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Enantioseparation of uncharged compounds by capillary electrophoresis using mixtures of anionic and neutral β-cyclodextrin derivatives

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Abstract

The use of the polyanionic sulfobutyl-β-cyclodextrin in combination with a neutral cyclodextrin derivative such as trimethyl-β-cyclodextrin (TMCD) or dimethyl-β-cyclodextrin (DMCD) in a pH 3 phosphoric acid–triethanolamine buffer has proved to be very suited to the enantioseparation of acidic compounds such as non-steroidal anti-inflammatory drugs. In this paper, the usefulness of such dual cyclodextrin systems was evaluated for the enantioseparation of weakly acidic and neutral compounds. Fairly good results with respect to chiral resolution were obtained at pH 3 for weak acids in these dual systems. However, no complete enantioseparation could be achieved under these conditions for the neutral drug chlormezanone. Another ionizable derivative, carboxymethyl-β-cyclodextrin, was then investigated together with TMCD or DMCD at pH 3 and 5. After optimisation of the concentration of the neutral cyclodextrin derivative, high enantioresolution could be obtained at pH 5 for chlormezanone as well as for all the other compounds tested. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Capillary electrophoresis (CE) has proved to be an effective technique for the enantioseparation of a large variety of molecules and several reviews have been recently published in the field [1–5]. The most common chiral CE approach involves the addition of cyclodextrins (CDs) to the running buffer. Several modified CDs have been synthesized and are now commercially available. The modification of the native CDs leads to significant changes in their physicochemical properties and in their chiral recognition ability. In contrast with neutral CDs, charged CD derivatives have a self electrophoretic mobility which allows their use as carriers in electrokinetic chromatography for the separation of neutral or uncharged compounds.

Terabe et al. [6] used the first the anionic carboxymethyl-β-CD (CMCD) as a carrier for the achiral separation of neutral compounds. More recently, a number of other charged CD derivatives such as carboxyethyl-CD, phosphorylated CDs, sulfated CDs or amino- or alkylamino-CDs were also tested for the direct enantioseparation of uncharged compounds. For example, sulfated CDs have proved to be very effective selectors for the enantioseparation of a series of cationic compounds and neutral compounds [7]. Benzoin was enantioseparated by

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Lelièvre et al. [8] using a combination of mono-(6-amino-6-deoxy)-β-CD and trimethyl-β-CD and by Tanaka with different charged CDs (sulfobutyl-β-CD, carboxymethyl-β-CD and -γ-CD or γ-CD phosphate) [9].

The separation of barbiturate enantiomers was performed by Jakubetz et al. [10] with 6-*O*-(2-hydroxy-3-trimethylammoniopropyl)-β-CD or 6-*O*-(sulfo-*n*-propyl)-β-CD derivatives and also by Schulte et al. [11] using the same hydroxypropyltrimethylammonium β-CD derivative.

In previous work, complete enantiomeric separation was obtained in CE for a series of acidic compounds containing carboxy groups, such as non-steroidal anti-inflammatory drugs, by the simultaneous addition of anionic and uncharged β-CD derivatives to a phosphoric acid–triethanolamine pH 3 buffer [12,13]. The use of dual systems containing mixtures of charged and neutral CDs for selectivity improvement was also described by Sepaniak et al. [14], Lurie et al. [15], Anigbogu et al. [16], Lelièvre et al. [8] and Gahm et al. [17].

In the present work, several dual CD systems were tested for the enantioseparation of weakly acidic and neutral drugs. The nature and the concentration of CDs as well as the buffer pH were more particularly investigated.

2. Experimental

2.1. Apparatus

All experiments were performed on a Spectraphoresis 1000 CE instrument (Spectra-Physics, San Jose, CA, USA) equipped with an automatic injector, an autosampler, a variable-wavelength UV–visible absorbance detector (190–800 nm) and a temperature control system (15–60°C). The pH of running buffers were measured by means of a Delta 345 pH meter from Mettler (Halstead, UK).

2.2. Chemicals and reagents

Heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (dimethyl-β-cyclodextrin: DMCD) and heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (trimethyl-β-cyclodextrin: TMCD) were from Sigma (St. Louis, MO, USA). Carboxymethyl-β-cyclodextrin (CMCD) was from Cyclolab (Budapest, Hungary). Sulfobutyl-β-cyclodextrin (SBCD) was kindly provided by Professor Stobaugh (University of Kansas, Lawrence, KS, USA).

Phosphoric acid (85%) and triethanolamine were of analytical reagent grade from Merck (Darmstadt, Germany). Water was of Milli-Q quality (Millipore, Bedford, MA, USA) and methanol was of HPLC grade from Acros (Geel, Belgium). Hexobarbital, mephobarbital, pentobarbital, secobarbital, thiopental, chlormezanone, chlorthalidone and mephenytoin were from Sigma. The standard solutions were prepared by dissolving each compound at a concentration of about $5 \cdot 10^{-5}$ *M* (20 μg/ml) in a mixture of water–methanol (7:3).

2.3. Electrophoretic technique

Electrophoretic separations were carried out with uncoated fused-silica capillaries, 44 cm (37 cm to the detector)×50 μm I.D. provided by Supelco (Bellefonte, PA, USA). The capillary was pretreated successively with alkaline solutions (1 *M* NaOH, 0.1 *M* NaOH), water and running buffer. At the beginning of each working day, the capillary was rinsed with running buffer for 10 min. Between each injection, the capillary was rinsed with buffer for 3 min (about 6 volumes of the capillary). The applied voltage was −25 kV (detector at the anode end of the capillary). UV detection was performed at 210 nm and injections were made in hydrodynamic mode for a period of 5 s (corresponding to 13.3 nl). The capillary was thermostatted at 25°C. For the electrophoretic experiments, a buffer made of 100 m*M* phosphoric acid adjusted to pH 3.0 or 5.0 with triethanolamine was used.

The resolution (R_s) and plate number (N) were calculated according to the standard expressions based on the peak width at half height [18].

3. Results and discussion

In previous papers, the usefulness of buffers made of 100 m*M* phosphoric acid adjusted to pH 3 with triethanolamine and containing different kinds of β-cyclodextrin derivatives for the enantiomeric CE

separation of acidic and basic drugs was demonstrated [12,13,19–21]. In this work, the same kind of chiral selectors has been tested for the CE enantioseparation of neutral or very weakly acidic drugs: chlormezanone (anxiolytic), hexobarbital, mephobarbital, pentobarbital, secobarbital and thiopental (barbiturates), chlorthalidone (diuretic) and mephenytoin (antiepileptic). The chemical structures of these drugs are given in Fig. 1.

3.1. Systems containing only one cyclodextrin

The first experiments were carried out with the phosphoric acid triethanolamine buffer adjusted to pH 3. At this low pH, all analytes were present in uncharged form and therefore were migrating with the electroosmotic flow (EOF) (low but directed towards the anode [20]), the latter making their detection possible at the anodic side of the capillary (reversed polarity mode).

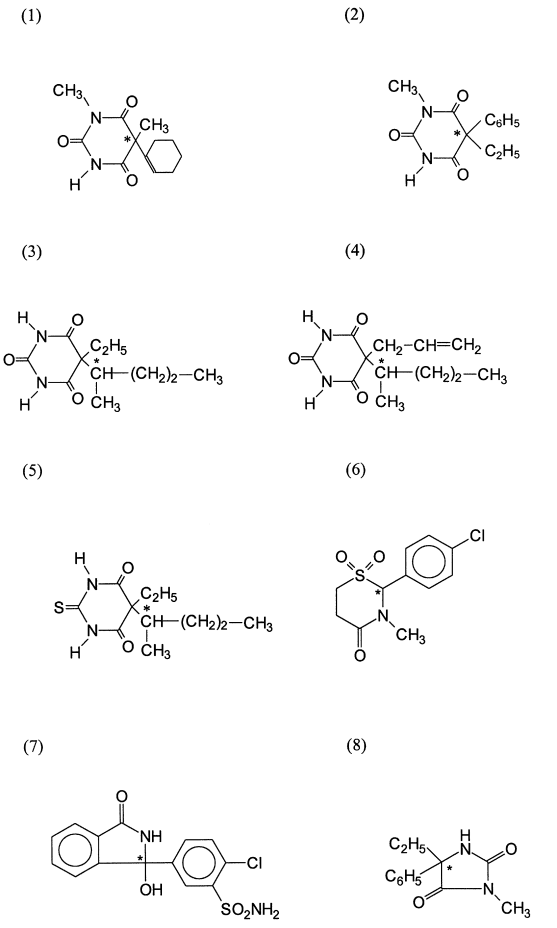

Fig. 1. Chemical structures: (1) hexobarbital (pK_a=8.2), (2) mephobarbital (pK_a=8.0), (3) pentobarbital (pK_a=8.0), (4) secobarbital (pK_a=7.9), (5) thiopental (pK_a=7.6), (6) chlormezanone, (7) chlorthalidone (pK_a=9.4), (8) mephenytoin (pK_a=8.1).

Under these conditions, no separation could be expected for these compounds by addition of a neutral cyclodextrin alone (e.g., TMCD). Indeed, even in the presence of selective interactions with this kind of cyclodextrin, no resolution would be observed, since the free and complexed forms of the analytes have no electrophoretic mobilities. On the contrary, the use of a negatively charged β-cyclodextrin derivative (SBCD or CMCD) was found to give rise to the formation of complexes migrating electrophoretically towards the anode and consequently allowed the achiral and chiral resolution of uncharged compounds. As can be seen in Table 1, complete enantiomeric resolution was obtained for hexobarbital (R_s=1.7) and mephenytoin (R_s=4.4) enantiomers by addition of SBCD (5 m*M*) to the buffer. Chlorthalidone (R_s=1.5) and mephenytoin (R_s=2.6) enantiomers were completely resolved using CMCD (10 m*M*) as chiral additive.

A pH 9 buffer (made of glycine and triethanolamine) was then tested. Under these conditions, the analytes, except chlormezanone, were partly negatively charged, while SBCD and CMCD were fully charged. Enantioselectivity was very poor at this pH, even with a neutral cyclodextrin such as TMCD (results not shown here). This clearly indi-

Table 1
Influence of the nature of the charged CD on chiral resolution (R_s) at pH 3

Analyte	R_s	
	SBCD	CMCD
Hexobarbital	1.7	–
Pentobarbital	<0.7	1.1
Secobarbital	–	1.1
Chlormezanone	–	0.9
Chlorthalidone	1.3	1.5
Mephenytoin	4.4	2.6

–=No detectable resolution (R_s<0.5); buffer: 100 m*M* phosphoric acid–triethanolamine (pH 3) containing SBCD (5 m*M*) or CMCD (10 m*M*); voltage: −25 kV; temperature: 25°C; wavelength: 210 nm; hydrodynamic injection: 5 s; samples: 20 μg/ml.

cates that the presence of a negative charge is unfavourable to the enantioseparation of these compounds, even though the EOF at such pH should have in principle a positive influence on resolution since it was moving in the opposite direction compared to the analytes.

The influence of the concentration of two anionic cyclodextrin derivatives (SBCD and CMCD) on the enantioseparation of uncharged drugs (especially barbiturates) was then studied at pH 3. Resolution values obtained with SBCD were found fairly constant in the whole concentration range studied (1–10 m*M*, results not shown here), while in the case of CMCD (cf. Fig. 2), chiral resolution for pentobarbital and secobarbital increased with cyclodextrin concentration before reaching a plateau in the 5–10 m*M* concentration range. Usually, maximum resolution values are obtained at a given cyclodextrin concentration, which depends on the affinity of the analyte enantiomers for the selector, and a further increase of cyclodextrin concentration results in a decrease of resolution. The unusual behaviour observed in this case with both anionic cyclodextrins could be explained by the poor enantiomeric purity of these cyclodextrin derivatives. Indeed, it has been shown by several authors that these anionic CD derivatives are in fact mixtures of CDs with different degrees of substitution and with substituents placed at different positions on glucopyranosyl residues [22,23].

With both cyclodextrins, a decrease in analyte migration times was observed with increasing selector concentration (cf. Fig. 3) since the proportion of negatively charged complexes was increased.

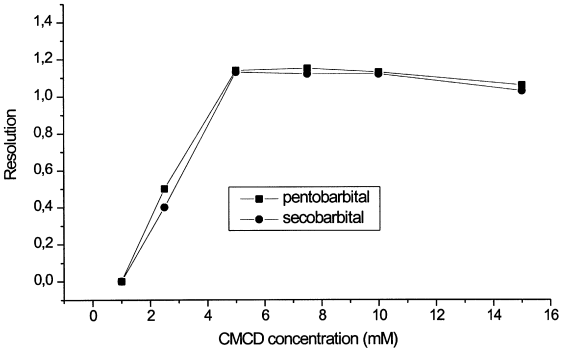

Fig. 2. Influence of CMCD concentration on chiral resolution. Buffer: 100 m*M* phosphoric acid–triethanolamine (pH 3) containing CMCD (0–15 m*M*). Other conditions as described in Table 1.

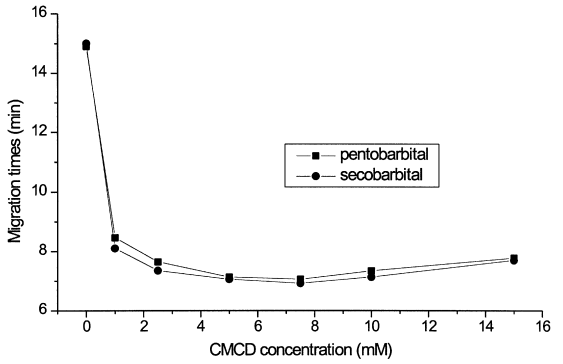

Fig. 3. Influence of CMCD concentration on the migration times of the second enantiomer. Buffer: 100 m*M* phosphoric acid–triethanolamine (pH 3) containing CMCD (0–15 m*M*). Other conditions as described in Table 1.

A 5 m*M* concentration for SBCD and a 10 m*M* concentration for CMCD were found to give the analytes appropriate migration times (less than 10 min) and these concentrations were kept constant in all further experiments.

3.2. Systems containing two cyclodextrins at pH 3

As can be seen in Table 2, resolution values obtained at pH 3 in CE systems containing two cyclodextrins, one charged (SBCD or CMCD) and one neutral (DMCD or TMCD), were higher in most cases than those achieved with buffers containing only SBCD or CMCD (cf. Table 1). This could be explained by a particularly high enantioselectivity resulting from the complexation of the uncharged compounds studied with the neutral cyclodextrins, compared to that obtained with SBCD or CMCD.

Among the different neutral β-CD derivatives tested (results not shown here), DMCD and TMCD were found to be particularly well suited to the enantioseparation of the compounds examined when they were used in combination with SBCD or CMCD, which play essentially the role of carriers in these systems.

Table 2 shows that all compounds studied (except chlormezanone) were completely enantioseparated in such dual systems, using a neutral CD (DMCD or

Table 2
Influence of the nature of CDs on chiral resolution (R_s) in dual systems at pH 3

Analyte	R_s			
	SBCD/DMCD	SBCD/TMCD	CMCD/DMCD	CMCD/TMCD
Hexobarbital	1.8	2.1	1.5	–
Pentobarbital	<0.7	1.6	<0.7	2.4
Secobarbital	<0.7	<0.7	–	1.6
Chlormezanone	1.1	0.9	1.1	1.4
Chlorthalidone	–	2.2	1.9	2.4
Mephenytoin	3.6	5.2	3.4	5.5

–=No detectable resolution (R_s<0.5); buffer: 100 m*M* phosphoric acid–triethanolamine (pH 3) containing a charged CD (5 m*M* SBCD or 10 m*M* CMCD) and a neutral CD (DMCD or TMCD); other conditions as described in Table 1.

TMCD) at 10 m*M* concentration, together with SBCD (5 m*M*) or CMCD (10 m*M*) at pH 3. For most compounds, higher resolution values were obtained with TMCD as the neutral cyclodextrin.

3.3. Systems containing two cyclodextrins at pH 5

As could be expected, an increase of pH from 3 to 5 in the phosphoric acid–triethanolamine buffer was of very limited interest with the SBCD/TMCD system (cf. Tables 2 and 3). Indeed, no significant changes can occur in the ionization of the strongly acidic SBCD, already fully charged at pH 3, and the very weakly acidic analytes in this pH range.

On the contrary, a resolution improvement was obtained for most compounds with the CMCD/TMCD system by increasing the buffer pH from 3 to 5 (cf. Tables 2 and 3, respectively), due to the significant increase of the negative charge of CMCD in this pH range. This favourable effect could be explained by an increase in the mobility difference between the free and complexed forms of the analyte enantiomers at pH 5. For very weakly acidic drugs, such barbiturates, which are undissociated in this pH range, the use of the CMCD/TMCD system at pH 5 seems to be particularly interesting (cf. Table 3).

The next step of the study was then to investigate the influence on enantioresolution of the concentration of the neutral cyclodextrin added to the pH 5 buffer containing CMCD. TMCD was more particularly investigated because it was found to lead to chiral resolution for all compounds studied within short migration times. As shown in Fig. 4, maximum resolution values were achieved in the 40–50 m*M* concentration range for most compounds.

Resolution values higher than 4 were obtained for mephobarbital, pentobarbital, secobarbital and thiopental. The corresponding analysis times were lower than 20 min.

Table 3
Influence of the nature of the charged CD on chiral resolution (R_s) in dual systems at pH 5

Analyte	R_s	
	SBCD/TMCD	CMCD/TMCD
Hexobarbital	2.3	<0.7
Mephobarbital	ND	1.7
Pentobarbital	1.5	2.7
Secobarbital	1.0	1.7
Thiopental	ND	2.7
Chlormezanone	ND	3.3

Buffer: 100 m*M* phosphoric acid–triethanolamine (pH 5) containing SBCD (5 m*M*) or CMCD (10 m*M*), and TMCD (10 m*M*); ND=not determined; other conditions as described in Table 1.

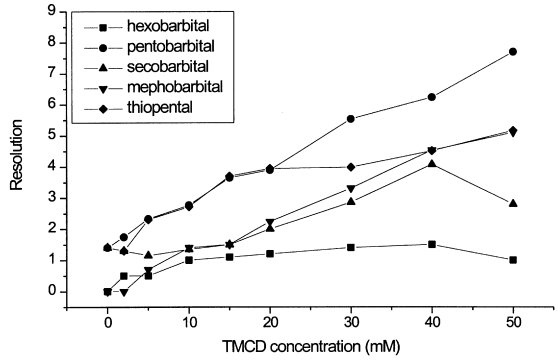

Fig. 4. Influence of TMCD concentration on chiral resolution in dual CD systems at pH 5. Buffer: 100 m*M* phosphoric acid–triethanolamine (pH 5) containing CMCD (10 m*M*) and TMCD (0–50 m*M*). Other conditions as described in Table 1.

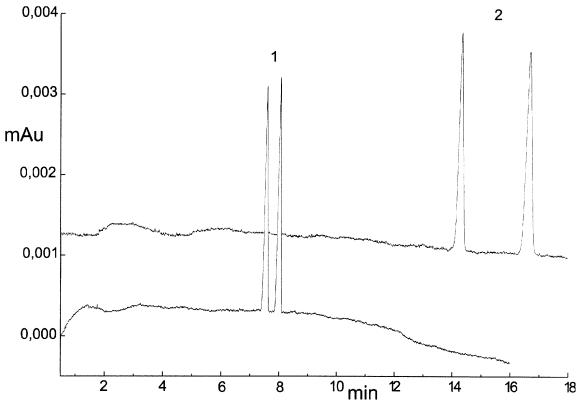

Fig. 5. Enantioseparation of pentobarbital in dual CD systems. Buffers: (1) 100 m*M* phosphoric acid–triethanolamine (pH 3) containing SBCD (5 m*M*) and TMCD (30 m*M*); (2) 100 m*M* phosphoric acid–triethanolamine (pH 5) containing CMCD (10 m*M*) and TMCD (50 m*M*). Other conditions as described in Table 1.

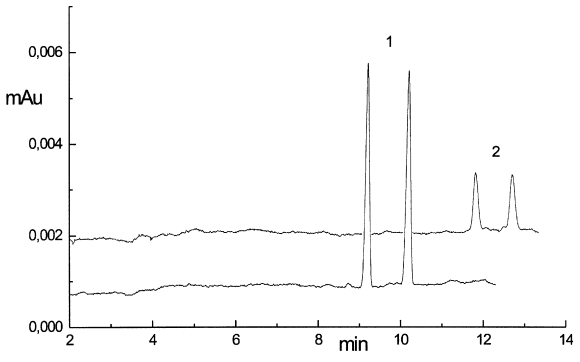

Fig. 6. Enantioseparation of chlorthalidone and mephenytoin in dual CD systems. (1) Mephenytoin, (2) chlorthalidone. Buffer: 100 m*M* phosphoric acid–triethanolamine (pH 3) containing CMCD (10 m*M*) and TMCD (10 m*M*). Other conditions as described in Table 1.

After optimisation of the TMCD concentration, higher resolution values were obtained at pH 5 for all barbiturates studied in the CMCD/TMCD system, compared to those given by the SBCD/TMCD or CMCD/TMCD systems at pH 3. Fig. 5 illustrates the enantioseparation of pentobarbital enantiomers at optimum TMCD concentrations using the SBCD/TMCD system at pH 3 and the CMCD/TMCD system at pH 5.

3.4. Conditions for maximum enantiomeric resolution

The composition of the buffers giving rise to the highest chiral resolution values for the different uncharged drugs studied is given in Table 4. For all compounds tested, impressive resolution values, ranging from 2.4 to 7.7, were obtained using dual systems with CMCD as the charged cyclodextrin.

The combination of CMCD with TMCD was found to provide the most effective dual CD system, except for hexobarbital, for which better resolution was achieved with DMCD than with TMCD. Fig. 6 illustrates the excellent enantioseparation of mephenytoin and chlorthalidone using the CMCD/TMCD system at pH 3.

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Table 4
Conditions for maximum enantiomeric resolution for uncharged drugs

Analyte	Type of CD	pH	Concentration (m*M*)	R_s
Hexobarbital	CMCD/DMCD	5	10/10	3.2
Mephobarbital	CMCD/TMCD	5	10/50	5.1
Pentobarbital	CMCD/TMCD	5	10/50	7.7
Secobarbital	CMCD/TMCD	5	10/50	4.0
Thiopental	CMCD/TMCD	5	10/50	5.2
Chlormezanone	CMCD/TMCD	5	10/10	3.3
Chlorthalidone	CMCD/TMCD	3	10/10	2.4
Mephenytoin	CMCD/TMCD	3	10/10	5.5

Buffer: 100 m*M* phosphoric acid–triethanolamine (pH 3 or 5) containing CMCD (10 m*M*) and a neutral CD (DMCD or TMCD); other conditions as described in Table 1.

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