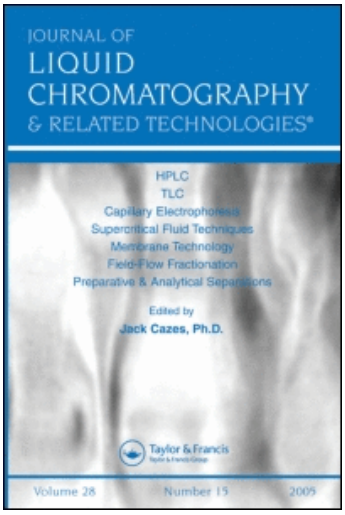

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CAPILLARY ELECTROPHORESIS: A GOOD ALTERNATIVE FOR THE SEPARATION OF CHIRAL COMPOUNDS OF ENVIRONMENTAL INTEREST

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ABSTRACT

Capillary electrophoresis potential for the separation of the enantiomers of chiral compounds of environmental interest is described. Applicability of capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) to achieve these separations is discussed.

INTRODUCTION

The importance of chirality in biological phenomena, chemical synthesis, toxicology, environmental control, and design of pharmaceutical agents has given rise to the search for new and effective means to resolve enantiomeric mixtures.[1] These separations have become particularly interesting in pharmacological and environmental fields. In the case of the pharmaceutical industry, many of the most widely prescribed drugs contain at least one chiral center, and an important percentage are marketed as racemates; thus, the study of the properties of individual drug enantiomers is important.[2]

Also, an enantiomer of a given drug may be physiologically active, whereas the other enantiomer may be totally useless, or even toxic.[3] Concerning chiral compounds detrimental to the environment, enantiomers may have different toxicities or one of them may not be toxic at all.[4] On the other hand, many herbicides and pesticides are produced as racemic mixtures and, often, only one enantiomer is biologically active. The extent to which various agricultural chemicals are being applied in the environment will soon make it urgent to monitor such potential ecological hazards, enantioselectively.[5]

Chromatographic techniques are important tools to carry out enantiomeric separations, since chiral stationary phases are easily accessible and commercially available today. High performance liquid chromatography (HPLC) and gas chromatography (GC) are the most common techniques used routinely and, in complementary form, in these types of separations. Various gas and liquid chromatographic methods have been used to resolve numerous enantiomeric mixtures.[6-10] Nevertheless, in recent years, another instrumental technique, —considered an interesting alternative to HPLC and GC techniques,— has been developed, i.e., capillary electrophoresis (CE).[11] The utilization of capillary tubes, of inside diameter generally ranging from 10 to 200 μm to achieve electrophoretic separations, has enabled obtaining high efficiencies and an enormous resolving power.[12-14] Even though CE was initially limited to the separation of charged compounds, the introduction of a micellar system into the separation buffer in 1984[15], which resulted in what is generally called micellar electrokinetic chromatography (MEKC), enabled the application of CE to the separation of uncharged compounds.[16-20] The limitations of GC techniques to the separation of volatile compounds and the poor performance that HPLC techniques sometimes provide, causing loss in sensitivity, have led one to consider CE techniques as an interesting alternative. In fact, they combine the high performance of GC techniques with the versatility of HPLC techniques as to selectivity and range of application.[21] The use of the principles and additives originally developed for the separation of chiral mixtures in HPLC has enabled the wide use of CE to perform enantiomeric separations.[1,2,5,22-26] Aside from all other considerations mentioned here, which show the great potential of CE to achieve enantiomeric separations, CE can also be considered a part of the so-called group *clean analytical techniques*. This is due to the small volumes of mobile phase (electrolyte solution) and sample required to perform separations by this technique. This property confers to CE a great environmental interest.

CE applications to the separation of chiral mixtures have been described mainly for drugs.[27-35] The aim of this work is to describe this technique's potential for the separation of the enantiomers of chiral compounds of

environmental interest. Although other CE techniques have been used to achieve the separation of chiral compounds (isotachophoresis, gel capillary electrophoresis and electrochromatography),[23] only the applicability of capillary zone electrophoresis (CZE) and MEKC will be discussed in this work, since they are primarily used to achieve chiral separations of environmental interest.

CHIRAL SEPARATIONS BY CAPILLARY ZONE ELECTROPHORESIS (CZE)

CZE is the easiest mode of CE to use since, in this working mode, the capillary contains only the electrolyte solution. Assuming that the capillary surface is negatively charged, there will be an electroosmotic flow which, if strong enough, will sweep the negatively charged substances towards the cathode.[36] The different electrophoretic migrations of the analytes enable their separation in the capillary, eluting, initially, the positively charged substances, later the neutral ones (not separated) and, finally, the negatively charged solutes.

Chiral separations are usually performed by CZE by adding a chiral selector to the electrolyte solution. The complexation reaction between enantiomer and chiral selector can be compared to the partition of an analyte between a mobile phase and a pseudophase. Whereas, in HPLC, the efficiency is limited by the profile of the laminar flow, transfer mass term, and possible additional interaction with the residual silanol groups of the stationary phase, in CE, high efficiencies are generally obtained. This is due to the flat profile originated and to an homogeneous partition of the chiral selector in the electrolyte which, in turn, minimizes the mass transfer term.[22]

Although several CZE chiral separation approach principles exist,[37] the most frequently used in the chiral separation of pollutants is the formation of inclusion complexes. They are molecular compounds of specific structural arrangements, in which one compound (the host molecule) spatially encloses another (the guest molecule), or at least part of it. The inclusion phenomena have found the widest use in separation methods such as chromatographics.[38] Two types of compounds are basically used in CZE to form inclusion complexes with enantiomers: cyclodextrins or their derivatives, and chiral crown ethers being the cyclodextrins the ligands most widely employed in the separations of compounds of environmental interest. Cyclodextrins are cyclic oligo-saccharides that consist of six, seven, or eight glucopyranose units and which are named α-, β-, and γ-cylodextrins, respectively. Their structure is unique; it resembles a truncated cone with both ends open. The top of the torus corresponds to the more open side which is rimmed with secondary hydroxyl

groups on carbons 2 and 3 of each glucose unit, all rotated to the right. The smaller opening of the cones is rimmed with the more polar primary hydroxyl groups on carbon 6 of the glucose unit. The interior of the cyclodextrin cavity contains two rings of C-H groups with a ring of glycosidic oxygens in between.[37] Their surface is relatively hydrophilic, whereas their cavity is of a hydrophobic nature. The exceptional properties of cyclodextrins have been described,[38-40] and their ability to selectively include a wide variety of guest molecules into their hydrophobic cavity is remarkable. Derivatives of the cyclodextrins have been synthesized in order to enhance their water solubility and to modify their cavity size.[41-50] Chiral recognition is based on the inclusion of an aryl or alkyl group in the cavity, in addition to hydrogen-bonding between secondary hydroxyl groups of the cyclodextrin and substituents of the enantiomer.

The separation of different phenoxy acids into their respective enantiomers was done by CZE.[51-53] Phenoxy acids are widely used in agriculture as selective herbicides. Phenoxy-propionic acid herbicides are racemic mixtures and d-isomers are the only active ingredients. Chiral separations of these herbicides are required in order to assess the enantiopurity of formulations and to optimize enantioselective production processes. The experimental conditions in which separations were achieved are grouped in Table 1. Results obtained in the separation of different herbicides (chiral and non chiral) with three types of cyclodextrins (α-cyclodextrin, β-cyclodextrin and a derivative of the latter) were compared. The addition of heptakis-(2,6-di-O-methyl)-β-cyclodextrin to the electrolyte solution improves the results obtained with β-cyclodextrin, since it enables the separation of all herbicides studied and the chiral separation of two of them (2-(2-methyl-4-chlorophenoxy)propionic acid and 2-(2,4-dichlorophenoxy)propionic acid). The use of α-cyclodextrin further improves the results and changes the selectivity obtained entirely. A 0.01 M concentration of this cyclodextrin allows the separation of all herbicides and the chiral separation of three of them (those mentioned above and 2-(2-methyl-4,6-dichlorophenoxy)propionic acid). Excessively high concentrations of α-cyclodextrin in the separation buffer resulted in the co-migration of several peaks close to the electroosmotic flow marker peak. The results obtained concerning the identification and determination of impurities are comparable to those obtained by chromatographic methods. However, the authors found CZE simpler, more flexible and cost-effective. By changing the chiral selector in the electrolyte solution, complete changes in selectivity can be obtained which may be used as a criterion to confirm impurities. The methods developed were successfully applied to the analysis of real production samples and the determination of their enantiopurities.[51]

Table 1

Experimental Conditions used on Enantiomeric Separations by CZE

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
2-(2-methyl-4-chlorophenoxy) propionic acid;	α-cyclodextrin	0.05 M LiOAc	30 kV	Hydrodynamic (pressure) (20 mbar x 6 s)	UV, 200 nm	Determination of impurities in production samples at levels as low as 1 mg/g relative to the	51
2-(2-methyl-4,6-dichlorophenoxy) propionic acid;	β-cyclodextrin					main component.	
2-(2,4-dichlorophenoxy) propionic acid	heptakis-(2,6-di-O-methyl-β-cyclodextrin						
2-(2-methyl-4-chlorophenoxy)-propionic acid; (MCPP)	heptakis-(2,6-di-O-methyl)-β-cyclodextrin	0.03 M LiOAc (pH = 4.8)	30 kV	Hydrodynamic (pressure) (20 mbar x 6 s)	UV, 200 nm	Chiral sep'n in river and drinking water samples spiked with 5 ppb MCPP and DP. Preconcentration by field	52
2-(2,4-dichlorophenoxy)-propionic acid (DP)						amplification (sample volume, 20 mL or 40 mL).	

(continued)

Table 1 (continued)

Experimental Conditions used on Enantiomeric Separations by CZE

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
2-(2-methyl-4-chlorophenoxy) propionic acid;	α-cyclodextrin	0.05 M NaOAc (pH = 4.5)	20 kV	Hydrodynamic (pressure)	UV, 230 nm	Study of the influence of cyclodextrin nature and the addition of organic midifiers upon the sep'n of phenoxy acid herbicides.	53
2-(2,4-dichlorophenoxy) propionic acid;	di-O-methyl-β-cyclodextrin						
2-(2,4,5-trichlorophenoxy) propionic acid	tri-O-methyl-β-cyclodextrin						
1,1'-bi-2-naphthol; 1,1'-binaphthyl-2,2'-dicarboxylic acid;	α-cyclodextrin	0.01 M Phosphate + 0.006 M Borate (pH = 9)	20 kV	Hydrodynamic (gravity) (10 cm x 10 s)	UV, 254 nm	Study of the influence of the nature and conc'n of the cyclodextrin on enantiomeric resolution. Molecular modeling is used to calculate interaction energies between enantiomers and cyclodextrins	54
	β-cyclodextrin						
1,1'-binaphthyl-2,2`-diyl hydrogen phosphate	γ-cyclodextrin						

Table 1 (continued)

Experimental Conditions used on Enantiomeric Separations by CZE

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
1,1'-bi-2-naphthol (BN); 1,1'-binaphthyl-2, 2'-dicarboxylic acid (BNC); 1,1'-binaphthyl-2,	α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD)	0.025 M Phosph. (pH=10.5) cntng 0.010 M α-CD + 0.010 M TM-β-CD (BN, BNP, and BNC)	15 or 20 kV	Hydrodynamic (pressure) (30 mbar x 3-7 s)	UV, 214 nm	Successful separation of binaphthyl enantiomers is achieved by using >2 chiral selectors.	55
2'-diyl hydrogen-phosphate (BNP)	hydroxypropyl-α-cyclodextrin (HP-α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD), dextran, α-CD-phosphate, β-CD-phosphate, γ-CD-phosphate	0.025 M Phosph. (pH=7.0) cntng 3% dextran (BNP & BNC) 0.025 M Phosph. (pH=9) and 0.01 M β-CD phosphate + 0.01 M α-CD 0.025 M Phosph. (pH=10.5) and 0.005 M β-CD Phosph. + 0.010 M α-CD + 0.010 M TM-β-CD					

(continued)

Table 1 (continued)

Experimental Conditions used on Enantiomeric Separations by CZE

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
1,1'-binaphthyl-2-2'-dicarboxylic acid (BNC); 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (BNP); 2,2'-dihydroxy-1,1'-binaphthyl-3,3'-dicarboxylic acid (HBNP)	Non-cyclic-oligosaccharides (α-1,4-dextrins)	0.04 M Carbonate (pH = 9)	15 kV	Electromigration (15 kV x 10 s)	UV 225 nm (BNC) 215 nm (BNP) 235 nm (HBNC)	Hydrogen bonding as well as hydrophobic interaction ins suggested as an essential force for enantioselective complexation between saccharide and anionic binaphthyl.	56

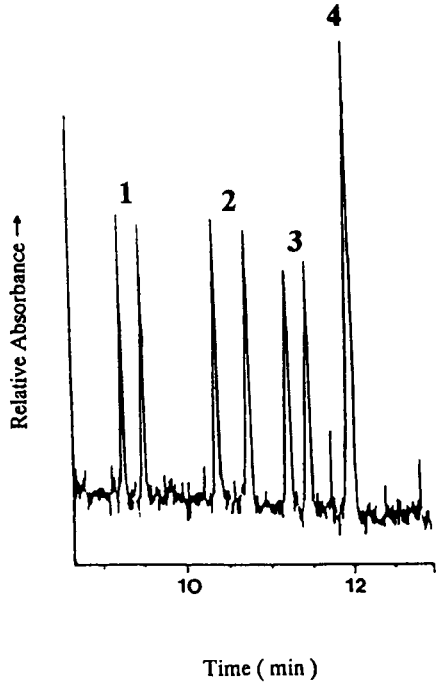

Figure 1. Chiral separation of three optically active phenoxy acid herbicides and another non-chiral phenoxy acid. Buffer: 0.05 M acetate, pH = 4.45, 0.025 M tri-O-methyl-β-cyclodextrin. Detection: 230 nm. Capillary: 50 cm (to the detector) x 0.075 mm I.D., fused silica. Separation voltage/current: 20 kV (400 V/cm)/41 μA. Hydrodynamic injection: 5 s. Temperature: 30 °C. Concentration of each analyte: 1 μg/mL. 1: 2-(2,4,5-trichlorophenoxy)propionic acid. 2: 2-(4-chloro-2-methylphenoxy)-propionic acid. 3: 2-(2, 4-dichlorophenoxy)propionic acid. 4: 2,4-dichlorophenoxyacetic acid. Reproduced with permission from ref. 53.

On the other hand, a comparison of the results obtained in the chiral separation of three phenoxy acid herbicides with different native and modified cyclodextrins by CZE[53] has shown that tri-O-methyl-β-cyclodextrin added to an acetate separation buffer is what ensures good separation of the two enantiomers of each of the three optically active herbicides, separately and in mixtures of the three. Di-O-methyl-β-cyclodextrin or α-cyclodextrin separated the enantiomers of two of the herbicides, β-cyclodextrin provided very little separation and γ-cyclodextrin gave no separation. Finally, it was shown that separations improved when methanol was added to the electrolyte solution, but due to the considerable increase in the analysis time, its use was not considered

helpful. Figure 1 shows the chiral separation of the three optically active phenoxy acid herbicides (2-(2, 4, 5-trichlorophenoxy)-propionic acid, 2-(4-chloro-2-methylphenoxy)propionic acid, and 2-(2, 4-dichlorophenoxy)-propionic acid) into their six enantiomers by CZE using a 0.05 M acetate buffer (pH = 4.45), 0.025 M in tri-O-methyl-β-cyclodextrin. They are separated from other non-chiral phenoxy acid (2,4-dichlorophenoxyacetic acid) at 1 μg/mL levels in an analysis time close to 12 min.

Enantiomeric separations of binaphthyl compounds have also been achieved by CZE[54-56] under the conditions detailed in Table 1. These solutes can be taken as typical examples of the separation of aromatic compounds. The effect of the type and concentration of cyclodextrin on the separation of the enantiomers of 1,1'-bi-2-naphthol, 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate and 1,1'-binaphthyl-2,2'-dicarboxylic acid has been studied.[54] Non-cyclic oligosaccharides have also been used as chiral selectors for the separation of the enantiomers of these compounds.[56]

Several works have appeared dealing with the optimization of enantiomeric separations by CZE using chiral mobile-phase additives. The elution order of enantiomeric pairs in CZE can be optimized by reversal of the migration order.[57-60] Three different approaches can be used to achieve migration reversal for cationic and anionic enantiomers:

i) electroosmotic flow can be reversed using different additives in the electrolyte buffer,

ii) migration order can be reversed selecting different cyclodextrins as chiral additives due to the change in the separation and complexation mechanisms, and

iii) through the variation of the pH value using chargeable cyclodextrins in the separation buffer.

The application of various radial electric potential gradients across the capillary wall has been proposed to control the zeta potential and the electroosmotic flow, which enhance chiral resolution in cyclodextrin-modified CZE.[61] Finally, several models have recently been developed to explain separation in chiral CE[62] or to describe the effect of different factors on the separation selectivity of enantiomers.[63-66] However, in spite of the interesting results obtained in these works, the optimization approaches and models were tested in every case with drugs and were not applied to compounds of environmental interest.

CHIRAL SEPARATIONS BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC)

The addition of a surfactant at a concentration above its critical micellar concentration (c.m.c.) to the separation buffer in CE has given origin to the micellar electrokinetic chromatography (MEKC). A surfactant is a molecule possessing two zones of very different polarity, therefore resulting in solutions of special characteristics. There is a non-polar zone, of a hydrophobic nature, constituted by a hydrocarbon chain. The other zone can be polar or even ionic and permits classification of the surfactants into three principal classes: ionic (cationic and anionic), non-ionic, and zwitterionic.

When surfactant molecules are present in solution at low concentrations, they exist as monomers, but, at a given temperature and concentration (c.m.c.), they associate spontaneously to form submicroscopic aggregates called micelles.[67-70] As a result of the combination of hydrophobic and hydrophilic properties in the surfactant molecules, micellar systems have exhibited very interesting properties, such as the capacity to solubilize hydrophobic solutes into aqueous solutions or the possibility of improving sensitivity and selectivity of different analytical methodologies.[40,71,72]

MEKC enables separations of neutral and ionic compounds with the advantages of CE.[73] The most widely used micellar systems in MEKC have been those of anionic nature, such as sodium dodecyl sulphate (SDS). In this case, if the capillary generates a cathodic electroosmotic flow whose velocity is superior to that of the migration of anionic micelles towards the anode, these will migrate towards the cathode, but at a velocity lower than that of the electroosmotic flow. Neutral solutes are distributed between the micellar and aqueous phases according to their solute-micelle association constants.

Thus, they elute at a time somewhere between the migration time of a solute that moves with the electroosmotic flow and the elution time of a very hydrophobic solute, always associated with the micelle (micelle migration time).[15,20,74] The separation selectivity in MEKC can be controlled through a great number of parameters, such as the buffer and surfactant concentrations in the electrolyte solution and the nature and concentration of organic additives such as alcohols.[75]

The two methods most frequently used in MEKC to perform enantiomeric separations are the employment of chiral surfactants and the addition of chiral selectors to the micellar solution.[76]

Chiral Separations by MEKC with Chiral Surfactants

Chiral surfactants originate chiral aggregates. Most analytes are adsorbed onto the surface of the micelle or interact with the polar groups of the surfactants. Therefore, surfactants with chiral polar groups can be used for chiral dis-crimination.[76] Although many chiral surfactants are available, only a few have been found useful for enantiomeric separations by MEKC. Included are some amino acid derivatives, bile salts, glycosides, and saponins.[76] Recently, some novel chiral surfactants based on (R,R)-tartaric acid and long-chain aliphatic amines have been synthesized and have been used to achieve some chiral separations.[77] In some cases, chiral surfactants are used with achiral micelles (mixed micelles) in order to enhance the selectivity of the chiral separation.[78,79] Mixed micelles of bile salts and polyoxyethylene ethers have also been used.[80]

Bile salts have been considered as promising pseudo-phases in MEKC. Their structures and aggregation behaviour allow chiral recognition and the reduction of capacity factor values with respect to those obtained with SDS. These systems respond to organic modifiers in the same general manner as the SDS system.[81] In spite of these interesting properties, the usefulness of chiral surfactants in enantiomeric separations of pollutants has been investigated in very few works.[82] Separation of the two optical isomers of a silvex phenoxy acid herbicide (2-(2,4,5-trichlorophenoxy)propionic acid) was achieved by using a novel "in-situ" charged micelle, having chiral selectivity, named N,N-bis-(3-D-gluconamidopropyl)deoxycholamide, and using a high borate concentration in the electrolyte solution (0.4 M) (pH = 10) and a relatively high surfactant concentration (0.05 M). The addition of organic modifiers did not improve the chiral separation of the silvex phenoxy acid but, rather, decreased it.[82] This surfactant, together with others containing a cholic moiety (N,N-bis-(3-D-gluconamidopropyl)-cholamide), were evaluated in MEKC for the separation of enantiomers of other compounds.[82] These chiral, steroidal glycoside surfactants combine both the structural features of bile salts (chiral surfactants) and glycosidic surfactants (in-situ charged, and also chiral surfactants) through the steroidal portion and the polyolic polar groups, respectively. These two neutral surfactants could be charged readily via borate complexation.

As an example of enantiomeric separation of aromatic compounds, the chiral separation of bi-naphthyl derivatives can be cited.[80-83] The experimental conditions in which these separations were achieved are presented in Table 2, along with the results obtained by MEKC with chiral selectors.

Table 2

Experimental Conditions used on Enantiomeric Separations by MEKC

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
1,1'-bi-2-naphthol	Sodium deoxycholate Sodium deoxycholate + Polyoxyethylene ethers	0.016 M NaCl + Methanol (pH = 8.1-8.3)	20 kV	Hydrodynamic (gravity) (15 cm x 1-6 s)	UV, 210 nm	Determination of the effect of three variables on chiral resolution: (1) the type of ether; (2) the mole fraction of ether in solutions with bile salt; and (3) the percentage of methanol in the mobile phase.	80
1,1'-bi-2-naphthol; 1,1'-binaphthyl dicarboxylic acid; 1,1'-binaphthyl diyl hydrogen phosphate	Sodium cholate Sodium deoxycholate Sodium tauro-deoxycholate	0.01 M Phosphate + 0.06 M Borate + Methanol (pH = 9) Sodium acetate (pH = 4.7)	15-20 kV	Syphoning	UV (Laser-etched flow cells and a modified commercial detector)	Optimiztion of the enantiomer separation and study of poss. mechanism of chiral recognition	81

(continued)

Table 2 (continued)

Experimental Conditions used on Enantiomeric Separations by MEKC

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
Silvex phenoxy acid herbicide (2-(2,4,5-)-trichlorophenoxy propionic acid	N,N-bis-(3-D-gluconamidopropyl deoxycholamide	0.4 M Na Borate (pH = 10)	20 kV	Hydrodynamic (pressure) (34 mbar for various lengths of time	UV, 240 nm	Chiral separation is not improved by add'n of organic modifiers	82
1,1'-bi-2-naphthol 1,1'-binaphthyl-2, 2-diyl hydrogen phosphate;	N,N-bis-(3-D-gluconamidopropyl)-cholamide (Big Chap)	0.025 M-0.200 M Na borate (pH=8-11)	20 kV	Hydrodynamic (pressure) (34 mbar for various lengths of time	UV, 240 nm	Surface charge density of micelles is adjusted by varying the brate conc'n and the pH of the electrolyte. Resolution incr5eases with increasing pH and borate conc'n and decreasing temperature. Deoxy Big Chap micelles were more enantioselective than Big Chap micelles.	82
1,1'-binaphthyl-2, 2'-diamine	N,N-bis-(3-D-gluconamidopropyl)-deoxycholamide (Deoxy Big Chap)	Na borate + 15% methanol					

Table 2 (continued)

Experimental Conditions used on Enantiomeric Separations by MEKC

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
1,1'-bi-2-naphthol; 1,1'-binaphthyl-2, 2'-diyl hydrogen phosphaye	n-Dodecyl-β-D-glucopyranoside-4,6-hydrogen phosphate, Na salt n-Dodecyl-β-D-glucopyranoside-6-hydrogen sulfate, mono Na salt	0.03 M Na dihydrogen phosphate + 0.01 M Na borate (pH = 8)	20 kV or 28 kV	Hydrodynamic (pressure) 30 mbar x 1 s)	UV, 214 nm 254 nm	The 2 chiral micelles allows resolution of the enantiomers of 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate. Chiral separation of 1,1'-bi-2-naphthol is only possible with the phosphate derivative.	55
1,1'-bi-2-naphthol; 1,1'-binaphthyl-2, 2'-dicarboxylic acid; 1,1'-binaphthyl-2, 2-diyl hydrogen phosphate	Na deoxycholate (SDC) Na deoxycholate + α-cyclodextrin	0.025 M Phosphate (pH = 8)	15 kV	Hydrodynamic (pressure) (30 mbar x 3-7 s)	UV, 214 nm	Enantioseparation of the three compounds is achieved with two chiral selectors (SDC + α-cyclodextrin.	

(continued)

Table 2 (continued)

Experimental Conditions used on Enantiomeric Separations by MEKC

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
Diniconazole; Uniconazole	γ-Cyclodextrin + 5% 2-Me-2-propanol	0.1 M SDS* 2 M Urea 0.1 M Borate (pH = 9)	15 kV	Hydrodynamic (pressure)	UV, 254 nm	Study of the influence of the nature and conc'n of cyclodextrin and add'n of organic modifiers on enantiomeric separation.	88
Polychlorinated biphenyls (PCB's) (45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183, & 196)†	γ-Cyclodextrin	0.10 M CHES‡).11 M SDS 2 M Urea (pH = 10)	15 kV	Hydrodynamic (pressure) (20 mbar x 1.2 s)	UV, 235 nm	Optimization of the chiral separation of a mixture of 9 PCB's. (18 enantiomers)	90
Polychlorinated biphenyls (PCB's) (45, 64, 88, 91, 95, 132, 136, 139, 149, 171, 183, & 196)	β-Cyclodextrin γ-Cyclodextrin	0.09 M CHES 0.11 M SDS 2 M Urea (pH = 10)	15 kV	Hydrodynamic (pressure) (20 mbar x 1.2 s)	UV, 235 nm	Optimization of the chiral separation of a mixture of 8 PCB's (16 enantiomers)	92

Table 2 (continued)

Experimental Conditions used on Enantiomeric Separations by MEKC

Compounds	Chiral Selector	Buffer	Voltage	Injection	Detection	Observations	Ref.
1,1'-bi-2-naphthol; 2,2,2-trifluoro-1-(9-anthryl)ethanol; 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate	α-Cyclodextrin β-Cyclodextrin γ-Cyclodextrin 2,6-di-O-methyl-β-cyclodextrin 2,3,6-tri-O-methyl-β-cyclodextrin	0.05 M SDS 0.02 M Phosphate/Borate (pH = 9) Na d-camphor-10-sulphonate	20 kV		UV, 220 nm	Study of the influence of the add'n of different cyclodextrins, organic modifiers, and chiral additives.	93
1,1'-bi-2-naphthol; 1,1'-binaphthyl-2,2'-diyl hydrogen p0hosphate	γ-Cyclodextrin and poly-(Na N-undecylenyl-D-valinate)	0.025 M Borate (pH = 9)	12 kV		UV, 280 nm	A synergistic effect on the enentioselectivity was observed by use of both chiral selectors. The add'n of acetonitrile to the buffer reduces the enentioselectivity. The effect of the add'n of methanol is solute-dependent.	94

* SDS: Sodium dodecylsulphate. † Ballschmiter nomenclature.[89] ‡ CHES: 2-(N-cyclohexylamino)-ethanesulphonic acid)

Chiral Separations by MEKC with Chiral Selectors

Enantiomeric separation by MEKC with achiral micelles is possible if a chiral selector is added to the separation buffer. Chiral selectors most used in this MEKC method are cyclodextrins; this technique has been called "cyclodextrin modified micellar electrokinetic chromatography (CD-MEKC). Enantiomeric separation in CD-MEKC is based on the chiral recognition of cyclodextrins being important the choice of the type of cyclodextrin and its concentration. A mixture of different cyclodextrins may be helpful in order to increase selectivity. Addition of methanol also affects, not only the width of the migration time window and the capacity factors, but also selectivity.[84]

This technique is also considered very interesting for the separation of high hydrophobic compounds tending to be totally incorporated into the micelle and, hence, migrate at the same velocity as that of the micelle. The partition of the solutes among the micelle and the aqueous phase is affected by the presence of the cyclodextrin, which increases the fraction of the solute in the non-micellar aqueous phase, enhancing the resolution.[85-87] Likely for these reasons, CD-MEKC has been the technique mostly used to achieve enantiomeric separations of pollutants, especially those of a highly hydrophobic character.

The enantiomers of diniconazole and uniconazole, which are vinyl triazoles, were separated by CD-MEKC. They have fungicidal and plant growth regulating activities and their enantiomers are known to differ significantly in their biological properties. In both cases, the R-enantiomer demonstrates stronger fungicidal activity than the S-enantiomer, whereas the S-enantiomer is more active than the R-enantiomer with regard to plant growth regulating activity. Furthermore, uniconazole has a higher plant growth regulating activity than diniconazole, but it is less active as a fungicide. Consequently, diniconazole-M, containing a high proportion of the R-enantiomer, and uniconazole-P, containing a high proportion of the S-enantiomer, have been developed as a high activity fungicide and an effective plant growth regulator, respectively.[88] This production requires an efficient analytical method to separate the enantiomers. This was found to be possible using CD-MEKC with SDS micelles.

The study of the influence of the nature and concentration of the cyclodextrin and the addition of organic modifiers to the electrolyte solution has shown that the optimum conditions for the separation of enantiomers pertain to the use of γ-cyclodextrin at a 0.05 M concentration in the presence of a 5% 2-methyl-2-propanol as organic modifier.

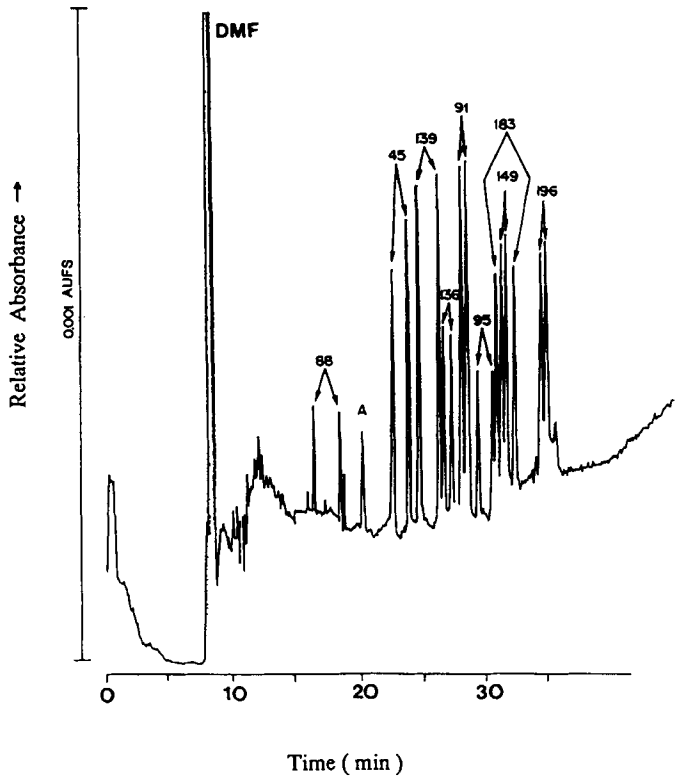

Figure 2. Electropherogram of the separation of a mixture of nine chiral PCB's. Each pair of enantiomers is identified by a number according to Ballschmiter nomenclature.[89] A: unknown peak. Separation buffer: 0.10 M CHES (pH = 10), 2 M urea, 0.11 M SDS and 0.05 M γ-cyclodextrin. Injection by pressure, 0.02 min at 20 mbar. Temperature, 45 °C. UV detection, 235 nm. Capillary, 65 cm length x 50 μm I.D. Applied voltage, 15 kV, current, 56 μA. Reproduced with permission from ref. 90.

CD-MEKC has also enabled the chiral separation of twelve polychlorinated biphenyls (PCB's) (45, 84, 88, 91, 95, 132, 136, 139, 149, 171, 183, and 196, Ballschmiter nomenclature[89]) that were individually separated into their two enantiomers by using γ-cyclodextrin as chiral selector in the separation buffer, which contained 2-(N-cyclohexylamino)ethanesulphonic acid (CHES) and SDS micelles. The multicomponent separation of the eighteen enantiomers of a mixture of nine chiral PCB's (containing one octachlorinated biphenyl) was also performed in an analysis time close to 35 min. (Figure 2).[90] This analysis time can be considered excellent, taking into account that the separation of a mixture of one hexachlorinated biphenyl and four

heptachlorinated biphenyls in their enantiomers by GC required about 110 min.[91] Chiral separation of PCB's was also studied by using mixtures of β- and γ-cyclodextrins in the separation buffer. The same twelve PCB's were individually separated into their two enantiomers but, in the separation of multicomponent mixtures, only the separation of a mixture of the sixteen enantiomers of an eighth PCB mixture was reported.[92] The only change in selectivity observed when using mixtures of β- and γ-cyclodextrins to achieve multicomponent separations of PCB's with respect to the use of γ-cyclodextrin alone in the separation buffer,[90] is the reversal in the elution order for PCB's 136 and 139. The use of β and γ-cyclodextrin mixtures reduces the cost of the technique with respect to γ-cyclodextrin-modified MEKC. However, the use of γ-cyclodextrin alone as modifier in MEKC to perform chiral separation of PCB's seems to have more potential in the separation of multicomponent mixtures or the separation of some individual, highly hydrophobic PCB's in their enantiomers.[90]

The effect of the addition of a chiral additive to the electrolyte solution in CD-MEKC with SDS micelles has been studied. The presence of sodium d-camphor-10-sulphonate in the SDS solution containing γ-cyclodextrin or 2,3, 6-tri-O-methyl-β-cyclodextrin improved enantioselectivity in the separation of aromatic compounds such as 1,1'-bi-2-naphthol, 2,2, 2-trifluoro-1-(9-anthryl)ethanol and 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate.[93] Also, chiral separation of the enantiomers of 1,1'-bi-2-naphthol and 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate by use of a combination of a polymerized chiral micelle (poly-(sodium N-undecylenyl-D-valinate)) and γ-cyclodextrin was superior to the use of either chiral selector alone. A synergistic effect on the enantioselectivity was observed when using both chiral selectors. Since surfactant monomers do not exist in the covalently bonded polymerized micelle, the interference of surfactant monomers on the enantioselectivity of γ-cyclodextrin is eliminated. Therefore, the use of a combination of both chiral selectors was considered to be a promising alternative for enantiomeric separations.[94]

The experimental conditions concerning all mentioned CD-MEKC separations are detailed in Table 2.

APPLICATION TO REAL SAMPLES

In spite of the enormous possibilities that CE techniques possess as applied to the separation of pollutants, the principal drawback, in view of the analysis of these compounds in environmental samples, is their lack of

sensitivity, particularly when trace analysis is required. Commercial CE instrumentation is equipped with UV detectors capable of detecting quantities in the order of 600 femtograms. However, in CE, injection volumes are generally in the nanolitre range to avoid the loss of the high separation efficiency that this technique ensures. This implies low sensitivity, expressed in concentration terms (about 10^{-6} M). Capillary geometry and improved detector cell design influence sensitivity in CE since, according to Beer's law, the optical absorbance of a sample is directly proportional to the optical pathlength through which the absorbance measurement is performed. Bent capillaries (Z-cell), alternative capillary shapes (rectangular), multi-reflection flow cells or end-column detection[96] have been used in order to increase the sensitivity obtained. A sensitivity enhancement ranging from 3- to 40-fold has been obtained.

Since the determination of lower and lower levels of pollutants in environmental samples is necessary nowadays, other methods to increase the sensitivity in CE have been developed. Detection limits in CE can be improved by using more sensitive detection systems, or by performing on-line concentration methods.[52,95,96] Laser-induced fluorescence, mass spectrometry and electrochemical or radiochemical detection have been employed. Excellent results have been obtained with laser-induced fluorescence which gives a 10^{-12} M sensitivity under favourable conditions. The problem is that this detection method cannot be used in a general form for environmental analysis. Methods based on indirect fluorescence have more applicability, but a 10^{-7} M sensitivity is obtained.

Different methods exist to achieve on-line concentration in CE. Sample *stacking* with discontinuous buffer systems has been used extensively. When a sample is dissolved in a solvent with electrical conductivity lower than that of the electrophoresis running buffer, a concentration, or *stacking*, occurs upon electrokinetic sample injection. The electric field strength in the low conductivity sample medium is higher than that in the running buffer, and ions rapidly migrate to the interface between the lower and higher conductivity zones. Upon reaching the interface, the analytes then slow (stack), causing contraction of the sample zone.[96] When *field amplification* is used, a large sample plug is hydrodynamically injected onto the column in a low-conductivity buffer. Then, the sample is focused at the cathodic end of the capillary, at the sample buffer–running buffer interface, using a voltage polarity opposite to that employed for the electrophoresis. Finally, when focusing is complete (as indicated by a change in current), the polarity is again reversed and the separation is performed. For a negatively charged silica surface, anions tend to stack at the back end of the sample buffer plug. Using a polarity opposite to the separation mode will result in driving out the sample buffer

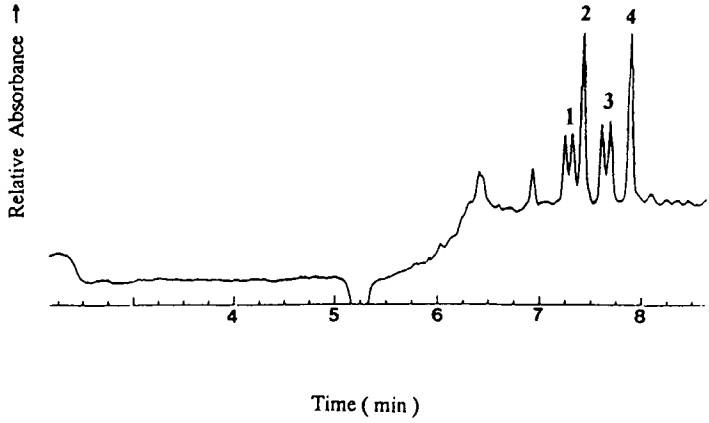

Figure 3. Chiral separation of a Rhine river water sample, spiked with 5 ppb of four herbicides and using cyclodextrin-modified CZE and C_{18} disks. Buffer: 0.03 M lithium acetate (pH = 4.8) with 20 g L^{-1} heptakis-(2,6-di-O-methyl)-β-cyclodextrin. Sample volume 20 mL. Voltage: 30 kV. Temperature: 30° C. UV detection: 200 nm. 1: 2-(2-methyl-4-chlorophenoxy)-propionic acid. 2: 2-methyl-4-chlorophenoxyacetic acid. 3: 2-(2,4-dichlorophenoxy)-propionic acid. 4: 2,4-dichlorophenoxyacetic acid. Reproduced with permission from ref. 52.

ahead of the negatively charged analytes. This method can be used to separate cationic analytes by coating the capillary.[96] On-column concentration of neutral molecules was achieved in MEKC by using field-amplification sample stacking. Neutral analytes were dissolved in a low-concentration micellar solution that was still above the critical micelle concentration. The lower total ionic strength in the sample buffer, compared to the electrophoresis buffer, allowed the negatively charged micelles to migrate rapidly into the boundary between the sample and the running buffer where they slowed down. The technique was performed by using normal or reverse electrode polarity and enabled a 75-85-fold increase in sensitivity for 1,2,4,7- and 1,2,4,8-tetrachlorodibenzo-p-dioxins.[97] *Isotachophoresis* may act as a concentration method for dilute samples, as their concentration is adapted to that of the leading zone according to Kohlrauch's regulating function.[98] This preconcentration step can be performed either in a dual- or single-column mode. Isotachophoretic concentration is performed in dual-column mode in a pre-capillary. The sample ions, which are concentrated in a sharp, narrow zone, are transferred to the analytical capillary where they are separated by CZE. In single-column mode, the use of buffers in a discontinuous system allows the use of this method with commercially available CE instruments.[95]

Another method for on-line concentration implies loading large volumes of solutes onto microcolumns of *chromatographic* material followed by elution onto a CE capillary.[96] The determination of phenoxy acid herbicides in drinking and river water samples at sub-ppb levels was performed by using field-amplification and sample pretreatment with C_{18} membrane disks for simultaneous filtering and solid phase extraction. Desorption from disks directly into the CZE vials was performed by employing acetonitrile-buffer mixtures, thus providing a sample matrix with a sufficiently low and constant conductivity.[52] Figure 3 shows the electropherogram corresponding to the chiral separation of a Rhine river water sample spiked with 5 ppb of four herbicides, two of them chiral, by using CZE and a derivative of β-cyclodextrin as chiral selector in the separation buffer (sample volume 20 mL). Changes in selectivity of the four herbicides were observed when using α-cyclodextrin; this change in elution order can be useful as confirmation criteria for the presence of these herbicides.

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