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Review

Achiral selectivity in cyclodextrin-modified capillary electrophoresis

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Abstract

Torus-shaped, circular, and hydrophilic cyclodextrins (CD) have been frequently used in capillary electrophoresis (CE) as buffer modifiers to effect chiral separation of enantiomers of drugs and specialty chemicals. Although less common, both neutral and charged cyclodextrins have also been exploited in CE to optimize the achiral separations of peptides, proteins, small molecules and a variety of positional isomers. Nonionic CDs are only useful for separations of net charged analytes through judicious partitioning of such guest molecules into their hydrophobic cavity of the former. However, they can be used with a surfactant for an effective resolution of neutral solutes as a result of a differential partitioning of such solutes in the micellar and the cyclodextrin-modified buffer phase. Ionic cyclodextrins, particularly, negatively charged derivatives with their own electrophoretic mobilities, increase the separation window and enable better resolution of analytes which weakly complex with or are poorly differentiated by neutral cyclodextrins. © 1997 Elsevier Science B.V.

Keywords: Reviews; Buffer composition; Selectivity; Cyclodextrins

Contents

1. Introduction

Capillary zone electrophoresis (CZE) and other capillary electrophoresis (CE)-based methods employ narrow-bore (20–200 μm I.D.) capillaries to perform high efficiency separations of both large and small molecules. Because of the large surface-to-volume ratio and the high electrical resistance, the walls of the fused-silica capillary permit efficient heat dissipation and thus the use of high field strengths (30 kV or higher). CE offers several advantages over slab-gel electrophoresis with respect

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to speed, efficiency, sensitivity, automation and ease of quantitation. Since the gel acts as a sieving medium, small molecules cannot be normally well separated by slab-gel techniques. CE can be considered complementary to liquid chromatography (LC) since most separations are based on charge or size. However, the technique provides efficiencies up to several orders of magnitude greater than high-performance liquid chromatography with column efficiencies of up to one million theoretical plates frequently reported. Since only very small amounts of buffer are needed for CE separations, it is possible to use expensive solvents that could not be normally considered as LC mobile-phase modifiers.

The simplicity of CE lends itself to the development of various separation modes. Resolution and efficiency of separation can be simply effected through the use of buffer additives that exhibit specific chemical or physical interactions with target analytes. Several forms of CE have been developed extensively over the last decade as powerful tools for rapid and efficient separation of charged and uncharged components present in small sample volumes. Cyclodextrin-based complexation phenomena have received considerable attention in analytical separation science and particularly in capillary electrophoresis. Cyclodextrins are torus-shaped, circular, and non-reducing oligosaccharides prepared by enzymatic degradation of starch [1]. Enhancement of selectivity by the use of cyclodextrins in CE can be attributed to their ability to selectively include a wide variety of water-insoluble organic and inorganic molecules into their hydrophobic cavity. The ability of cyclodextrins to act stereoselectively upon complexation with a guest molecule has also been well recognized in liquid chromatography, both as a mobile-phase additive and as a chiral stationary phase. The most frequent use for CDs in capillary electrophoresis has been as modifiers to effect chiral separation of enantiomers of charged compounds. Together with a micelle agent, neutral CDs can be extended for the chiral resolution of small neutral and hydrophobic compounds as a result of their partition between the micellar phase and the cyclodextrin-modified buffer. Negatively charged CDs with their own electrophoretic mobility can also be prepared and used in CE to increase the separation window which enables better resolution or enantioresolution of analytes which weakly complex with or are poorly differentiated by neutral CDs.

While not as common, neutral and charged cyclodextrins have been used as additives in CE to optimize the separations of peptides, proteins, and small achiral molecules such as plant regulators, food colors, positional isomers, and toxic pollutants. Native CDs and their nonionic derivatives are very effective in enhancing both selectivity and resolution through judicious partitioning of net charged guest molecules into their hydrophobic cavity. Together with a micelle agent, they have proven very useful for resolving numerous neutral and hydrophobic compounds in biological or environmental samples. Several forms of ionic cyclodextrins, particularly negatively charged derivatives with their own electrophoretic mobilities, have been prepared and used in CE to separate small and water-insoluble molecules such as aromatic and polyaromatic hydrocarbons. The objective of this review is to provide the reader with a better appreciation of how cyclodextrin-modified CE can be applied to achiral separation/detection of both charged and non-charged compounds.

2. Cyclodextrins and their derivatives

2.1. Natural cyclodextrins

Natural and nonionic cyclodextrins (CDs) comprise a family of macrocyclic oligosaccharides, formed by α-1,4-linked glucopyranose subunits, and shaped as truncated cones (Fig. 1). Crystal structure analyses of CD inclusion compounds proved that all glucose residues in the ring possess the thermodynamically favored $^4C_1$ chair conformation because all substitutions are in the equatorial position. The most common and commercially available CDs are α-CD, β-CD and γ-CD, with six, seven and eight subunits, respectively [2]. In recent years, CDs can be produced in large quantities and marketed at a reasonable price. Each D-glucose unit in the CD structure contains five chiral carbon atoms and as a result the CD macrocycle is chiral. CDs have no reducing end-groups and give positive reactions with tests characteristic of non-reducing carbohydrates. At temperatures above 200°C, CD starts to decompose,

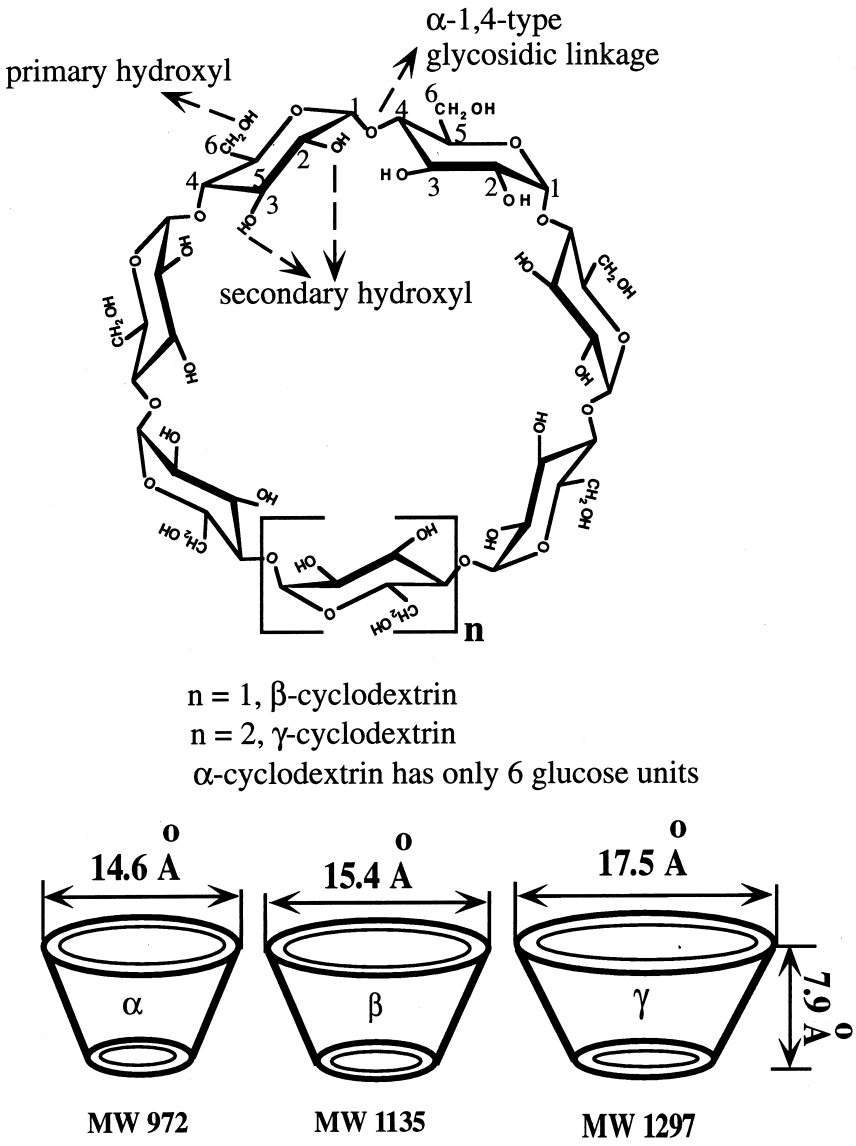

Fig. 1. Chemical structure and schematic model of cyclodextrins: α-CD ($n=0$), β-CD ($n=1$) and γ-CD ($n=2$). MW, molecular mass.

however, they are not sensitive to alkalis. The viscosity of aqueous cyclodextrin solutions differs only insignificantly from that of water. The viscosity of 0.95 m*M* α-CD is 8.88×10^{-4} Pa s and for 9.98 m*M* β-CD it is 9.46×10^{-4} Pa s, respectively, in comparison to 8.93×10^{-4} Pa s for water at 25°C [3]. Some important physical data of these three CDs are given in Table 1.

Like aqueous micelles, cyclodextrins possess a hydrophilic exterior and a hydrophobic interior or cavity. Hydroxyl groups occupy both rims of the truncated cone and render the CDs soluble in aqueous solution. The relatively hydrophobic cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges. This is accompanied with a high electron density and Lewis-base properties. The cavity of β-CD, which is intermediate between those of α and γ-CD (Table 1), is the most efficient host

Table 1
Some important characterists of cyclodextrins

	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Number of glucose units	6	7	8
Number of hydroxyl groups	18	21	24
Number of primary hydroxyls	6	7	8
Number of secondary hydroxyls	12	14	16
Molecular mass	972	1135	1297
Internal cavity diameter (nm)	0.47–0.52	0.60–0.64	0.75–0.83
External cavity diameter (nm)	1.46±0.05	1.54±0.04	1.75±0.04
Cavity height (nm)	0.79–0.80	0.79–0.80	0.79–0.80
Cavity volume (nm^3)	0.176	0.346	0.510
Number of water molecules inside the cavity	5	11	17
Melting point (K)	551	572	540
Solubility in water at 25°C (g/100 ml)	14.5	1.85	23.20
pK_a range of hydroxyl groups	12.1–12.6	12.1–12.6	12.1–12.6
Specific rotation, $[\alpha]_D^{25}$	150.5±0.5	162.5±0.5	177.4±0.5
Density (g/cm^3)	1.42–1.45	1.42–1.45	1.42–1.45

for many kinds of water-insoluble compounds or hydrophilic ions. The hydrophobic cavity can form inclusion complexes with alkyl or aromatic moieties of proteins and peptides, aromatic hydrocarbons and other hydrophobic compounds as long as there is a match between the size and shape of the entering solute and the CD cavity size. In general, the interaction between CDs with a very small or very large molecule may not be sufficient to affect complexation. However, in certain cases complexation can occur owing to hydrogen bonding of such guest molecules and the hydroxyls at the base of the cavity [4]. CDs are typical host molecules and include rather unspecifically a great variety of molecules having the size of one or two benzene rings, or even larger ones carrying a side chain of comparable size, to form inclusion complexes. On this basis, it is not surprising to find that noble gases, paraffins, alcohols, carboxylic acids, aromatic dyes, benzene derivatives, salts, etc., are included, just to name a few of a long list of potential guest substances. Five-ring polynuclear aromatic hydrocarbons such as benzo[*a*]pyrene and benzo[*e*]pyrene can also be solubilized using CD derivatives [5]. Inclusion complexes are defined as entities comprising two or more molecules, in which one of the molecules, the host, includes a guest molecule totally or partially by only physical forces, i.e., without covalent bonding. In the past, CD inclusion complexation has considered only hydrophobicity and steric properties (molecular dimensions) of the CD and inclusate. Modern descriptions include other complex geometries, which may not be one-to-one molecular ratios, and entropic effects, which are especially linked to the role of water molecules in the CD cavity. Upon complexation, the relative stabilities of the CD inclusion compounds may also be influenced by other factors such as hydrogen bonding, hydrophobic interactions, dipole–dipole interaction and London dispersion, solvent effects, extrusion of high energy water from the CD cavity, and size and shape of the guest molecule [6].

2.2. Cyclodextrin derivatives

The hydroxyl groups on both the primary and the secondary sides of the CD rim are the most common reaction sites and have been extensively derivatized. α, β, and γ-cyclodextrins contain 18, 21, and 24 hydroxyl groups, respectively, of which the primary ones, i.e., C-6 (six, seven and eight, respectively) are the most active. Twelve, 14, and 16 hydroxyls located on the secondary side (C-2 and C-3) also show reactivity, however, the hydroxyls at C-2 are much more active than those at C-3. Selective substitution can be achieved directly by selective reactions or indirectly by nonselective reactions accompanied with selective protection. The C-2 and C-3 hydroxyl groups of the adjacent glucopyranose units also form hydrogen bonds which stabilize the

shape of the molecule as well as affect its solubility in water. Due to the polyfunctional character of the hydroxyl groups, chemical modification leads to heterogeneous mixtures of CD derivatives that have to be separated and purified by suitable separation procedures. The higher reactivity of the C-2 and C-6 over C-3 has been supported by the fact that the hydroxyethyl derivative is primarily attached at the C-2 and C-6 positions with the C-3 position primarily open [1]. However, the hydroxypropyl derivative is distributed among all three position: C-2, C-3, and C-6 [1]. When one of the hydrophilic edges of cyclodextrin is transformed by substitution to form a hydrophobic cap on the molecule, the stability of the complex formed increases significantly. For instance, methylated cyclodextrins exhibit greater stability than their parent cyclodextrins. Methylation also increases solubility, presumably due to the distribution of the hydrogen bond system. Increased solubility by appropriate substitution is also well exemplified by the hydroxypropyl, acetyl and sulfopropyloxy derivatives.

Derivatization can lead to different degrees and patterns of substitution, and the degree of substitution significantly influences the complex formation. Most of CD derivatives are mixtures of differently substituted CD rings, therefore an average degree of substitution number must be given for the characterization of the CD derivatives. The average molar degree of substitution expresses the average number of moles of the substituting agent per mol glucose [7]. It has been speculated that the chemical modification of CDs effects changes in the shape and size of their cavities, in their hydrogen-bonding ability and in other physical properties. However, no physical data are available to confirm or refute this hypothesis. Most of the cyclodextrin derivatives known presently are derived from β-CD, while the derivatives of α- and γ-CD remain less understood and underexploited. One of the main reasons could be the significantly lower solubility of β-CD in comparison to α- and γ-CD. Several important ionic cyclodextrin derivatives have been prepared and commercialized. Depending on the functional group substituted, the derivatized cyclodextrin may be charged at low pH (quaternary amine), high pH (carboxylic) or across virtually the entire pH range (sulfated). Cationic CDs such as amino, alkylamino, nitrogen, phosphorus and sulfur-containing CDs have been synthesized and/or commercially available. More than 15 anionic CD derivatives are currently commercially available and their applications in CE are also more widespread than the cationic derivatives. They include sulfated-, carboxymethyl-, carboxyethyl-, succinyl-, sulfoethyl- and sulfobutyl-cyclodextrins.

3. CE using nonionic cyclodextrins

Neutral CDs such as α, β, and γ and their derivatives are only useful to separate solutes with a net charge. In general, CDs enhance both selectivity and resolution through judicious partitioning of guest molecules into the host hydrophobic cavity. When the solute forms a complex with CD, its mobility is greatly reduced owing to a change in the apparent molecular weight. The separation is due to an alteration in electrophoretic mobility (a function of charge (Z) and the molecular mass (M_r), ZM_r^{α}, α is a constant) of the complexed and free molecules. Formation of the inclusion complex is facile on the electrophoretic time scale and the apparent mean mobility of a solute can be related to the intrinsic mobility of the solute (μ_A) and the solute–cyclodextrin inclusion complex (μ_{ACD}) as $(\mu_A + \mu_{ACD}K[CD])/(1+K[CD])$. The formation constant of the inclusion complex or K is defined as [ACD]/([A]+[CD]) where [CD], [A], and [ACD] are the concentrations of cyclodextrin, solute, and inclusion complex, respectively. This model was originally developed for optimization of cyclodextrin-modified capillary electrophoresis for separations of enantiomers [8]. Therefore, the quality of a resolution and sensitivity are governed by the cyclodextrin concentration in the electrolyte solution, and the selectivity is dependent upon whether the solute will form a strong or weak complex with the cyclodextrin.

One of the important applications of neutral cyclodextrins in CE is the separation of peptides using different types of additives, including α- and β-CD. In general, the separation by CE of large peptides and proteins with equivalent or similar mass-to-charge ratios has proved to be difficult, as minor structural differences in such molecules often result in essentially identical electrophoretic mobili-

ties. Using 20 m*M* α-CD as a buffer additive, good resolution and peak shapes are achieved for six model peptides derivatized with 3-(4-carboxybenzoyl)-2-quinoline-carboxaldehyde [9]. With 20 m*M* β-CD in the running buffer, very narrow peaks and enhancement in sensitivity were also observed for a mixture of nine peptides derivatized with fluorescamine [9]. CDs have also been shown to enhance the fluorescence intensity of peptides and amino acids derivatized with naphthalene-2,3-dicarboxaldehyde/cyanide. The excellent peak shapes and high efficiencies can be attributed to the formation of inclusion complexes or by prevention of protein adsorption to the capillary wall.

The use of all three cyclodextrins together (8 m*M* α-CD, 1 m*M* β-CD, and 1 m*M* γ-CD in 0.05 *M* phosphate–0.1 *M* borate buffer, pH 8.09) was considered optimal and effected baseline separation for nine plant growth regulators [10]. As shown in Fig. 2, the addition of 12.5 m*M* β-CD to a buffer system containing 40 m*M* citric and 80 m*M* Na_2HPO_4, pH 5.5, was essential for the separation of several major alkaloids (berberine, chelidonine, coptisine, homochelidonine, protopine and stylopine) produced from the cultivation of *Chelidonium majus* [11]. Capillary electrophoretic separation of 15 auxins (plant hormones) using cyclodextrins as modifiers was also reported recently [12]. Optimal separation was attained in less than 19 min using a mixed buffer containing 5.8 m*M* α-CD, 1.8 m*M* γ-CD in 25 m*M* sodium tetraborate–50 m*M* sodium dihydrogenphosphate, pH 8. It should be noted that when each of the cyclodextrins (α-, β- and γ-CD) was used individually, satisfactory separation of the 15 auxins could not be obtained.

In food analysis using CE, food dyestuffs can be separated by taking advantage of inclusion complexation with β-CD. The complexation with β-CD was reported to decrease the charge density and the electrophoretic mobility of the dyestuffs. In particular, the resolution between the isomeric pairs Amaranth and New Coccine was significantly improved by selective complexation with β-CD [13]. A CE method for the determination of synthetic tar

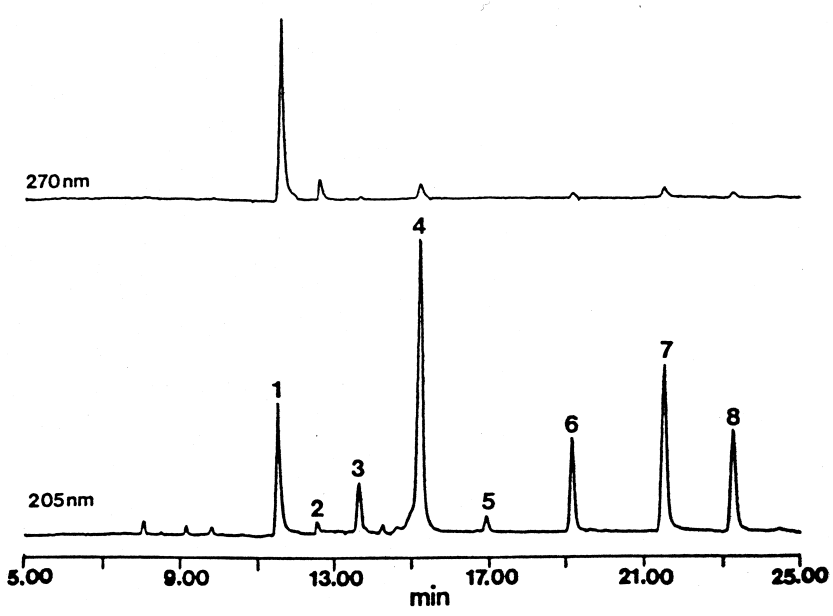

Fig. 2. Electropherograms of a methanolic extract of the aerial parts of *C. majus*. Running electrolyte, 40 m*M* citric acid–80 m*M* Na_2HPO_4, pH 5.5, and 12.5 m*M* β-cyclodextrin. Column, fused silica (70 cm×50 μm I.D.); voltage, 25 kV; UV at 205 and 270 nm; temperature, 25°C (adapted from Ref. [11]). Peak numbers: (1) coptisine, (2) berberine, (3) unidentified, (4) protopine, (5) unidentified, (6) chelidonine and (7) stylopine.

dyes used as food additives using cyclodextrin-modified capillary electrophoresis with photodiode-array detection was attempted. The electrophoresis buffer used was a mixture of 25 m*M* sodium phosphate and 25 m*M* sodium borate buffer, pH 8, containing 10 m*M* β-CD to separate seven red dyes including Amaranth and New Coccine [14]. The merit of β-CD as an additive (20 m*M* β-CD in 10 m*M* sodium tetraborate, pH 7.5) was also its ability to counteract the adverse wall effect. Recently, CE methods for the analysis of domoic acid in the tissue of mussels, clams and anchovies were developed, and the performance of CE was evaluated to be superior to that achieved in liquid chromatography [15]. This acid is the main toxic agent associated with incidents of amnesic shellfish poisoning on the east and west coasts of North America. The highest performance with respect to separation efficiency and analysis time was achieved with phosphate or borate buffers, pH 9. The addition of 20 m*M* β-CD in a buffer containing 22.5 m*M* sodium tetraborate, pH 9.2, allowed a separation of domoic acid and several of its isomers (isodomoic acids).

In pharmaceutical and drug analysis, several cyclodextrin modified CE methods have been developed for separation and quantitation of important drugs in biological samples. The use of CE to determine drug-related impurities has also become established within industrial pharmaceutical analysis laboratories, and this subject was recently reviewed and discussed by Altria [16]. A full baseline separation of pilocarpine, an antiglaucoma and miotic agent, from its epimer, isopilocarpine was achieved by adding 10 m*M* β-CD to a running buffer at pH 6.9 [17]. A buffer containing 20 m*M* α-CD and 10% acetonitrile in 30 m*M* borate buffer, pH 8.5, gave completely resolved peaks for each isomer of 13-*cis* and all-*trans* retinoic acid and their photodegradation products in less than 6 min [18]. Eight sulfonamides were resolved in less than 8 min using a buffer containing 2 m*M* β-CD in 0.05 *M* phosphate–0.05 *M* borate, pH 8.6 [19]. Capillary zone electrophoresis was attempted for separation of some major gangliosides (glycosphingolipids containing sialic acid) such as G_{M1}, G_{D1a}, G_{D1b}, and G_{T1b} in mammalian brains [20]. Enhancement of selectivity and efficiency of separation was obtained by adding 16.5 m*M* α-CD to a 50 m*M* borate–phosphate buffer, pH 9.3. It has also been suggested that the addition of α-CD to the electrolyte buffer may also act as an inhibitor of ganglioside micelle formation.

Cyclodextrins have proven to possess a remarkable capability of separating certain types of aromatic positional isomers. Through the use of α-, β-, γ- and heptakis(2,6-di-*O*-methyl)-β-CD, isomeric compounds such as benzene- and naphthalene-based structures were resolved [21]. It should be noted that the addition of soluble alkylhydroxyalkylcellulose was also required for the improvement of separation efficiency. CZE separation of *o*-, *m*-, *p*-, and α-methoxyphenylacetates was not attained unless 10 m*M* β-CD was added to a carrier buffer containing 50 m*M* Tris and tricine, pH 8.06, and 0.1% methylhydroxyethylcellulose [21]. Similarly, α- and β-naphthylphosphate ions were only separable when 10–20 m*M* α-CD were added to the running buffer. Separation of chlorophenoxy acid herbicides by cyclodextrin-modified capillary electrophoresis was feasible in less than 7 min by adding 4 m*M* α-CD and 1 m*M* β-CD in the phosphate buffer, pH 5.6. This condition allowed the separation of seven herbicides and the enantiomers of 2-(2,4-dichlorophenoxy) propionic acid [22]. A capillary electrophoresis–indirect UV absorbance detection method was developed for separation and analysis of five organotin compounds: trimethyltin, triethyltin, tripropyltin, tributyltin, and triphenyltin [23]. Although such compounds were efficiently separated by CE in 10 min with a 5 m*M* acetate buffer, pH 4.5, containing 3 m*M* 4-aminopyridine, the addition of 15 m*M* α-CD to the running buffer allowed simultaneous separation of di- and triorganotins. Organotin compounds have been widely used in the plastics industry as stabilizers and in agriculture as insecticides, fungicides and biocides.

4. CE using nonionic cyclodextrins and a micelle agent

Analysis of uncharged species through micellar electrokinetic chromatography (MEKC) is feasible by adding a negatively charged phase to a standard CZE buffer [24,25]. Micelles formed from surfactants, alkylammonium salts and bile salts have all been used in MEKC, and have the added advantage

of being able to solubilize water-insoluble analytes in the aqueous buffer. Surfactants are long-chain molecules (10–50 carbon units) with a long hydrophobic tail and a hydrophilic head. Above a surfactant concentration known as the critical micelle concentration (CMC), the aggregate is fully formed with the hydrophobic tails pointing inward and the hydrophilic heads pointing outward into the aqueous solution. When added to the carrier buffer, micelles compete with the negatively charged capillary walls for the positively charged sites on the analyte. Because of this reason, MEKC is not generally useful for protein separations because of strong binding of the protein molecules to surfactant molecules (1.4 g SDS for each gram of protein).

The MEKC system consists of an aqueous phase and a micellar phase. MEKC is most commonly performed with anionic surfactants such as sodium dodecyl sulfate (SDS). SDS micelles possess a negative charge and, therefore, exhibit an electrophoretic migration that opposes the electroosmotic flow. Under normal electrophoretic conditions, the micellar mobility is of a smaller magnitude than the electroosmotic flow; thus, both the running buffer and micellar phases are transported at different velocities towards a common outlet of the separation capillary. Normally, the negatively charged micelle migrates at a significantly slower rate than the buffer phase, and functions as a pseudo-stationary phase. Both charged and neutral compounds can partition between the two phases, resulting in retention based on differential solubilization by the micelles. Neutral CDs can be added as an additive in micellar solutions for CD-MEKC separations to resolve lipophilic compounds which migrate close to the micelle and cannot be separated by the micellar solution alone. Since CDs are electrically neutral and their outside surface is hydrophilic, they do not interact with the micelle and move along with the buffer phase. When a hydrophobic solute is introduced to the CD-MEKC system, it will be incorporated by either the micelle or CD. The capacity factor, k^*, of a hydrophobic solute in CD-MEKC is expressed as $\alpha V_{mc}/V_{CD}$, where V_{mc} and V_{CD} are the volumes of micelle and CD, respectively. The distribution coefficient α indicates the relative affinity of the solute between the micelle and CD. The ratio of the solute incorporated in the micelle largely depends on its hydrophobicity, but the inclusion complex formation of the solute with CD will depend on the size of the solute and the internal cavity of CD in addition to the hydrophobicity. Therefore, the selectivity in CD-MEKC is mostly governed by the tendency of the hydrophobic solute to form an inclusion complex with CD. A solute included more strongly in the CD cavity is expected to have a shorter migration time than a solute included less strongly in the CD cavity.

Adding CDs to the MEKC system has been shown to increase the versatility of the CE technique, particularly in separation of geometrical isomers of water-insoluble compounds. Several types of CDs: α-CD, β-CD, 2,6-di-*O*-methyl-β-CD, 2,3,6-tri-*O*-methyl-β-CD and γ-CD were used together with SDS to manipulate the selectivity in CD-MEKC [26]. CD was introduced to a micellar phase to improve both efficiency and resolution for the separation of 12 chlorinated benzene congeners (Fig. 3). Otherwise, the separation of such congeners by MEKC in the absence of CD was inefficient and not complete. The addition of 40 m*M* γ-CD to the

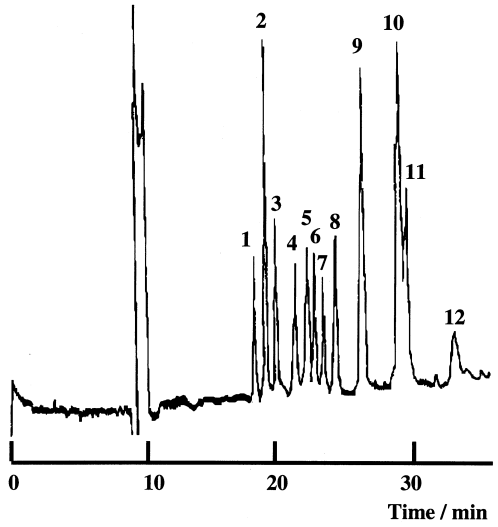

Fig. 3. Separation of chlorinated benzene congeners. Running solution, 100 m*M* SDS in 100 m*M* borate buffer, pH 8, containing 40 m*M* γ-CD. Voltage, 15 kV; current, 23 μA (adapted from Ref. [26]). Peak numbers: (1) 1,2,3,5-tetrachlorobenzene, (2) 1,2,3-trichlorobenzene, (3) 1,3,5-trichlorobenzene, (4) 1,2-dichlorobenzene, (5) 1,2,4-trichlorobenzene, (6) monochlorobenzene, (7) 1,3-dichlorobenzene, (8) 1,2,3,5-tetrachlorobenzene, (9) 1,2,3,4-tetrachlorobenzene, (10) pentachlorobenzene, (11) 1,4-dichlorobenzene and (12) hexachlorobenzene.

running buffer (100 m*M* SDS, 2 *M* urea, 100 m*M* borate, pH 8) allows a complete separation of these 12 congeners, except for pentachlorobenzene and 1,4-dichlorobenzene [27]. A 100 m*M* borate buffer, pH 9, containing 30 m*M* γ-CD, 100 m*M* SDS and 5 *M* urea was capable of separation of a mixture of naphthalene and four tricyclic and three tetracyclic hydrocarbons (acenaphthene, anthracene, fluorene, phenanthrene, chrysene, pyrene and fluoranthene) [27]. Eleven trichlorobiphenyl isomers were resolved using a 50 m*M* phosphate–100 m*M* borate buffer, pH 8, containing 100 m*M* SDS, 60 m*M* γ-CD and 2 *M* urea [27]. Photodiode-array detection has been utilized in CE separations of several groups of compounds, including aromatic hydrocarbons to facilitate peak identification [28].

Isomer identification and separation of chlorinated dibenzo-*p*-dioxins (ClDD) was another important application of CD/MEKC (100 m*M* SDS, 40 m*M* γ-CD, 5 *M* urea in 100 m*M* borate, pH 9) in environmental and food analysis [29]. All of the ClDD isomer pairs tested were baseline-separated by CD/MEKC, with electropherograms showing 11 peaks for the 12 mono- and dichlorinated isomers, 14 peaks for the trichlorinated isomers, 21 peaks for the 22 tetrachlorinated isomers and 14 peaks for the pentachlorinated isomers.

Cyclodextrin-modified MEKC has found a variety of important applications in food, pharmaceutical, clinical analysis and biotechnology. A mixture of seven water-soluble and two fat-soluble vitamins was separated in a single analysis by CE using 3 m*M* γ-CD and 30 m*M* SDS in 0.1 *M* borate–0.05 *M* phosphate, pH 7.6 [30]. A mixed buffer containing 10 m*M* β-CD and 10 m*M* SDS in 0.05 *M* borate–0.05 phosphate and 10 m*M* tetrabutylammonium hydrosulfate successfully resolved nine antihistamines including (±)-chloropheniramine [31]. All these compounds were satisfactorily baseline separated within 6 min and the method was applicable for analysis of such compounds in commercial pharmaceutical samples.

Separation of testosterone metabolites in microsomal incubates was effected using a buffer containing both SDS and β-CD. With SDS alone (51 m*M* in Tris–borate buffer, pH 8) 16α- and 16β-hydroxyestosterone and 2α- and 2β-hydroxyestosterone comigrated [32]. Addition of ethanol resulted in resolution of 16α and 16β whereas 2α and 2β were not affected. β-CD (12.75 m*M*) exhibited little effect on the testosterone metabolites, except for 2α and 2β. Consequently, the addition of β-CD in the carrier buffer containing also SDS and ethanol allowed resolution of all hydroxyestosterones [32]. Partial separation of estrogens, sex hormones formed from the precursors androstenedione and testosterone, was effected by MEKC using a 10 m*M* borate buffer, pH 9.2, containing 50 m*M* SDS [33]. However, the addition of 20 m*M* γ-CD to the running buffer improved resolutions significantly and allowed faster separation of ten tested estrogens [33].

There has been a noticeable increase in the use of CE for determination of drug impurities. Satisfactory separation of ondansetron and its drug-related impurities was obtained with a 25 m*M* phosphate–borate buffer, pH 6.3, containing 30 m*M* sodium cholate and 15 m*M* hydroxypropyl-β-CD [34]. The introduction of 20 m*M* γ-CD in a buffer containing 50 m*M* SDS and 25 m*M* phosphate–borate, pH 7.5, effected the separation of the *E* and *Z* isomers of tamoxifens [1-(4-hydroxyphenyl)1,2-diphenyl-but-1-ene) [34]. Tamoxifens are triphenylethylene derivatives which are non-steroidal and anti-estrogens used for the treatment of hormone-dependent breast cancer.

5. CE using ionic cyclodextrins

Like micelles, the negatively charged CD migrates at a significantly slower rate than the buffer phase, and functions as a pseudo-stationary phase in analogy with MEKC to increase the ‘separation window’. Optimization of separation conditions can be easily achieved by increasing CD concentrations or altering the running buffer pH. Terabe et al. [35] first reported on electrokinetic chromatography, using 2-*O*-carboxymethyl-β-cyclodextrin as a buffer additive. In view of electrostatic repulsion and aqueous solubility, more water-soluble and charged solutes would favor the buffer phase, whereas less water-soluble and neutral solutes would complex more strongly to the charged CD. Separation will be effected based on differential partitioning of solutes between the aqueous buffer and the charged cyclodextrin phase. The introduction of a charged CD as a

buffer additive enables the analysis of mixtures containing both charged and neutral compounds. However, the longer migration times and higher currents accompanying higher concentrations of charged CD may limit the utility of the charged CD to separate both weakly and strongly interacting analytes at the same time. The separation and detection of neutral analytes are particularly important in environmental analysis, since many pollutants and toxins are water insoluble and uncharged, such as polychlorinated biphenyls (PCBs), polychlorinated dioxins, and polycyclic aromatic hydrocarbons (PAHs) and their derivatives.

One of the elegant applications of CE using a negatively charged cyclodextrin in CE is the separation and detection of phenolic compounds in contaminated soil and water [36]. Phenols behave as weak acids, and buffer pH strongly affects the electrophoretic mobility of weak acids and bases in CZE. Therefore, phenols can be electrophoretically separated by adjusting their mobilities as a function of pH. It is difficult, however, to find an optimal overall pH that will resolve a complex phenol mixture with widely varying pK_a values. Often the neutral forms of the compound are observed to elute at or close to the electroosmotic front. The pH can be increased to the point where all of the analytes are charged (pH 12 in the case of most phenols) and the resulting increase in the electroosmotic flow results in fast separation. Unfortunately, the silica capillary walls are degraded under these conditions and it is difficult to maintain reproducibility. In 50 m*M* phosphate buffer, pH 7.5, containing as low as 1 m*M* sulfobutyloxy β-CD (SBβCD), 25 phenolic compounds including 11 EPA priority phenols were well separated with theoretical plate numbers well above 100 000, for 50 cm of effective length in most cases (Fig. 4). The SBβCD-mediated capillary electrophoresis system was also applicable for separating and quantifying pentachlorophenol in contaminated soil samples [36].

Another important application of cyclodextrin-modified CE in environmental analysis is the detection of PAHs. Environmental contaminants often occur as complex mixtures of very similar compounds in low concentrations, imposing stringent requirements on the method chosen for analysis. One such class of contaminants are the polycyclic aromatic hydrocarbons (PAHs), for which the US Environmental Protection Agency (EPA) has designated 16 PAHs as priority pollutants in view of their suspected carcinogenicity. Tetrahexylammonium salts have been used in MEKC for analysis of PAHs. However, PAHs were so strongly associated with the hydrocarbon phase that separation was not efficient [37]. Separation of benzo[*a*]pyrene (BaP), benzo[*e*]pyrene (BeP) and several derivatives of BaP using SDS/MEKC was effected after addition of γ-CD. SDS was also mixed with α-CD and β-CD additives to achieve separation of some smaller PAHs but not chrysene, BaP and perylene [37]. Addition of γ-CD resulted in separation of those latter components, but BaP and BeP were still not separated. Although β-CD derivatives are usually regarded as better complexers than γ-CD, this was not observed in MEKC systems suggesting the micelle components might interact with β-CD to inhibit PAH inclusion. Terabe et al. [38] reported that

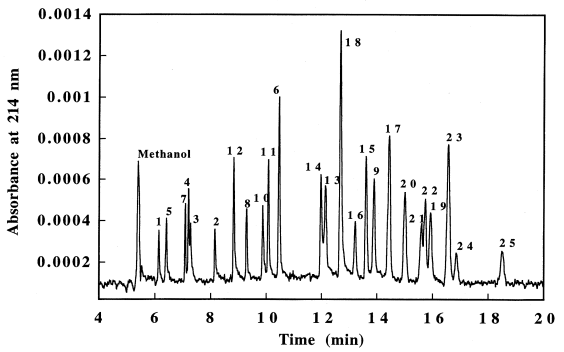

Fig. 4. Electropherograms for the separation of 25 substituted phenols using 50 m*M* phosphate buffer, pH 7.5, containing 1 m*M* sulfobutylether-β-cyclodextrin. Fused-silica capillary internal diameter, 50 μm; total length, 57 cm; length to detector, 50 cm; temperature, 30°C; separation voltage, 20 kV; resultant current, 80 μA; detector wavelength at 214 nm; pressure injection time, 2 s (adapted from Ref. [35]). Peak numbers: (1) phenol, (2) 4-chloro-3-methylphenol, (3) 4-chlorophenol (4) 3-chlorophenol, (5) 2,4-dimethylphenol, (6) 3,4-dichlorophenol (7) 2-chlorophenol, (8) 2,4-dichlorophenol, (9) 3,4,5-trichlorophenol (10) 2,3-dichlorophenol, (11) 2,5-dichlorophenol, (12) 3,5-dichlorophenol (13) 2,3,4-trichlorophenol, (14) 2-nitrophenol, (15) 2,4,5-trichlorophenol (16) 4-nitrophenol, (17) 2,3,5-trichlorophenol, (18) 2,6-dichlorophenol (19) pentachlorophenol, (20) 2,4,6-trichlorophenol, (21) 2,3,4,6-tetrachlorophenol (22) 2,3,5,6-tetrachlorophenol (23) 2,3,6-trichlorophenol, (24) 4,6-dinitro-*ortho*-cresol, and (25) 2,4-dinitrophenol.

the addition of β-CD to a SDS buffer was not very successful since certain PAHs with similar structures were not separated. A mixture of 8 m*M* β-CD, 1 m*M* γ-CD and 10 m*M* carboxymethyl-β-CD with 30% methanol was used for separation of a mixture of four PAHs: pyrene, chrysene, benzo[*a*]pyrene, and anthracene [39]. Such PAHs were not separable using a running buffer containing 50 m*M* SDS, 8 m*M* β-CD and 8 m*M* γ-CD.

Recently, the separation of PAHs using CE with a buffer containing a mixture of a neutral and anionic β-CD derivatives was demonstrated. The mixture of hydroxypropyl-β-CD (HPβCD) or methyl-β-CD (MβCD) with SBβCD or carboxymethyl-β-CD (CMβCD) provided separation based on differential partitioning of the various PAHs between the two CD phases [40]. Solutes are separated based on their differential partitioning between the neutral CD phase, which virtually migrates with the electro-osmotic flow, and the negatively charged phase, which migrates very slowly at a different velocity. If the mobility of the neutral CD_1 form is taken as zero and the aqueous concentration of the PAHs is negligible (i.e. all PAHs are complexed with CDs), then the mobility of each PAH (μ_i) can be related to the mobility of the negatively charged CD_2 (μ_2) as $\mu_2 K_i[CD_2]/([CD_1]+K_i[CD_2])$. The selectivity, therefore, is dependent upon the type of negatively charged cyclodextrins although it can be manipulated by varying the concentrations of both neutral and charged cyclodextrins to a certain extent [40].

The SBβCD/MβCD system was superior to the HPβCD/CMβCD mixture with respect to resolution, separation efficiency and speed of analysis. The migration times of all six PAHs separated with the 20 m*M* MβCD/25 m*M* SBβCD were reproducible within 3% and provided much better separation of PAH isomers, including the BaP/BeP components. The use of CDs exclusively, without micelles, resulted in dramatically reduced sensitivity to parameters such as temperature and separation potential when compared with MEKC [40]. It should be noted that as running buffer additives, CDs are fixed reagents, while micelles are dynamic aggregates that are in equilibrium with the surfactants used. Also in SDS/MEKC a limited amount of organic modifier such as acetonitrile (1–20%) [40] or methanol (maximum 30%) [39,41] can be added to the running buffer before disruption of the micellar phase occurs. In the dual CD system, up to 60% organic modifier can be used for expansion of the elution range [42].

From a practical viewpoint, the dual MβCD and SBβCD system was also tested for separation/detection of 16 EPA priority PAHs in contaminated soil [43]. Satisfactory separation of all 16 PAHs was achieved in under 20 min with 35 m*M* SBβCD and 15 m*M* MβCD (Fig. 5), with efficiencies for all components greater than 10^5 theoretical plates. Laser-induced fluorescence detection provided sensitive detection of 11 of the 16 components, with detection limits measured typically in the low μg/l (ppb) range. The separation was sufficient and applicable for analysis of several PAHs and a variety of other compounds in soil extracts and the results agreed well with the EPA-certified procedure [43]. Due to the complexity and often low levels of PAHs

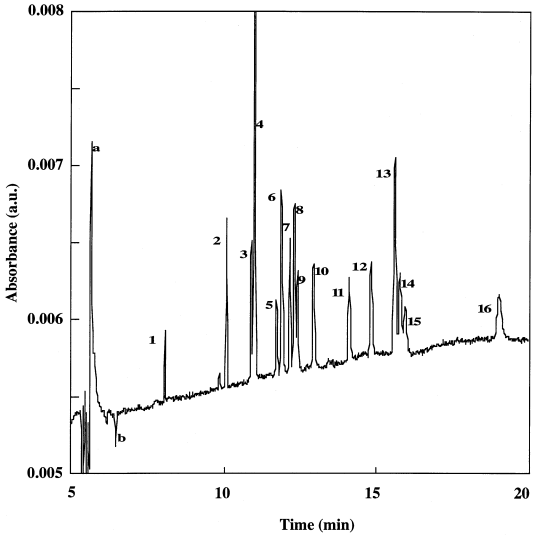

Fig. 5. Electropherograms of a mixture of 16 EPA priority PAHs, using 35 m*M* SBβCD, 15 m*M* MβCD, 50 m*M* borate buffer, pH 9.2, 30°C, 30 kV, absorbance detection at 254 nm. Peaks (a) and (b) indicate methanol and dichloromethane, respectively. Separation was done using a fused-silica capillary with an inlet to detector length of 50 cm and a total length of 57 cm (adapted from Ref. [41]). Peak numbers: (1) dibenz[*a*,*h*]anthracene, (2) naphthalene, (3) fluorene, (4) anthracene, (5) acenaphthene, (6) acenaphthylene, (7) chrysene, (8) phenanthrene, (9) benz[*a*]anthracene, (10) benzo[*k*]fluoranthene, (11) benzo[*a*]pyrene, (12) fluoranthene, (13) benzo[*b*]fluoranthene, (14) indeno[123-*cd*]pyrene, (15) pyrene, and (16) benzo[*ghi*]perylene.

in contaminated soil, analysis of these extracts perhaps represents one of the most difficult environmental matrices.

A recent study confirmed that the 16 EPA priority PAHs could be satisfactorily separated using a buffer containing 35 m*M* SBβCD and 15 m*M* MβCD in 50 m*M* borate, pH 9.2. However, under this operating condition, eight PAHs exhibited very close migration times to form a cluster, whereas a second cluster was formed by another three compounds [44]. In an attempt to improve separation, SBβCD was increased to 70 m*M* while decreasing MβCD to 10 m*M* to delay migration of most PAH components. With this buffer, 13 PAHs were well resolved, but unfortunately four others migrated as one new cluster. Attempts to introduce γ-CD (up to 10 m*M*), hydroxypropyl-β-CD (up to 10 m*M*) as well as urea (up to 5 m*M*) in the running buffer containing 35 m*M* SBβCD and 15 m*M* MβCD did not result in any improvement in separation resolution. The negative results obtained with γ-CD were somewhat surprising since one may expect that this eight-glucose cyclodextrin with its fairly large cavity (7.9 Å) should be able to interact with most of the 16 PAHs to improve separation. In contrast, better resolution was effected with 4 m*M* α-CD which in general was not expected to interact with the PAHs in view of its smaller cavity size of 4.9 Å. Therefore, one must concede that cyclodextrin-aided capillary electrophoresis is still a trial and error procedure and the interaction mechanism between cyclodextrins and PAHs is yet to be deciphered.

Recently, the dual MβCD and SBβCD system was also extended for the analysis of small aromatic hydrocarbons such as benzene, toluene, ethyl benzene and three xylene isomers [45]. In 80 m*M* phosphate buffer containing 15 m*M* SBβCD and 5 m*M* HPβCD, benzene, toluene, ethyl benzene and three xylene isomers were well resolved with number of theoretical plates well above 100 000, for 50 cm of effective length. Some halogenated benzenes were also observed to separate well from the BTEX components to indicate their suitability as an internal standard for BTEX analyses.

Native β-CD was used in conjunction with CMβCD or sulfated-βCD (SuβCD) to separate pyrene from six alkyl-substituted anthracenes and the results were compared with MEKC [46]. These seven PAHs (anthracene, 1-methylanthracene, 2-methylanthracene, 9-methylanthracene, 2-ethylanthracene, 9,10-dimethylanthracene and pyrene) were completely separated using a buffer containing 5 m*M* SuβCD, 1 m*M* β-CD, 10% methanol, pH 6. This dual CD system equipped with laser-induced fluorescence provided detection limits in the low-to-sub-parts per million range. Although applicable, less satisfactory separation was observed when 5 m*M* SuβCD was replaced with 6 m*M* CMβCD. However, these seven PAHs could not be resolved using MEKC with a buffer containing 20 m*M* SDS, 10% methanol and 10 m*M* γ-CD, pH 9.3.

Although less common, the negatively charged SBβCD was used together with a surfactant for analysis of impurities in heroin [47]. The separation via MEKC (42.5 m*M* SDS and 15% acetonitrile in 8.5 m*M* phosphate and 8.5 m*M* borate, pH 9) of acidic and neutral phenanthrene-like impurities in illicit heroin was significantly improved by adding either a neutral β-CD (6.5 m*M*) or a negatively charged β-CD, 6.5 m*M* SBβCD to the running buffer. Although similar selectivity changes occurred with either CD, there was a significant increase in resolution for SBβCD vs. β-CD, particularly for the hydrophobic compounds eluting after 20 min. However with SBβCD, the latest peak only emerged after 42 min instead of 26–27 min as observed when separations were carried out using SDS alone or in combination with 6.5 m*M* β-CD. The addition of β-CD to the buffer containing SDS only resulted in a slight decrease in migration times for most solutes.

6. Discussion and concluding remarks

In food, pharmaceutical, and environmental analysis, measurement of trace levels of both charged and uncharged compounds is important and there is always a continuing need to develop better and faster separations using less and less materials. Besides the importance of cyclodextrins in chiral separation, there is little doubt that various forms of CE using cyclodextrins will be developed and become one of the most useful separation techniques to address ‘real world’ problems in achiral separation and detection of small and neutral compounds.

Solid-phase microextraction has been coupled with

CD-modified capillary electrophoresis to improve detection limit [44]. In brief, a glass fiber with an appropriate coating is used for absorbing target analytes from diluted samples. The glass fiber is then connected to a separation capillary via an adapter and the absorbed analytes are directly released into the CE buffer stream. On the basis of this principle, a recent study illustrated that a glass fiber could be prepared and used for absorbing 16 EPA priority PAHs from diluted samples until equilibrium was reached. After connecting the glass fiber to a separation capillary, the absorbed analytes were directly released into the CE buffer stream and electrophoretic separation was effected using a 50 m*M* borate buffer, pH 9.2, containing 35 m*M* SBβCD, 10 m*M* MβCD and 4 m*M* α-CD. Under 30 kV applied potential, separation was achieved in less than 15 min with high resolution and theoretical plates. Pyrene as low as 8 ppb was detected while the highest limit of detection was 75 ppb for acenaphthene [44]. Very satisfactory reproducibility with respect to migration time and peak area was obtained for repetitions using the same separation capillary and adapter, whereas only the extraction fiber was discarded after each analysis.

Electrochemical detectors, which promise high sensitivity, simplicity and low cost, have been coupled with CE to detect carbohydrates, amino acids, and neurotransmitters. Recently, a simple end-column amperometric detector that is compatible with a commercial CE instrument was realized for separation of chlorophenols [48]. To minimize electrode fouling due to phenol oxidation, as well as to improve detection sensitivity, metallic tin is electrochemically deposited on the platinum surface of the detecting electrode. The modified electrode (0.127 mm in diameter) in combination with a 20-μm I.D. separation capillary yielded sharp peaks and enabled the detection of phenol as low as 0.10 μ*M*. To date, none of the commercially available CE systems has been equipped with an electrical detector.

Capillary electrophoresis is still evolving as a separation technique for analysis of a variety of analytes of varying complexity and cyclodextrin-modified CE is a rapidly growing area within capillary electrophoresis, largely due to its capability to simultaneously separate charged, neutral, and chiral solutes in a very efficient manner. To date, a wide range of cyclodextrins have been developed and can be used in CE to resolve both chiral and achiral compounds. More widely and commercially available neutral cyclodextrins can only separate neutral compounds unless they are added as buffer additives together with a micelle agent or a charged cyclodextrin. Negatively charged cyclodextrins can effectively resolve both neutral and charged small molecules except that there is only a limited choice of charged cyclodextrins. It is anticipated that the development and commercialization of various cyclodextrin derivatives will grow exponentially to enable widespread applications of these elegant buffer additives in CE analysis.

References

[1] J. Szejtli, Cyclodextrins and their Inclusion Complexes, Akademjai Kiado, Budapest, 1982.
[2] K. Takeo, T. Kuge, Agric. Biol. Chem. (Tokyo) 33 (1969) 1174.
[3] K. Flohr, R.M. Paton, E.T. Kaiser, J. Am. Chem. Soc. 97 (1975) 1209.
[4] S. Zhao, J.H.T. Luong, Chem. Commun. 19 (1994) 2307.
[5] K.B. Male, R.S. Brown, J.H.T. Luong, Enzyme Microb. Technol. 17 (1995) 607.
[6] J. Emert, R. Breslow, J. Am. Chem. Soc. 97 (1975) 173.
[7] E. Albers, B.W. Muller, Crit. Rev. Ther. Drug Carrier Syst. 1 (1995) 311.
[8] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 603 (1992) 235.
[9] J. Liu, K.A. Cobb, M. Novotny, J. Chromatogr. 519 (1990) 189.
[10] S.K. Yeo, C.P. Ong, S.F.Y. Li, Anal. Chem. 63 (1991) 2222.
[11] H. Stuppner, M. Ganzera, J. Chromatogr. A 717 (1995) 271.
[12] J. Liu, S.F.Y. Li, J. Liq. Chromatogr. Rel. Technol. 19(11) (1996) 1697.
[13] S. Razee, A. Tamura, T. Masujima, J. Chromatogr. A 715 (1995) 179.
[14] S. Suzuki, M. Shirao, M. Aizawa, H. Nakazawa, K. Sasa, H. Sasagawa, J. Chromatogr. A 680 (1994) 541.
[15] J.-Y. Zhao, P. Thibault, M.A. Quilliam, Electrophoresis 18 (1997) 268.
[16] K.D. Altria, J. Chromatogr. A 735 (1996) 53.
[17] W. Baeyens, G. Weiss, G. Van Der Weken, W. Van Den Bossche, J. Chromatogr. 638 (1993) 319.
[18] D.K. Bempong, I.L. Honigberg, N.M. Meltzer, J. Pharm. Biomed. Anal. 11 (1993) 829.
[19] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Applications, Elsevier, Amsterdam, 1992, p. 321.
[20] Y.S. Yoo, Y.S. Kim, G.-J. Jhon, J. Park, J. Chromatogr. A 652 (1993) 431.
[21] J. Snopek, H. Soini, M. Novotny, E. Smolkova-Keulemansova, I. Jelinek, J. Chromatogr. 559 (1991) 215.

[22] Y.-Z. Hsieh, H.-Y. Huang, J. Chromatogr. A 745 (1996) 217.
[23] K.-S. Whang, C.-W. Whang, Electrophoresis 18 (1997) 241.
[24] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[25] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 59 (1985) 834.
[26] H. Nishi, T. Fukuyama, S. Terabe, J. Chromatogr. 553 (1991) 503.
[27] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexandra, D.G. Patterson, B.L. Karger, K. Hosaya, N. Tanaka, J. Chromatogr. 516 (1990) 23.
[28] S. Kobayashi, T. Ueda, M. Kikumoto, J. Chromatogr. 480 (1989) 179.
[29] J. Grainger, P. McClure, Z. Liu, B. Botero, S. Sirimanne, D.G. Patterson, K. Kimata, K. Hosoya, T. Araki, N. Tanaka, S. Terabe, Chemosphere 12 (1996) 13.
[30] C.P. Ong, C.L. Ng, H.K. Lee, S.F.Y. Li, J. Chromatogr. 547 (1991) 419.
[31] C.P. Ong, C.L. Ng, H.K. Lee, S.F.Y. Li, J. Chromatogr. 588 (1991) 335.
[32] C. Fernandez, G. Egginger, I.W. Wainer, D.K. Lloyd, J. Chromatogr. B 677 (1996) 363.
[33] K.C. Chan, G.M. Muchik, H.J. Issaq, P. Siiteri, J. Chromatogr. A 690 (1995) 149.
[34] C.L. Ng, C.P. Ong, H.K. Lee, S.F.Y. Li, J. Chromatogr. A 680 (1994) 579.
[35] S. Terabe, H. Ozaki, K. Otsuka, T. Ando, J. Chromatogr. 332 (1985) 211.
[36] C. Groom, J.H.T. Luong, Electrophoresis 18 (1997) 1166.
[37] S. Nie, R. Dadoo, R.N. Zare, Anal. Chem. 65 (1993) 3571.
[38] S. Terabe, Y. Miyashita, Y. Ishihama, O. Shibata, J. Chromatogr. 636 (1993) 47.
[39] M.J. Sepaniak, C.L. Copper, K.W. Whitaker, V.C. Anigbogu, Anal. Chem. 67 (1995) 2037.
[40] O.H.J. Szolar, R.S. Brown, J.H.T. Luong, Anal. Chem. 67 (1995) 3004.
[41] J. Gorse, A.T. Balchunas, D.F. Swaile, M.J. Sepaniak, J. High Resolut. Chromatogr. Chromatogr. Commun. 11 (1988) 554.
[42] M.J. Sepaniak, A.C. Powell, D.F. Swaile, R.O. Cole, in: P.D. Grossman, J.C. Colburn (Eds.), Capillary Electrophoresis, Theory and Practice, Academic Press, San Diego, CA, 1992, pp. 159–189.
[43] R.S. Brown, J.H.T. Luong, O.H.J. Szolar, A. Halasz, J. Hawari, Anal. Chem. 68 (1996) 287.
[44] A.-L. Nguyen, J.H.T. Luong, Anal. Chem. 69 (1997) 1726.
[45] A.-L. Nguyen, J.H.T. Luong, Electrophoresis 18 (1997) 247.
[46] K.W. Whitaker, C.L. Copper, M.J. Sepaniak, J. Microcol. Sep. 8 (1996) 461.
[47] I.S. Lurie, K.C. Chan, T.K. Spratley, J.F. Casale, H.J. Issaq, J. Chromatogr. B 669 (1995) 3.
[48] A. Hilmi, J.H.T. Luong, A.-L. Nguyen, J. Chromatogr. A 761 (1997) 259.