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Review

Separation selectivity in chiral and achiral capillary electrophoresis with mixed cyclodextrins

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Abstract

The use of mixed cyclodextrins (CDs) as run buffer additives for capillary electrophoresis (CE) is reviewed. Mixtures of neutral CDs or neutral and charged CDs are advantageous for both chiral and achiral separations. The unique selectivities offered by these novel combinations allow separations that are often problematic to obtain by other CE techniques. © 1997 Elsevier Science B.V.

Keywords: Buffer composition; Enantiomer separation; Reviews; Selectivity; Cyclodextrins

Contents

1. Introduction

Cyclodextrins (CDs), which consist of 6 (α-), 7 (β-) or 8 (γ-) glucopyranose units linked together by α (1→4) linkages, are now widely used as run buffer additives for CE analyses [1]. Their use is advantageous because they provide excellent selectivities, especially for chiral separations. Separations are enhanced due to the multitude of possible differential interactions between the solutes and CDs, including hydrophobic, hydrophilic and steric. CDs consist of a relatively hydrophobic cavity and polar exterior (the latter consisting of primary and secondary hydroxyl groups.) Steric effects can predominate, depending on the size of the CD (i.e. the number of glucopyranose units). In addition, the exterior hydroxyl groups can be chemically modified to produce altered neutral or anionically or cationically charged CDs. Derivatized, neutral CDs are useful for their increased solubilities in buffer solutions (as well as modified selectivities), while charged CDs are useful for separations of neutral solutes (owing to their

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migration counter to osmotic flow). In addition, counter migration can lead to significantly increased resolution of charged compounds.

In many cases, however, the addition of a neutral or charged CD alone does not adequately resolve the solutes of interest. In these cases, the use of mixtures of CDs have proven highly successful, due in large part to their unique and easily varied selectivities [2–14]. Neutral CDs have been employed in combination with either other neutral [2,3] or charged CDs [4–14] for both chiral [2–8] and achiral separations [9–14]. Applications for chiral analyses include anionic binapthyl compounds [2], anionic derivatives of phenoxy acid herbicides [3], cationic drugs of forensic interest [4,5] and acidic drugs in their neutral forms [6–8]. For achiral separations, mixed CDs have been applied to the analyses of polycyclic aromatic hydrocarbons (PAHs) [9–12] and isomeric impurities present in illicit cocaine [13,14].

2. Chiral separations

Although neutral CDs have been widely used for chiral separations, determining the optimal experimental conditions can be time consuming. Enantiomeric selectivity depends not only on the size of the CD (i.e. the number of glucopyranose units) and the type of chemical modification of the primary and secondary hydroxyl groups, but also on the relative concentration of the CD. Other factors include ionic strength, buffer type, pH, organic modifiers and temperature. Determining the ''best'' CD and correct CD concentration for a given solute mixture can therefore involve a multitude of experiments.

Charged CDs have the capability of providing increased chiral recognition for oppositely charged solutes, and therefore the potential of more rapid method development. In some cases, however, a charged CD may provide little or no enantiomeric selectivity. In others, charged CDs may provide strong enantiomeric selectivity; however, the solutes may never migrate past the detector, due to a combination of the high mobility of the solute/charged CD complex counter to osmotic flow, and the development of strong inclusion complexes.

The addition of a second CD to the run buffer can therefore provide a powerful means for rapidly obtaining satisfactory enantiomeric resolution. The additional CD can either provide enantioselectivity in itself, compete with a charged CD to allow detection of an already enantiomerically resolved mixture (i.e. to permit migration of the solute(s) past the detector), or provide a differential migration rate.

Mixed CDs have also been used to analyze combinations of enantiomeric solutes where each of the individual CDs resolved only some of the compounds of interest [2–4]. Mixtures of neutral CDs or neutral and charged CDs have both been used for this purpose. For example, Nishi [2] resolved a mixture of three racemic anionic binaphthyl compounds using a combination of α-CD and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD). Only the enantiomers of 1,1′-binaphthyl-2,2′-dicarboxylic acid (BNC) were resolved using 20 m*M* α-CD alone (Fig. 1A). In another example using 20 m*M* TM-β-CD, the enantiomers of 1,1′-bi-2-naphthol (BN) and 1,2′-binaphthyl-2,2′-diyl hydrogenphosphate (BNP) were resolved, but not from each other (Fig. 1B). However, all 6 enantiomers of BNC, BN and BNP were resolved using a mixture of 10 m*M* α-CD+10 m*M* TM-β-CD (Fig. 1C). This same enantiomeric mixture was also resolved using a mixture of 10 m*M* β-CD phosphate and 10 m*M* α-CD [2]. In this latter case, in separate experiments, BN and BNP were resolved using β-CD phosphate, while BNC was separated using α-CD.

In another example, Mechref and El Rassi [3] separated a mixture of the 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) derivatives of enantiomeric phenoxy acid herbicides using mixtures of β-CD and TM-β-CD. In this case, Silvex 2-(2,4,5-trichlorophenoxy)propionic acid, 2-(4-chlorophenoxy)-propionic acid (2,4-CPPA), 2-(3-chlorophenoxy)-propionic acid (2,3-CPPA) and 2-phenoxypropionic acid (2-PPA) were enantiomerically separated using 5 m*M* β-CD (Fig. 2a), while Silvex, 2-(4-chloro-2-methylphenoxy)propionic acid (Mecoprop), 2-(2,4-dichlorophenoxy)propionic acid (Dichlorprop), 2,4-CPPA and 2-PPA were enantiomerically resolved with 5 m*M* TM-β-CD (Fig. 2b); as shown in Fig. 2c, all enantiomeric pairs (except Silvex) were resolved. Using a mixture of 5 m*M* β-CD and 30 m*M* TM-β-CD, all enantiomeric pairs were also separated; however, the enantiomers of dichlorprop and 2,4-CPPA co-eluted (Fig. 2e).

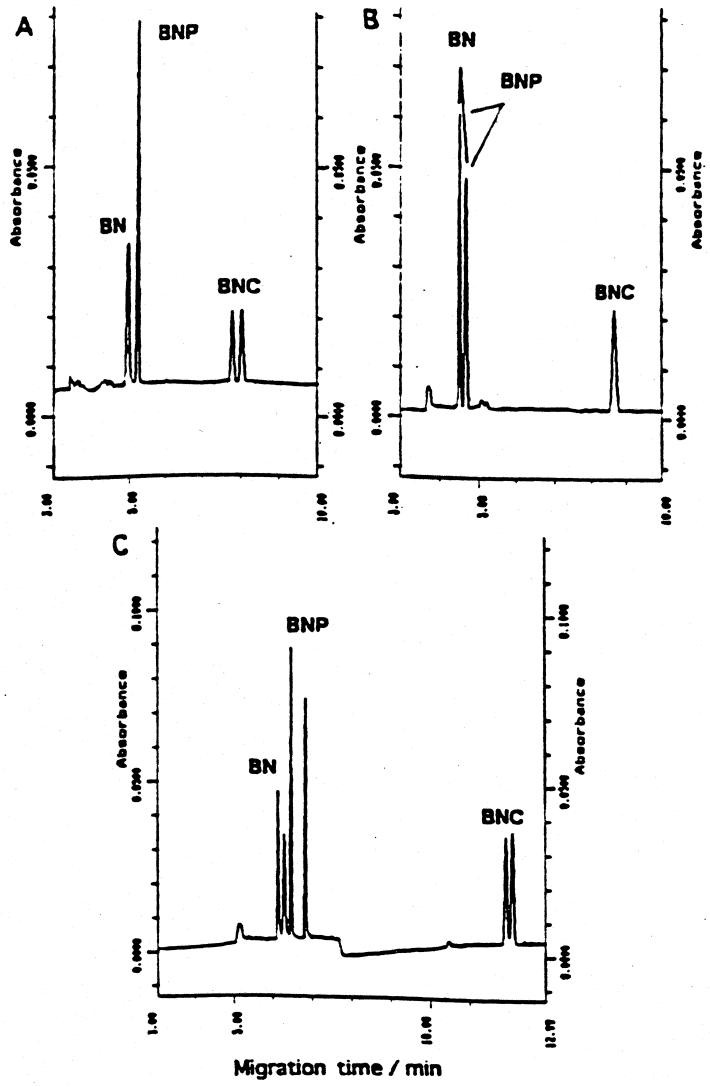

Fig. 1. Separation of binapthyl enantiomers by CD-CZE with the uncoated capillary. Conditions: buffer, 25 m*M* phosphate pH 10.5 containing: (A) 20 m*M* α-CD; (B) 20 m*M* TM-β-CD; (C) 10 m*M* α-CD+10 m*M* TM-β-CD. Separation tube, 40 cm (effective length 33 cm)×75 μm I.D., uncoated capillary: applied voltage, +20 kV: detection, 214 nm: temperature, 23°C. From Ref. [2].

Lurie et al. [4] demonstrated the utility of using mixtures of the neutral CD heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD) and the charged CD β-cyclodextrin sulfobutyl ether IV (β-CD-SBE(IV)) for obtaining chiral resolution of a number of cationic primary amine drugs of forensic interest. For the primary amines, 5 m*M* DM-β-CD alone failed to adequately resolve (±)-amphetamine or (−)-norpseudoephedrine from (−)-norephedrine (Fig. 3A). In addition, (±)-norephedrine, co-eluted when using 1 m*M* β-CD-SBE(IV) alone (Fig. 3B). However, a mixture of 5 m*M* DM-β-CD plus 1 m*M* β-CD-SBE(IV) resolved all solutes except (±)-cathinone (Fig. 3C). The inability to resolve this latter solute was not significant, however, since (±)-cathinone has (to date) never been clandestinely manufactured. For a mixture of the secondary amines (±)-ephedrine, (±)- pseudoephedrine, (±)-meth-

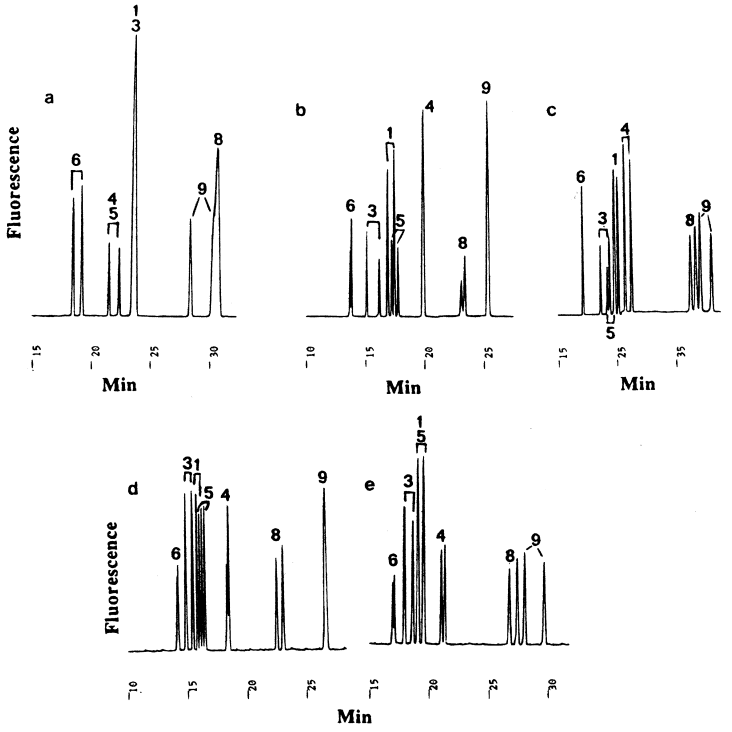

Fig. 2. Electropherograms of ANDSA-phenoxy acid herbicides by CD-CZE mixed CD-CZE using lamp-operated fluorescence detector (excitation 315 nm). Running electrolyte, 25 m*M* sodium phosphate, 600 m*M* borate, pH 5.0 containing: (a) 5 m*M* β-CD, (b) 5 m*M* TM-β-CD, (c) 5 m*M* of each TM-β-CD and β-CD, (d) 30 m*M* TM-β-CD and (e) 30 m*M* TM-β-CD and 5.0 m*M* β-CD. Capillary, 80 cm (50 cm to detection)×50 μm I.D.; voltage, 20 kV. Peaks: (1) dichlorprop, (3) mecoprop, (4) 2,3-CPPA, (5) 2,4-CPPA, (6) silvex, (8) 2,2-CPPA, (9) 2-PPA. From Ref. [3].

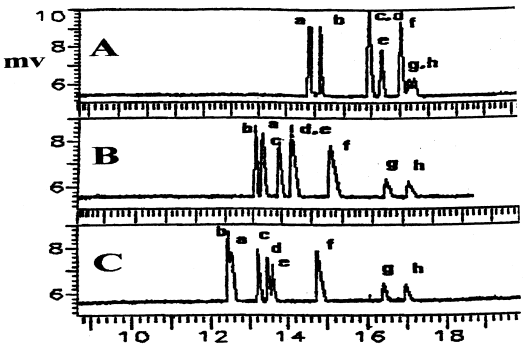

Fig. 3. Electropherograms of primary amine standards. Conditions: (A) 1.2% methanol, 98.8% 5 m*M* DM-β-CD; (B) 1.2% methanol, 98.8% 1 m*M* β-CD-SBE(IV); (C) 1.2% methanol, 98.8% 5 m*M* DM-β-CD and 1 m*M* β-CD-SBE(IV). Capillary, 82 cm (60 cm effective length)×50 μm I.D.; voltage 30 kV; detection UV 210 nm; temperature 30°C. Key (a) (+)-cathinone, (b) (−)cathinone, (c) (−)-norpseudoephedrine, (d) (−)-norephedrine, (e) (+)norephedrine, (f) (+)-norpseudoephedrine, (g) (−)-amphetamine, (h) (+)-amphetamine.

cathinone and (±)-methamphetamine, both 5 m*M* DM-β-CD and 1 m*M* β-CD-SBE(IV) alone adequately resolved the solutes of interest; however, a mixture of 5 m*M* DM-β-CD plus 1 m*M* β-CD-SBE(IV) gave an improved separation.

In other cases, mixed CDs gave improved enantiomeric separations when both of the individual CDs did not [4–8]. For example, Sepaniak et al. [6] was unable to resolve (±)-aminoglutethimide in its neutral form using the charged CD carboxymethyl-β-cyclodextrin (CM-β-CD). Use of a neutral CD alone (e.g., β-CD) also provided no enantiomeric resolution for these neutral solutes, since the solutes in both free solution and complexed forms migrated with the osmotic flow. However, (±)-aminoglutethimide was partially resolved using a mixture of 5 m*M* CM-β-CD and 1 m*M* β-CD. Apparently, β-CD provided enantioselectivity, while CM-β-CD provided a differential migration rate (which allowed

the separation to occur), This separation was also greatly improved by the addition of 50% methanol to the run buffer, which extended the elution window. Since the separation was performed at pH 9.0, a rapid enough osmotic flow still existed for reasonable migration times (less than 14 min).

Similarly Lelievre et al. [7] obtained baseline resolution for the arylpropionic acids calprofen, flurbiprofen, ketoprofen, naproxen and suprofen with a 20 m*M* amino-β-cyclodextrin (β-CD-NH_2)/TM-β-CD system at pH 2.3. The charged CD alone provided no enantioselectivity; in addition, the solutes are almost completely protonated at this pH, and the neutral CD alone therefore also provided no enantioselectivity. However, for the dual system, the positively charged CD (which migrated with osmotic flow) provided the differential migration rate which allowed the separation to occur. In addition, the positively charged CD allowed for reasonable migration times.

In an analogous situation, Fillet et al. [8] obtained vastly improved enantioresolution for various acidic drugs using a mixture of charged and neutral CDs (Table 1). At pH 3.0, the analytes are essentially uncharged, and are therefore not separated using neutral CDs. In this instance, the charged CD [β-CD-SBE(IV)] provided some enantioselectivity (which was vastly improved with the addition of the neutral CD.) Of the neutral CDs investigated (β-CD, methylated-β-cyclodextrin (M-β-CD), DM-β-CD, TM-β-CD and hydroxypropyl-β-cyclodextrin (HP-β-CD), DM-β-CD provided the best enantioselectivity.

For each of these systems, triethanolamine was added to provide a weak anodic electroosmotic flow; thus, the anionic CD moved with the osmotic flow. A dual CD system consisting of 7.5 m*M* β-CD-SBE(IV) and 30 m*M* TM-β-CD provided high resolution values and analysis times of under 10 min.

In another example, tertiary amine drugs such as (±)-propoxyphene and (±)-methorphan were not resolved using 5 m*M* DM-β-CD [4,5]. In addition, no peaks were observed with 1 m*M* β-CD-SBE(IV) for either solute. However, both enantiomeric pairs were separated using a mixture of 5 m*M* DM-β-CD and 1 m*M* β-CD-SBE(IV). Better separations in terms of improved peak shapes and greater speed of analysis were obtained by using 5 m*M* DM-β-CD and 0.5 m*M* β-CD-SBE(IV). This example illustrates a major drawback of the use of charged CDs such as β-CD-SBE(IV) (average degree of substitution 4), especially at low pH's; that is, significant band broadening caused by electrodispersion [4,15]. This phenomena results from a mobility mismatch between the β-CD-SBE(IV)–drug complex and the background electrolyte. The addition of the neutral CD improved peak shapes by reducing the residence time of the solute in the charged CD. In this example, although resolution decreased when using the smaller concentration of β-CD-SBE(IV), baseline resolution was still obtained for the enantiomeric solutes.

In certain instances, an enantiomeric pair is resolved using an individual CD but not with a CD mixture [3,4]. This is illustrated in Fig. 2a–c for Silvex, and Fig. 3A–C for (±)-cathinone. This arises when the enantiomeric selectivities of the individual CDs oppose each other. For Silvex, a separation was obtained with the CD mixture by diminishing enantiomeric resolution for one of the chiral selectors (see Fig. 2d,e).

Interestingly, enantiomers may switch migration order when using mixed CDs versus one of the

Table 1
Influence of the addition of a neutral β-cyclodextrin in a buffer containing SPE on enantioresolution

	Enantiomeric resolution (R_s)					
	SBE	SBE/CD	SBE/MCD	SBE/DMCD	SBE/TMCD	SBE/HPCD
Sulindac	1.4	2.3	3.5	3.8	1.6	<0.7
Fenoprofen	<0.7	1.0	1.5	2.8	2.8	<0.7
Ketoprofen	1.1	1.5	2.1	4.6	2.1	1.3
Warfarin	2.2	5.1	9.2	9.3	4.3	2.7
Hexobarbital	1.7	0.8	1.2	3.0	1.9	<0.7

Buffer: 5 m*M* SBE in 100 m*M* phosphoric acid adjusted to pH 3.0 with triethanolamine containing no additional cyclodextrin or CD, MCD, DMCD, TMCD and HPCD (10 m*M*). Other conditions as described in Ref. [8].

individual CDs. This occurred for (±)-ephedrine, (±)-methcathinone and (±)-cocaine using the experimental conditions described in Fig. 3. In addition, although no peaks were observed for cocaine with 1.0 m*M* β-CD-SBE(IV), it is presumed that the charged CD exhibited enantioselectivity opposite to that of DM-β-CD.

Since enantioselectivity depends on the differences in the apparent mobilities of the individual enantiomers, it is pertinent to examine equations that formally describe this parameter. A better understanding of the factors that influence mobility allows insight as to why mixed CDs provide improved enantioselectivity for certain chiral separations. For a mixture of two CDs, the apparent mobility μ_a can be expressed as follows [4]:

$$\mu_a = (\mu_f + \mu_1 K_1[C_1] + \mu_2 K_2[C_2])/(1 + K_1[C_1] + K_2[C_2]) \quad (1)$$

where μ_f, μ_1 and μ_2 are the mobilities of the uncomplexed, first CD and second CD, respectively, and K_1, K_2, C_1, C_2 are the equilibrium constants for the inclusion complexes, CD concentrations, first (neutral), and second (neutral or charged) CDs, respectively. It should be noted that for neutral solutes, μ_f and $\mu_1 K_1[C_1]$ are zero.

Eq. (1) indicates that the increased enantioselectivity resulting from mixed CDs arises from differing *K* values for the individual enantiomers. For example, for the separation shown in Fig. 1A, K_1 (α-CD) differs for the enantiomers of BNC, but is identical for the enantiomers of BN and BNP. On the other hand, K_2 (TM-β-CD) differs for the enantiomers of BN and BNP, but is identical for the enantiomers of BNC. Thus, the use of both CDs resolves all three solutes.

To date, the choice of the individual CDs has been largely empirical. Obviously, the ability to better predict K_1 and K_2 would be extremely beneficial. To this end, molecular modelling and NMR [16] approaches appear promising.

In order to increase the likelihood that the K_2 values for individual enantiomers will differ from the K_1 values for the same enantiomers, it would appear to be more desirable to use a charged CD as the second additive. Not only will a charged CD provide counter migration (which will give a separation more time to occur), but it can provide additional selectivity (due to ionic interactions). The large difference in enantioselectivity for charged versus neutral CDs is evidenced by the reversal in elution order for certain enantiomers [4].

However, the K_2 values for certain charged complexes can be extremely large; in these cases, elution will occur only if a neutral CD is added. This effect can be explained by examining the numerator of Eq. (1). Elution toward the cathode will occur when:

$$\mu_f + \mu_1 K_1[C_1] > \mu_2 K_2[C_2]$$

For the examples presented earlier for (±)-propoxyphene, (±)-methorphan and (±)-cocaine, it appears that the primary effect of the neutral CD is not enantioselection, but rather modulation of migration toward the anode by the charged CD complex.

Lelievre et al. [7] derived two expressions which show the strong dependence of selectivity on the complex formation constants. Assuming 1:1 complexation and no mixed complexes for two enantiomers A and B, the selectivity of the second CD in the presence of a given concentration of the first CD can be described as follows:

$$\alpha = ([K_2(B)]/[K_2(A)])[(1 + K_1(A)[C_1])]/[1 + K_1(B)[C_1])]. \quad (2)$$

When $K_1[C_1] >> 1$, i.e., when the solute concentration in free solution is negligible compared to the concentration of complexed solute (a good approximation for un-ionized hydrophobic solutes), Eq. (2) can be re-written as:

$$\alpha = [(K_2(B)][K_1(A)]/[(K_2(A)][K_1(B)]. \quad (3)$$

It is apparent from Eq. (3) that for separation to occur, not only must $K_1(A) \neq K_1(B)$ or $K_2(A) \neq K_2(B)$, but $K_1(A) \neq K_2(A)$ and $K_1(B) \neq K_2(B)$ (i.e. the complexation characteristics of both CD phases must be different).

3. Achiral separations

For achiral separations, the greatest challenges involve the separation of uncharged hydrophobic

solutes and the differentiation of structurally similar compounds (such as positional isomers).

For example, hydrophobic solutes such as PAHs can be difficult to analyze via MECC, even in the presence of organic modifiers [17]. Although separation of these compounds can be obtained using CDs in combination with micelles, insufficient selectivity can be observed for structurally similar solutes [18]. Capillary electrochromatography (CEC) appears to be a promising approach for these separations, as shown by Yan et al. [19]; however, the number of theoretical plates that was obtained by CEC in this case was considerably less than that obtained by electrokinetic techniques. An excellent alternative to these approaches is the use of a dual CD system. A charged/neutral CD mixture not only allows the separation of neutral solutes, but also provides unique selectivity. In addition, a dual CD system can tolerate large amounts of organic solvent [6] (which can be problematic in MECC.)

Sepaniak et al. first demonstrated the utility of a dual CD phase for the separation of uncharged hydrophobic solutes [9]. In this capillary electrophoretic technique, which the author dubbed cyclodextrin distribution capillary electro-chromatography, a negatively charged CD (CM-β-CD) acted as a counter-migrating phase, while the neutral CD(s) (β-CD, γ-CD and HP-β-CD, which migrate with the osmotic flow) acted as competing phases. In this separation mode, the solubility of the solute in the background electrolyte (run buffer minus CD) is considered negligible.

The great utility of this technique is illustrated in Fig. 4, where the separation of anthracene, pyrene, chrysene and benzo[*a*]pyrene (B[*a*]P) via CD-modified MECC is compared with cyclodextrin distribution capillary electro-chromatography [9]. In contrast to the dual CD system, where all solutes were well resolved, the MECC-CD system was non-selective for pyrene, chrysene and B[*a*]P. The addition of γ-CD in addition to β-CD (in the dual CD system) was required to separate B[*a*]P and chrysene. In contrast to CD-MECC, there are no interactions between the various additives when dual CDs are used. Therefore, the effects of the individual CDs on the mixed system can be predicted a priori via single CD experiments, NMR experiments or molecular modelling.

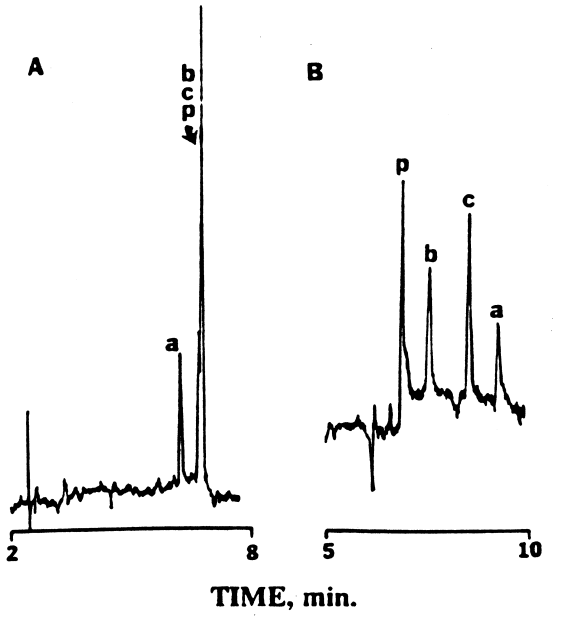

Fig. 4. Separations of (a) anthracene, (p) pyrene, (c) chrysene and (b) B[*a*]P) using standard capillary, 50 cm (40 cm effective length)×50 μm I.D.. Running buffer: (A) 50 m*M* SDS, 8 m*M* γ-CD, 8 m*M* β-CD, CD modified MECC system; and (B) 8 m*M* β-CD and 1 m*M* γ-CD: 10 m*M* CM-β-CD with 30% organic modifier, complex CD-CZE system. Separations performed at 20 kV at ambient temperature using laser induced fluorescence (LIF) detection (excitation 325 nm). From Ref. [9].

For the analysis of anthracene and pyrene using 10 m*M* CM-β-CD and a neutral CD, significantly greater selectivity was obtained when using 5.0 m*M* β-CD versus 5.0 m*M* HP-β-CD [9]. It is also interesting to note that the migration order of these solutes is reversed via MECC.

Szolar et al. [10] also used mixtures of a charged and neutral CDs for the separation of PAHs, including phenanthrene, anthracene, pyrene, chrysene, B[*a*]P and B[*e*]P. Two neutral CDs (HP-β-CD and M-β-CD) and two anionic CDs [CM-β-CD and β-CD-SBE(IV)] were employed in this study. The effects of varying the concentration of the charged CD with constant neutral CD is shown in Fig. 5 and Fig. 6, respectively. As expected, the neutral CD alone did not resolve any of the neutral PAHs. In contrast, the migration times and overall selectivity increased with higher concentrations of either CM-β-CD or β-CD-SBE(IV). The order of migration in the HP-β-CD/CM-β-CD system was approximately cor-

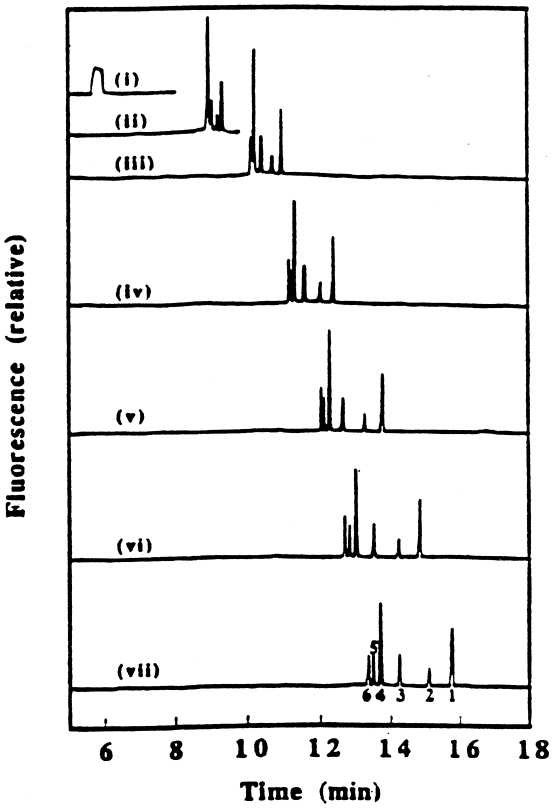

Fig. 5. Electropherograms of PAH mixture at 23 kV, 20°C in pH 9 buffer containing 50 m*M* borate and 30 m*M* HP-β-CD with CM-β-CD concentrations of (i) 0, (ii) 15, (iii) 25, (iv) 35, (v) 45, (vi) 55 and (vii) 60 m*M*. Capillary 57 cm (50 cm effective length)×50 μm I.D. with LIF detection (excitation 325 nm). Peak labels correspond to (1) anthracene, (2) phenanthrene, (3) chrysene, (4) pyrene, (5) B[*a*]P and (6) B[*e*]P. From Ref. [10].

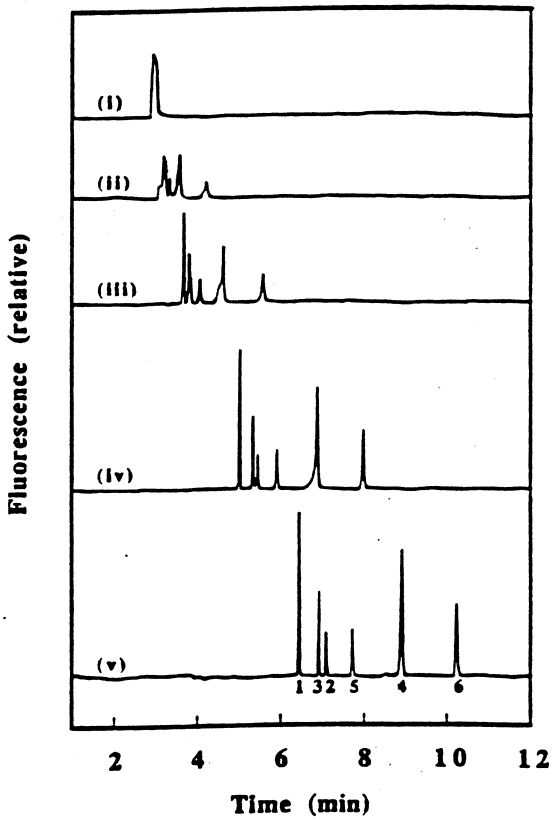

Fig. 6. Electropherograms of PAH mixture at 30 kV, 30°C in pH 9 buffer containing 50 m*M* borate and 20 m*M* M-β-CD with β-CD-SBE(IV) concentrations of (i) 0, (ii) 1.5, (iii) 5, (iv) 15 and (v) 25 m*M*. For additional experimental conditions and identity of peaks, see Fig. 5. From Ref. [10].

related with molecular size, i.e. the largest solutes migrated faster. This indicated that the larger PAHs favored HP-β-CD over CM-β-CD. In contrast to these results, a different migration order for the PAHs was obtained for the M-β-CD/β-CD-SBE(IV) combination (see Figs. 5 and 6). The selection of HP-β-CD and M-β-CD was serendipitous, since replacing either CD decreased resolution. Since a smaller concentration of β-CD-SBE(IV) versus CM-β-CD is required for the resolution of these solutes, the former CD is preferred because of the lower current generated.

Brown et al. [11] used a mixture of 35 m*M* β-CD-SBE(IV) and 15 m*M* M-β-CD to resolve a mixture of 16 US Environmental Protection Agency (EPA) priority PAHs in under 20 min. It is of interest to note that this same mixture required nearly 60 min for an equivalent resolution using CD-modified MECC [20], and well over an hour using CEC [19]. In a subsequent experiment, Brown et al. [11] used identical conditions for the separation of 11 of the EPA priority PAHs, except that a mixture of 25 m*M* β-CD-SBE(IV) and 20 m*M* M-β-CD was used. The lower concentration of the charged CD allowed for a reduced current and faster migration times.

Whitaker et al. [12] compared the separation of various alkyl-substituted anthracenes on two dual CD systems and a CD-modified MECC system (see Fig. 7). The MECC system was unable to resolve the mixture; however, both dual CD systems (i.e. 6 m*M* CM-β-CD/1 m*M* β-CD or 5 m*M* sulfated β-CD

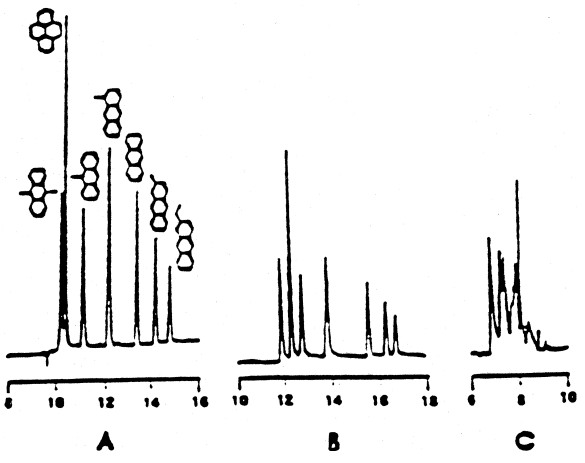

Fig. 7. Separation of various PAHs (structures shown) with (A) running buffer (pH 6) containing 30% methanol with 6 m*M* CM-β-CD and 1 m*M* β-CD; (B) same as (A), with CM-β-CD replaced with 5 m*M* Su-β-CD; and (C) pH 9.3, 10% methanol, 20 m*M* SDS with 10 m*M* γ-CD. Capillary 50 cm(40 cm effective length)×50 μm I.D., voltage 15 kV at ambient temperature; LIF detection (excitation 325 nm). From Ref. [12].

(Su-β-CD)/1 m*M* β-CD) resolved the solute mixture. CM-β-CD and Su-β-CD gave similar selectivities, with the former additive giving shorter migration times. In this example, the charged CD alone resolved the solute mixture, while the neutral CD served to reduce migration times and improve efficiency. This same study used molecular modelling to predict the cavity geometry of CM-β-CD, which has a significant effect on the selectivities observed in Fig. 7.

Capillary zone electrophoresis (CZE) is not viable for charged compounds with similar charge-to-size ratios. Although MECC and cyclodextrin-modified capillary zone electrophoresis (CD-CZE) with single CD additives are useful alternatives for the analysis of these solutes, the obtainable selectivities are not always optimal. The use of dual CD additives can provide the additional selectivity needed for these difficult separations.

Lurie et al. [13] employed a mixture of β-CD and β-CD-SBE(IV) for the baseline resolution of a mixture of 5 truxilline diastereomers. These di-cationic impurities, which are present in illicit cocaine, were not fully resolved using either MECC or CD-CZE with a run buffer containing only a single CD (charged or uncharged). Additional work was performed on the effect of using different neutral CDs with the charged CD β-CD-SBE(IV). The neutral CDs included α-CD, β-CD, γ-CD, DM-β-CD and TM-β-CD. Significant selectivity changes were observed, varying on the neutral CD used. For the CDs examined, the best results were obtained with β-CD.

In a related study by Lurie [14], a mixture of 6- and 7-hydroxycocaine endo, exo, isomers were fully resolved using a mixture of DM-β-CD and β-CD-SBE(IV). These singly charged cationic impurities, which are also found in illicit cocaine, were not baseline resolved using either MECC or CD-CZE with a run buffer containing only a single CD (neutral or anionic.) In addition, using β-CD-SBE(IV) alone, the 6- and 7-hydroxy cocaine endo isomers do not migrate toward the cathode.

As was the case for chiral solutes, the selectivity effects of the mixed CDs on these solutes can be explained by examining Eq. (1). Again, the magnitudes of K_1 and K_2 for the individual solutes are the critical parameters effecting selectivity.

For neutral hydrophobic solutes, if the aqueous concentration of the solute is considered negligible, and the mobility of the neutral CD is considered zero, the following expression can be written [10]:

$$\mu_{nh} = \mu_2 K_i [C_2]^{\omega_2} / ([C_1]^{\omega_1} + K_i [C_2]^{\omega_2}) \quad (4)$$

where μ_{nh} is the mobility of the neutral hydrophobic solute, μ_2 is the mobility of the charged complex, C_1 and C_2 are the concentrations of the neutral and charged CDs, respectively, ω is the stoichiometry of the respective inclusion complexes, and K_i is the ratio of the inclusion complexes, for each CD (i.e. $K_i = K_2/K_1$). From Eq. (4), it is readily apparent that K_i is the fundamental parameter effecting selectivity.

Eq. (4) can be rearranged to give:

$$[C_2]^{\omega_2}/\mu_{nh} = [C_2]^{\omega_2}/\mu_2 + [C_1]^{\omega_1}/\mu_2 K_i \quad (5)$$

so that a plot of $[C_2]^{\omega_2}/\mu_{nh}$ vs. $[C_2]^{\omega_2}$ can be employed to determine K_i for each solute in the electropherogram. K_i values were determined for individual PAHs from the experimental data shown in Fig. 5 and Fig. 6. A fit of the data obtained with various values of ω_1 and ω_2 indicated that a stoichiometry of 1:1 gave the best results. The results obtained for the K_i values indicated that PAHs prefer β-CD-SBE(IV) over the neutral CD over CM-β-CD.

It was concluded that inclusion into CM-β-CD is related to molecular size, while β-CD-SBE(IV) inclusion is more sensitive to molecular configuration.

For the achiral separation of two solutes, Eq. (2) and Eq. (3) can also be used to derive the strong dependence of selectivity on the complex formation constants. Eq. (3) predicts that a separation occurs for hydrophobic neutral solutes only when both phases complex differently; this is consistent with the observation of Sepaniak et al. [9].

Recently, Peng et al. [21] reported a quantitative description of migration behavior when one or more additives are present in the run buffer. Their model used a viscosity correction factor, which converts all mobilities to an ideal state where the viscosity remains constant.

4. Conclusions

Because of the unique selectivities obtainable and the ability to analyze neutral hydrophobic compounds, the use of mixed CDs as run buffer additives is highly advantageous for CE analyses. At present, there are a good selection of neutral CDs and an increasing number of charged CDs (both anionic and cationic) available. Proper choice of CDs (ideally by predictive means) allows careful control of selectivity and migration times. The use of charged CDs containing isolated charges would aid in separation control [15]. Finally, employing CDs with smaller charges could give better peak shapes because of lower electrodispersion.

5. Abbreviations

ANDSA	7-Aminonaphthalene-1,3-disulfonic acid
B[*a*]P	Benzo[*a*]pyrene
B[*e*]P	Benzo[*e*]pyrene
β-CD-NH_2	Amino β-cyclodextrin
β-CD-SBE(IV)	β-Cyclodextrin sulfobutyl ether IV
BN	1,1′-Bi-2-naphthol
BNC	1,1′-Binaphthyl-2,2′-dicarboxylic acid
BNP	1,2′-Binaphthyl-2,2′-diyl hydrogenphosphate
CM-β-CD	Carboxymethyl-β-cyclodextrin
CD	Cyclodextrin
CD-CZE	Cyclodextrin-modified capillary zone electrophoresis
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
2,3-CPPA	2-(3-Chlorophenoxy)propionic acid
2,4-CPPA	2-(4-Chlorophenoxy)propionic acid
CZE	Capillary zone electrophoresis
Dichlorprop	2-(2,4-Dichlorophenoxy)propionic acid
DM-β-CD	Heptakis(2,6-di-O-methyl)-β-cyclodextrin
EPA	US Environmental Protection Agency
HP-β-CD	Hydroxypropyl β-cyclodextrin
LIF	Laser induced fluorescence
M-β-CD	Methylated β-cyclodextrin
MECC	Micellar electrokinetic capillary chromatography
Mecoprop	2-(4-Chloro-2-methylphenoxy)-propionic acid
NMR	Nuclear magnetic resonance
2-PPA	2-Phenoxypropionic acid
PAH	Polycyclic aromatic hydrocarbon
SDS	Sodium dodecyl sulfate
Silvex	2-(2,4,5-Trichlorophenoxy)propionic acid
SU-β-CD	Sulfated β-cyclodextrin
TM-β-CD	Heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin

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