclodextrin; **DMCD,** dimethyl-β-cyclodextrin; **EKC,** electrokinetic chromatography; **HPCD,** hydroxypropyl-β-cyclodextrin; **NSAIDs,** nonsteroidal anti-inflammatory drugs; **SBCD,** sulfobutyl β-cyclodextrin; **TMCD,** trimethyl-β-cyclodextrin

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acidic drugs (fenoprofen, ketoprofen, hexobarbital, sulindac, and warfarin) by using the anionic sulfobutyl ether β-CD in combination with a neutral CD in a pH 3 phosphoric acid-triethanolamine buffer [23]. In this paper, the usefulness of the simultaneous addition of an anionic CD derivative (sulfobutyl ether β-CD or carboxymethyl-β-CD) and a neutral CD to the pH 3 phosphoric acid-triethanolamine buffer is further investigated for the enantioseparation of a series of NSAIDs. The influence of the nature and concentration of both CDs is studied with the aim of obtaining high enantiomeric resolution within short analysis times.

2 Materials and methods

2.1 Apparatus

All experiments were performed on a Spectraphoresis 1000 CE instrument (Spectraphysics, San Jose, CA, USA) equipped with an automatic injector, an autosampler, a variable wavelength UV visible detector (190–800 nm) and a temperature control system (15–60°C). Electrophoretic separations were carried out with uncoated fused-silica capillaries, 50 μm ID and 44 cm length (37 cm to the detector), provided by Supelco (Bellefonte, PA, USA). At the beginning of each working day, the capillary was washed with running buffer for 10 min, while after each injection the capillary was washed with buffer for 3 min. The injections were made at the cathodic side and the applied voltage was −25 kV (reversed polarity mode). The UV detection (at the anodic side) was performed at 280 nm for indoprofen, ketoprofen, sulindac, suprofen and tiaprofenic acid; 230 nm for carprofen, fenoprofen and flurbiprofen; and 210 nm for ibuprofen. Injections were made in the hydrodynamic mode for a period of 5s (corresponding to 13.3 nL) and the capillary was thermostated at 25°C. For the electrophoretic experiments, a buffer made of 100 mM phosphoric acid, adjusted to pH 3.0 with triethanolamine, was used (current produced in these conditions: 60 μA). The resolution (R_s) and the plate number (N) were calculated according to the standard expressions based on peak width at half-height [24].

2.2 Reagents

β-CD, heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (dimethyl-β-cyclodextrin: DMCD), heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (trimethyl-β-cyclodextrin: TMCD) were from Sigma (St. Louis, MO, USA). Hydroxypropyl-β-cyclodextrin (HPCD) was from Janssen Chimica (Geel, Belgium). Carboxymethyl-β-cyclodextrin (CMCD) was from Cyclolab (Budapest, Hungary). Sulfobutyl ether β-cyclodextrin (SBCD) was kindly provided by Prof. Stobaugh (University of Kansas, Lawrence, KS, USA). Phosphoric acid (85%) and triethanolamine were analytical quality from Merck (Darmstadt, Germany). Water was of Milli-Q quality (Millipore Corporation, Bedford, MA, USA) and methanol was of HPLC grade from Janssen Chimica. Carprofen, fenoprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, sulindac, suprofen, and tiaprofenic acid were from Sigma. The standard solutions were prepared by dissolving each compound at a concentration of about 5×10^{-5} M (20 μg/mL) in a mixture of water and methanol (9:1). In such a dissolution medium, sample stacking was obtained.

3 Results and discussion

In previous papers, buffers made of 100 mM phosphoric acid adjusted to pH 3 with triethanolamine and containing different kinds of β-CD derivatives have proved to be very well suited to the enantioseparation of basic drugs with uncharged and anionic CD as chiral selectors [5–7]. Under these conditions, triethanolamine is adsorbed to the fused silica capillary wall, giving rise to a weak anodic, fairly constant, electroosmotic flow (−7.2 10^{-5} cm^2/Vs; inter-day RSD: 1.5%) [23]. This was found to be favorable to the resolution of the enantiomers of these basic compounds, which migrate electrophoretically towards the cathode [7]. Since it has been shown by Vigh and co-workers [10, 11] that for several acidic compounds, only the nondissociated forms of the enantiomers give rise to selective interactions with CDs, the pH 3 phosphoric acid/triethanolamine buffer was also tested for the enantiomeric resolution of acidic drugs from different pharmacological classes, namely sulindac, fenoprofen, and ketoprofen (NSAIDs), hexobarbital (barbiturate) and warfarin (anti-coagulant) [23].

In such a low pH buffer, these compounds were mainly present in uncharged form and therefore either migrated with or very close to the electroosmotic flow, the latter making their detection possible at the anodic side of the capillary (reversed polarity mode). Under these conditions, no enantiomeric separation could be expected for these acidic compounds from the addition of a neutral CD alone. Indeed, even in the presence of enantioselective interactions with this kind of CD, no chiral resolution would be observed, due to the lack of significant mobility difference between the free and complexed forms of the analyte enantiomers.

However, the use of a negatively charged β-CD derivative SBCD as chiral additive was found to give rise to the formation of complexes migrating electrophoretically towards the anode; consequently, this could lead to the enantiomeric resolution of acidic compounds in uncharged form [23]. Here, such CD-EKC systems, in which an anionic CD is added to the pH 3 phosphoric acid/triethanolamine buffer, possibly together with another CD, were more specifically investigated with respect to their potential for the enantiomeric separation of NSAIDs (carprofen, fenoprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, sulindac, suprofen, and tiaprofenic acid), the structures of which are given in Fig. 1. All these compounds are arylpropionic acid derivatives (profens), except sulindac (an arylacetic acid derivative). The chirality of the latter is due to the presence of a sulfoxide group.

3.1 Systems containing an anionic CD as chiral selector

Two different anionic CD derivatives were tested as chiral additives for the enantioseparation of the NSAIDs at pH 3, namely SBCD and CMCD. SBCD possesses an

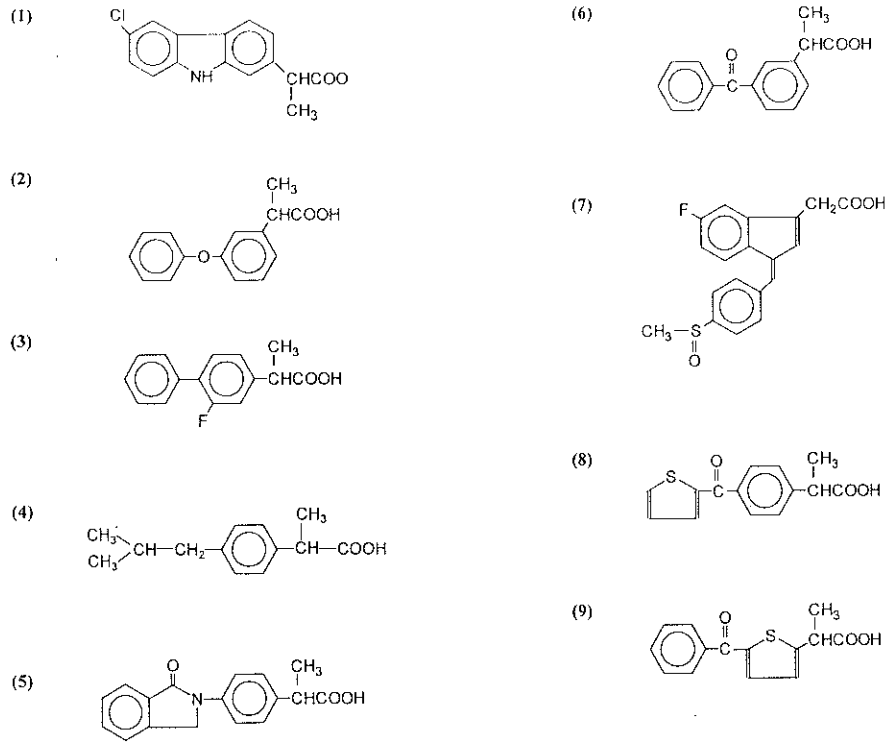

Figure 1. Chemical structures of the NSAIDs examined. (1) Carprofen, (2) fenoprofen, (3) flurbiprofen, (4) ibuprofen, (5) indoprofen, (6) ketoprofen, (7) sulindac, (8) suprofen, (9) tiaprofenic acid.

Table 1. Influence of the type of neutral CD on chiral resolution (R_s) for NSAIDs (dual CD systems with SBCD)[a]

Analyte	R_s				
	–	β-CD	DMCD	TMCD	HPCD
Carprofen	–	–	n.d.	16.6	1.1
Fenoprofen[b]	< 0.7	1.0	2.8	2.8	< 0.7
Flurbiprofen	–	< 0.7	2.0	3.2	–
Ibuprofen	–	–	1.5	1.3	–
Indoprofen	0.9	2.0	0.9	0.9	1.2
Ketoprofen[b]	1.1	1.5	4.7	2.1	1.3
Sulindac[b]	1.4	2.3	3.8	1.6	< 0.7
Suprofen	–	–	–	1.6	< 0.7
Tiaprofenic acid	–	1.1	–	< 0.7	–

a) Buffer: 5 mM SBCD in phosphoric acid/triethanolamine (pH 3) also containing a neutral CD (10 mM; current: 68 μA). Other conditions as described in Section 2.1; n.d., not determined; –, no visible resolution (R_s < 0.5)

b) Data from [23]

average of four butyl chains substituted by sulfonyl groups, which gives this CD a strong negative charge at any commonly used pH in CE. CMCD has an average degree of substitution of 3.5 and its carboxyl groups are only slightly dissociated at pH 3. Clearly, the negative charge of CMCD increases rapidly at higher pH. As can be seen in Table 1, the addition of SBCD at 5 mM concentration to the pH 3 phosphoric acid-triethanolamine buffer leads to incomplete enantiomeric resolution for fenoprofen, ketoprofen, indoprofen, and sulindac. For all the other compounds examined, no chiral resolution was observed, although their migration times were reduced, indicating a significant complexation of these compounds with the polyanionic SBCD.

Table 2 states the resolution values obtained by using CMCD at 10 mM concentration in the same buffer. Partial resolution was only observed for indoprofen enantiomers. Again, however, the migration times of all NSAIDs were found to decrease, although to a lesser extent than in the presence of SBCD. From these results, it can be concluded that both SBCD and CMCD are able to provide NSAIDs with adequate apparent electrophoretic mobility at pH 3, but they cannot by themselves lead to sufficient enantioselectivity for these compounds. By contrast, complete enantioseparation could be obtained earlier for two acidic drugs with widely different structures, warfarin (R_s = 2.2) and hexobarbital (R_s = 1.7), using SBCD as chiral selector in the same kind of buffer [23]. The influence of the concentration of both anionic CDs (SBCD and CMCD) on the enantiomeric resolution of indoprofen was also studied. As can be seen in Fig. 2, resolution values obtained with SBCD are fairly constant in the whole concentration range tested (1–10 mM), while in the case of CMCD, chiral resolution reaches a maximum value in the 5–10 mM concentration range. With both CDs, a decrease in analyte migration times was observed with increasing selector concentration. The effect of adding higher concentrations of these CD derivatives was not investigated because the currents generated were too high.

3.2 Dual systems

As an attempt to improve enantioselectivity for NSAIDs, four neutral cyclodextrins, native β-CD and three of its derivatives, DMCD, TMCD and HPCD, were successively added at 10 mM concentration to the pH 3 phosphoric acid/triethanolamine buffer containing SBCD. In such systems, the mobilities of the analyte enantiomers will

Table 2. Influence of the type of additional CD on chiral resolution (R_s) for NSAIDs (dual CD systems with CMCD)[a)]

Analyte	R_s			
	–	DMCD	TMCD	SBCD
Carprofen	–	1.2	6.0	–
Fenoprofen	–	–	2.5	< 0.7
Flurbiprofen	–	< 0.7	2.7	–
Ibuprofen	–	< 0.7	1.6	–
Indoprofen	1.1	–	1.0	1.0
Ketoprofen	–	< 0.7	1.4	1.3
Sulindac	–	–	1.2	1.9
Suprofen	–	–	1.6	–
Tiaprofenic acid	–	–	–	–

a) Buffer: 10 mM CMCD in phosphoric acid/triethanolamine (pH 3) also containing 5 mM SBCD or a neutral CD (10 mM; current: 75 μA). Other conditions as described in Section 2.1.

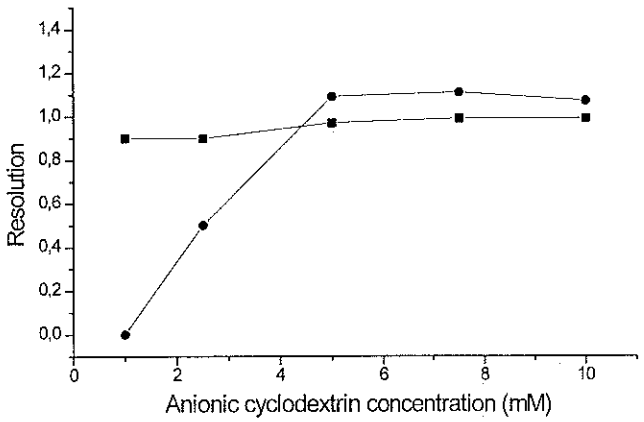

Figure 2. Influence of the concentration of anionic CD derivative on the enantioseparation of indoprofen. Buffer: phosphoric acid/triethanolamine (pH 3) containing an anionic cyclodextrin derivative. (■) SBCD, (●) CMCD.

depend on their individual affinities for the two CDs. Therefore the magnitude of the mobility difference between the enantiomers will in principle be influenced by the type and concentration of both the charged and the uncharged CDs.

The SBCD concentration has little influence on chiral resolution (Fig. 2). Earlier observations made in the presence and the absence of a neutral CD have also indicated that this parameter only plays a minor role in the optimization of the enantioseparation of acidic compounds in such systems [23]. A 5 mM concentration of SBCD was found to give NSAIDs appropriate migration times and this concentration was kept constant in all further experiments. Note that on addition of a neutral CD to a buffer containing an anionic CD, the apparent electrophoretic mobilities of the analyte enantiomers will decrease in the case of complexation with the neutral CD. Indeed, the neutral CD will then compete with the negatively charged selector for interactions with the analyte enantiomers, giving rise to complexes migrating with the electroosmotic flow. In the presence of stereoselective interactions between only the neutral CD and the analyte, a higher affinity of an enantiomer for this CD will, in principle, result in a lower mobility for this enantiomer. On the contrary, if the enantiomers interact selectively with the anionic CD, a higher mobility can be expected for the enantiomer with the higher affinity for this CD. Consequently, simultaneous selective interactions with the two CDs may have a favorable or a detrimetal effect on chiral resolution, depending on the chiral recognition mechanism involved.

Table 1 shows the influence of the type of neutral CD added to the enantioseparation of the compounds examined. In most cases, resolution values obtained with dual systems were higher than those achieved with the system containing only SBCD. This could be explained by a particularly high enantioselectivity resulting from the complexation of NSAIDs in uncharged form with the neutral CD while interactions with SBCD generally either give rise to no or to low chiral resolution. A noticeable exception is sulindac, for which the addition of the neutral HPCD leads to a decrease of enantiomeric resolution (*cf.* Table 1). Fairly high enantioselectivity was obtained for this compound with SBCD; this might have a negative influence on chiral resolution in the presence of HPCD. Similar behavior was observed with hexobarbital in systems where HPCD, β-CD, or methyl-β-CD were used in combination with SBCD [23]. For warfarin enantiomers, however, resolution was found to increase on addition of different neutral CDs, including HPCD, although complete resolution was already achieved with SBCD alone [23].

Among the four neutral CDs tested, DMCD and TMCD seem to be particularly well-suited to the enantioseparation of NSAIDs when they are associated with SBCD. As shown in Table 1, resolution values were significantly enhanced for most compounds tested in the presence of one of these two CDs. β-CD and particularly HPCD gave rise to lower resolution values in most cases. Figure 3 shows the separation of fenoprofen enantiomers in the five different EKC systems tested, using SBCD alone or in combination with different neutral CDs. As expected, the shortest migration times for fenoprofen enantiomers were obtained with the system containing only the negatively charged CD. The addition of a neutral CD led in all cases to an increase in migration times, indicating a significant complexation with this CD. The same effect was observed with all other NSAIDs on addition of a neutral CD, even if chiral resolution was not improved. Fenoprofen enantiomers seem to have a high affinity for DMCD, as demonstrated by the particularly long migration times obtained in the presence of this CD. These interactions with DMCD are enantioselective and lead to a complete chiral resolution for this compound (R_s = 2.8, *cf.* Table 1). On the other hand, Fig. 3 also shows that, although the addition of HPCD gives rise to longer migration times than TMCD and β-CD, indicating stronger complexation, it results finally in lower chiral resolution.

As can be seen from Table 1, all compounds tested, except tiaprofenic acid, were completely enantioseparated, in such dual systems, using a neutral CD at 10 mM concentration together with SBCD at pH 3. High resolution values were already obtained under these conditions for carprofen (R_s = 16.6 with TMCD), ketoprofen (R_s = 4.7 with DMCD), sulindac (R_s = 3.8 with DMCD) and flurbiprofen (R_s = 3.2 with TMCD).

The next step of our investigation was to study the influence on enantioresolution of the neutral CD con-

centration that was added to the pH 3 buffer containing SBCD. TMCD was studied in particular because it was found to give rise to chiral resolution for all NSAIDs (*cf.* Table 1) within short migration times (*cf.* Fig. 3). As shown in Fig. 4, maximum resolution values were achieved in the 30–40 mM concentration range for most compounds studied. For sulindac, however, the optimum TMCD concentration was 10 mM. Resolution values higher than 10 were obtained for fenoprofen, flurbiprofen and ketoprofen with a 30 mM concentration of TMCD.

Another kind of dual systems was also tested, in which CMCD at 10 mM concentration was used in combination with DMCD (10 mM), TMCD (10 mM) or SBCD (5 mM) in the pH 3 phosphoric acid/triethanolamine buffer. As can be seen in Table 2, dual systems based on the association of CMCD with DMCD or SBCD do not seem to be well-suited for the enantioseparation of the NSAIDs examined, except for sulindac, the enantiomers of which were completely resolved in the CMCD/SBCD system. On the contrary, the introduction of TMCD in these dual systems with CMCD leads to the complete enantioseparation of five NSAIDs. After optimization of TMCD concentration, the enantiomers of all compounds were completely resolved. However, maximum resolution values obtained with the CMCD/TMCD system were always lower than those obtained with the SBCD/TMCD system. The latter also presented the advantage of giving shorter analysis times, owing to the higher mobility of the analyte/SBCD complex at pH 3. Preliminary experiments have shown that an improvement in resolution can be obtained in the CMCD/TMCD system by increasing the buffer pH from 3 to 5. However, for compounds such as NSAIDs, which are significantly dissociated at pH 5, this improvement seems to be limited.

3.3 Conditions for maximum enantiomeric resolution of NSAIDs

The composition of the buffers giving rise to the highest resolution values for the different NSAIDs studied is given in Table 3. SBCD was found to be the most appropriate anionic CD in all cases. For most compounds, impressive resolution values, ranging from 3.8 to 30.6, were obtained in these dual systems. A typical electropherogram, showing the enantioseparation of carprofen, is given in Fig. 5.

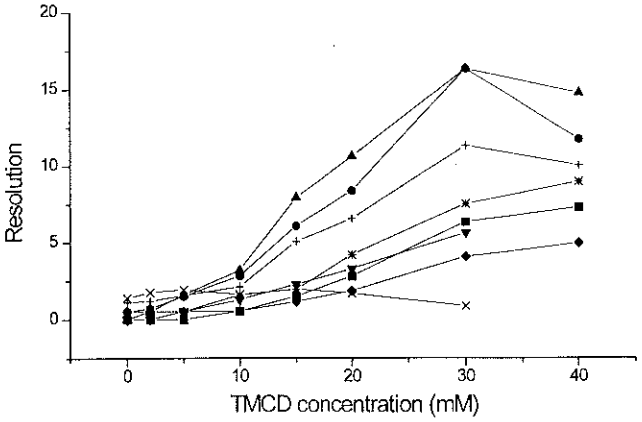

Figure 4. Influence of TMCD concentration on the enantiomeric resolution of NSAIDs. Buffer: 0–40 mM TMCD in phosphoric acid/triethanolamine (pH 3) containing 5 mM SBCD. Other conditions as described in Section 2.1. (■) tiaprofenic acid; (●) fenoprofen; (▲) flurbiprofen; (▼) ibuprofen; (♦) indoprofen; (+) ketoprofen; (×) sulindac; (★) suprofen.

Table 3. Optimal conditions for the enantioseparation of NSAIDs[a)]

Analyte	Type	Concentration mM	R_s
Carprofen	TMCD	15	30.6
Fenoprofen	TMCD	30	16.3
Flurbiprofen	TMCD	30	16.3
Ibuprofen	TMCD	30	5.6
Indoprofen	TMCD	40	4.9
Ketoprofen	TMCD	30	11.3
Sulindac	DMCD	10	3.8
Suprofen	TMCD	40	8.9
Tiaprofenic acid	TMCD	40	7.3

a) Buffer: 5 mM SBCD in phosphoric acid/triethanolamine (pH 3) also containing a neutral CD. Other conditions as described in Section 2.1.

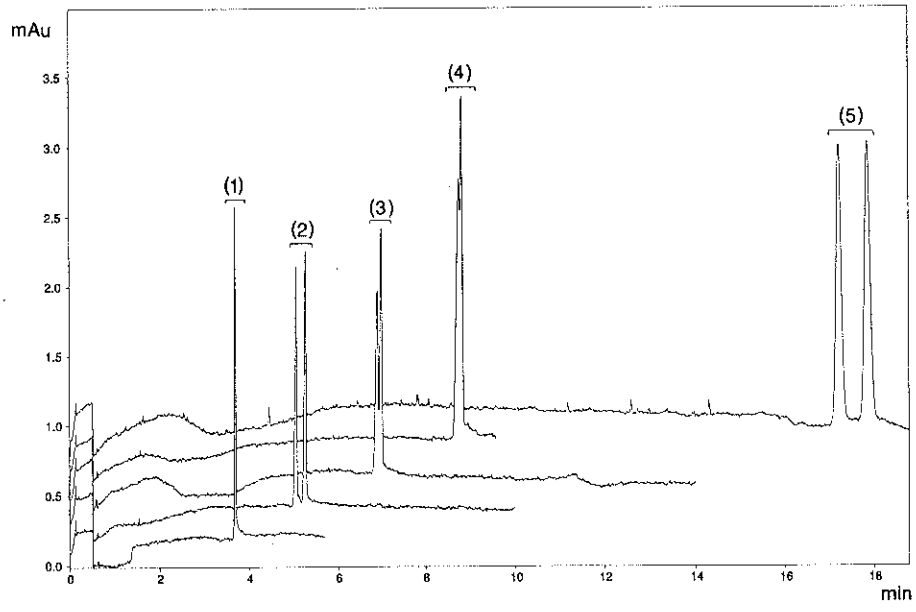

Figure 3. Enantioseparation of fenoprofen enantiomers. Buffer: phosphoric acid/triethanolamine (pH 3) containing: (1) 5 mM SBCD, (2) 5 mM SBCD and 10 mM TMCD, (3) 5 mM SBCD and 10 mM β-CD, (4) 5 mM SBCD and 10 mM HPCD, (5) 5 mM SBCD and 10 mM DMCD. Other conditions as described in Section 2.1.

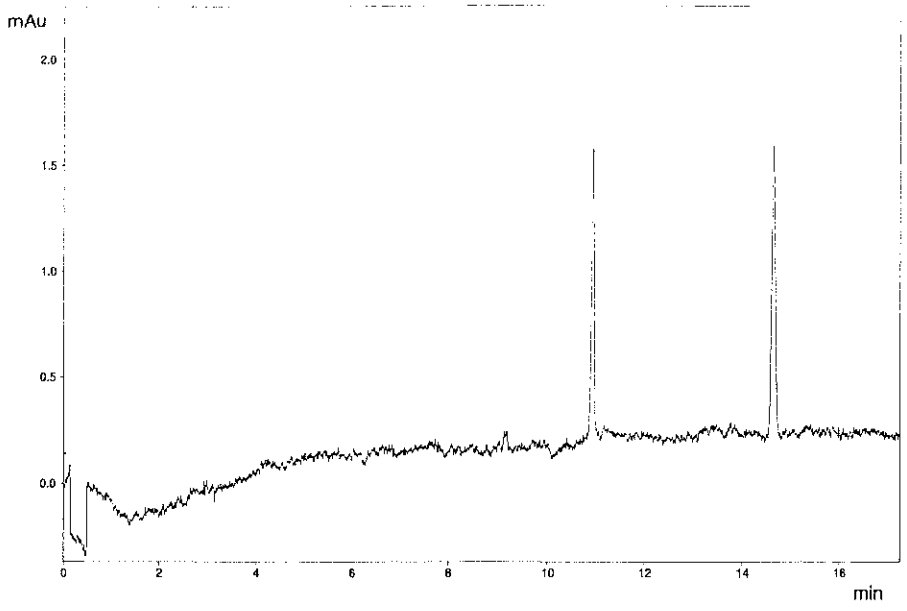

Figure 5. Enantioseparation of carprofen enantiomers, Buffer: phosphoric acid/triethanolamine (pH 3) containing 5 mM SBCD and 15 mM TMCD. Other conditions as described in Section 2.1.

4 Concluding remarks

EKC systems using the polyanionic SBCD in combination with a neutral CD, such as TMCD or DMCD in a pH 3 phosphoric acid/triethanolamine buffer, were found to be particularly useful for the enantioseparation of a series of NSAIDs. In such systems, the negatively charged CD was used essentially as carrier, giving the analytes, mainly present in an uncharged form at this pH, a suitable apparent electrophoretic mobility while the uncharged CD was added to provide high enantioselectivity. In these dual CD systems, particularly high resolution values, ranging from 4 to 30, could be obtained in short analysis times (less than 15 min) for all compounds examined. Further studies on the potential of such systems for the enantioseparation of other classes of acidic and neutral drugs are in progress.

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