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Mechanism of capillary electrophoresis enantioseparations using a combination of an achiral crown ether plus cyclodextrins

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Abstract

The addition of an achiral crown ether (18-crown-6) to a cyclodextrin-based separation can significantly affect the capillary electrophoresis (CE) enantioresolution of organic racemates that contain a primary amine functional group. In most cases an enhancement of the enantioseparation was observed. However, there are also cases where the addition of 18-crown-6 was detrimental to a cyclodextrin-based CE enantioseparation. The effect of concentration of the two complexing additives as well as the effect of pH and added potassium ion were examined. A specific three-body complex involving simultaneous, dual inclusion complex formation can be used to explain both the enhanced and diminished enantioselectivities observed when 18-crown-6 is added to the run buffer. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Buffer composition; Chiral selectors; Crown ethers; Amines

1. Introduction

Capillary electrophoresis (CE) has proven to be an effective technique for the resolution of a variety of enantiomeric molecules [1–9]. Originally, CE enantioseparations simply followed the lead of liquid chromatography (LC) in that the same chiral selectors were used to resolve the same or analogous racemic analytes. However, the most common and effective LC approach for the resolution of enantiomers is through the use of chiral stationary phases (CSPs), while the most common CE approach involves dissolving the chiral selector in the run buffer [1–9]. This basic difference in the two approaches has resulted in a somewhat different evolution of chiral selectors used in these methods, even though their ultimate goal (to effectively resolve a greater number and variety of enantiomers) often is the same. The solubility, charge and UV absorbance of chiral selectors used as a part of LC CSPs is not as important a consideration as it is for CE run buffer additives. Consequently many of the chiral selectors used in LC are not used in CE. This can greatly limit the number and type of compounds that are resolved by CE. The most effective classes of chiral selectors for CE enantioseparations have been cyclodextrins (CDs) [1–8], macrocyclic antibiotics [1,9–22] and chiral crown ethers [23–29]. The crown ethers have the most limited selectivity of these three classes of chiral selectors. Chiral crown ethers can be used to resolve enantiomers that contain primary amine functional groups [30,31]. An ionizable, water soluble 18-crown-6 chiral selector (i.e., 18-crown-6-tetracarboxylic acid) has been used for the CE resolution of some primary amine containing racemates [23–29].

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CDs tend to be more widely useful for CE enantioseparations. Two different approaches have been developed to expand the usefulness of CDs as chiral selectors for CE. The first has been to use a variety of different neutral and charged derivatives of CDs. Derivatizing a CD can substantially alter its enantioselectivity and solubility. However, it should be noted that the increased water solubility of neutral CDs (e.g., methylated, hydroxypropyl, hydroxyethyl, etc.) results from the fact that they are impure mixtures of homologues and isomers [32]. Indeed, highly purified, single component CD derivatives (few, if any of which are available commercially) usually are less soluble in water than the corresponding native CD. Neutral CDs have been used in CE to resolve a variety of charged analytes. Although native CDs or derivatized neutral CDs can enantioselectively complex with uncharged enantiomers (i.e., have different binding constants), no CE separation can occur since all neutral species travel at the same rate, with the electroosmotic flow (EOF). Ionizable or charged derivatives of CDs are used to overcome this problem [1–8]. Ionizable CDs also are mixtures of homologues and isomers except for a few monosubstituted compounds.

There is another approach that is used to expand the usefulness of CDs for CE enantioseparations. It consists of using another additive in the run buffer (in addition to the CD). The additional additive can be chiral or achiral and must interact via secondary equilibria with either the enantiomers or the primary complex. This allows the resolution of enantiomers that cannot be resolved by the CD itself. The secondary or additional run buffer additive can play different roles in the separation process. One role is to produce a charged analyte/additive complex (in an otherwise neutral system) that is amenable to electrophoresis. Ionic micelles have been used in this capacity [33–38] as have charged CD derivatives [39,40]. Another role of the dual additive approach is to enhance the enantioselectivity of a CE system that does not produce an acceptable separation [33–40]. Sometimes the secondary run buffer additive also can decrease analyses times, enhance efficiency, alter selectivity and enhance detection [18].

Recently it was shown that the combination of a chiral crown ether (18-crown-6-tetracarboxylic acid) and β-CD sometimes produced better CE enantioseparations than did either chiral selector alone [25,27,28]. Subsequently, it was noted that the combination of a nonchiral crown ether plus β-CD could produce enantioseparations of racemic amines that could not be resolved using only the CD [41]. In this work, we examine this phenomenon using a wider variety of analytes and CDs.

2. Experimental

2.1. Materials

α-, β-, γ-CDs were obtained from Advanced Separation Technologies (Whippany, NJ, USA). Hydroxypropyl-β-cyclodextrin was obtained from the Consortium fur Elektrochemische Industrie (Munich, Germany). Heptakis(2,6-di-O-methyl)-β-cyclodextrin, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin and tris(hydroxylmethyl)aminomethane (Tris) were also obtained from Advanced Separation Technologies. 18-Crown-6, sodium and potassium dihydrogenphosphate were purchased from Aldrich (Milwaukee, WI, USA). Citric acid was purchased from Fisher Scientific (St. Louis, MO, USA). All racemic solutes were obtained from Aldrich or Sigma or Fluka (Buchs, Switzerland). Filters (0.45 μm) used on the buffer and the solute solutions were purchased from Alltech (Deerfield, IL, USA).

2.2. Methods

A P/ACE 5000 instrument (Beckman, Palo Alto, CA, USA) was used for sodium and potassium phosphate buffer solutions. Data acquisition was done with System Gold software. A Waters Quanta 4000 CE apparatus interfaced to a Chromatopac CR-501 data station was used for the Tris–citric buffer solution. All chiral separations were performed using a 57 cm (50 cm to the detector)×75 mm I.D. fused-silica capillary, applying a potential of 15 kV. All analytes were monitored using UV detection at 254 nm. The capillary was conditioned with 0.1 *M* potassium hydroxide solution for 10 min. The capillary was further purged with doubly distilled water for 10 min followed by a 5 min equilibration with the desired composition and pH of the running buffer. Between runs, the capillary was

rinsed with 0.1 *M* potassium hydroxide, water and run buffer for 2 min each. The phosphate buffer solution was prepared using a 50 m*M* sodium dihydrogenphosphate solution and adjusted to the desired pH with phosphoric acid. The potassium phosphate buffer solutions were prepared by adjusting the pH of a solution containing 30, 50 or 100 m*M* potassium dihydrogenphosphate to a pH of 2.2 with phosphoric acid. The Tris–citrate buffer was prepared using a 10 m*M* Tris solution adjusted with citric acid to the pH 2.7. CD with or without 18-crown-6 was prepared by weighing the appropriate amounts of the compounds together in a volumetric flask and dissolving them in the desired concentration of buffer. All samples were dissolved in methanol or the running buffer at about 1 mg/ml. The samples were hydrostatically injected for 2 s and the separation temperature was 25°C. The EOF was determined using methanol or mesityl oxide as the neutral marker.

2.3. Mobility calculation

The electrophoretic mobility of analytes was calculated from the observed migration time using the equation:

$$\mu_{ep} = \mu_a - \mu_{eof} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eof}}\right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested. μ_a is the apparent mobility, μ_{eof} is the migration time of mesityl oxide or methanol, L_t is the total length of the capillary, L_d is the length of the capillary between the injection end and the detector, V is the applied voltage, t_m is the migration time of the analyte and t_{eof} is the migration time of a neutral marker which moves with the EOF.

3. Results and discussion

Over fifty racemic analytes (all of which contained a primary amine functional group) were evaluated using six different CDs. The effect of the presence and absence of 18-crown-6 in the run buffer was examined in each case. The results are summarized in Tables 1–3. Several things are apparent from this data. First, as seen in Table 1, there are a significant number of racemic compounds where the addition of achiral 18-crown-6 to a CD containing run buffer, either induces enantioresolution or significantly enhances a poor enantioseparation. These separations will be considered in more detail shortly. Also, it is apparent from the data in Tables 2 and 3 that this ''enantio-enhancement effect'' is not a universal phenomenon for this system. In cases where enantiorecognition exists for the CDs by themselves, the addition of 18-crown-6 can sometimes have no effect (Table 2) or a deleterious effect (Table 3) on the enantioseparation. Indeed the effect of 18-crown-6 can vary both with the type of analyte being resolved and the type of CD used in the run buffer. This is illustrated in Fig. 1 for racemic 2-amino-9-hydroxyfluorene. No enantioresolution is obtained with γ-CD in the run buffer, while a combination of γ-CD plus 18-crown-6 gives a substantial resolution (R_s) of 3.0 (Fig. 1A). When the analogous comparison is made using the smaller α-CDs (versus α-CD plus 18-crown-6), there is no difference in the relatively poor enantioresolutions (Fig. 1B). Fig. 1C shows that when using heptakis 2,6-di-O-methyl-β-CD as the run buffer additive, the addition the crown ether is detrimental to the enantioseparation. Clearly the CD/18-crown-6 system can produce diverse results (Fig. 1, Tables 1–3). However our main interest in the use of achiral 18-crown-6 in CE, is its ability to induce or enhance enantioseparations in the presence of certain CDs (Table 1, Fig. 1A) and the mechanism by which it affects CE enantioseparations.

Several studies have been done in an attempt to better understand the ''CD/18-crown-6 synergistic system''. In the first study a series of racemic analytes were chosen which required both the CD and 18-crown-6 in the run buffer in order to obtain any enantioresolution or optimum enantioresolution. The CD concentration was held constant (10 m*M*) and the crown ether concentration was varied between 0 and 30 m*M*. Results are shown in Fig. 2. When the 18-crown-6 plays a role in the separation, its effects are apparent even at low concentrations (Figs. 2 and 3). Three different separation trends were found. In some cases [e.g., racemic 1-aminoindan, 1-(1-naphthyl)ethylamine, 1,2,3,4-tetrahydronaphthylamine and *cis*-1-amino-2-indanol] the enantioresolution increases with 18-crown-6 concentration (Fig. 2). Some compounds (e.g., *trans*-2-

Table 1
CE enantioresolution data for racemic analytes in which the cyclodextrin-based separations were enhanced by the addition of 18-crown-6[a]

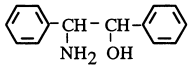
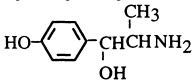
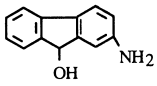
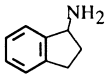
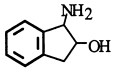
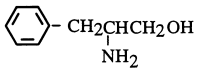
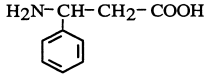
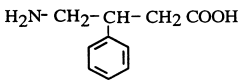
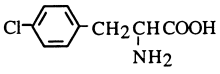
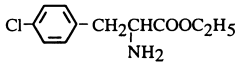
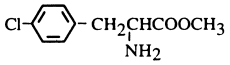
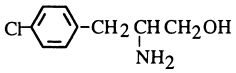
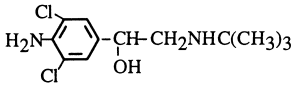
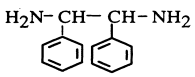
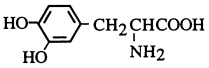
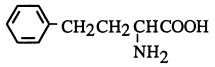
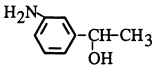
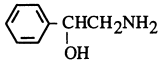
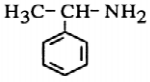
Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
(1*R*2*S*, 1*S*2*R*)-2-Amino-1,2-diphenylethanol	β-CD	8.9	8.5	17.5	18.1	1.9	8.1	7.7	20.9	21.8	2.7
	DM-β-CD	4.7	4.5	28.1	29.2	1.6	3.8	3.6	43.1	45.3	1.9
	TM-β-CD	9.7	9.4	16.2	16.6	1.5	6.9	6.5	24.9	26.1	2.9
	HP-β-CD	7.7	7.5	19.0	19.4	1.4	7.4	7.1	22.7	23.4	1.9
(±)α-(1-Aminoethyl)-4-hydroxybenzyl alcohol	TM-β-CD	10.9	10.8	14.7	14.8	0.6	11.1	10.9	16.0	16.2	1.2
											
2-Amino-9-hydroxyfluorene	γ-CD	10.5		14.7		0.0	9.2	8.82	18.97	19.7	3.0
											
1-Aminoindan	α-CD	14.2	14.0	11.7	11.8	1.1	12.4	12.0	14.2	14.5	1.6
	β-CD	15.0	14.8	11.2	11.3	0.9	9.6	9.0	17.8	19.0	2.5
	γ-CD	14.6		11.2		0.0	7.3	6.9	23.3	24.6	2.2
	DM-β-CD	11.1		14.5		0.0	5.8	5.1	29.5	32.9	3.6
	HP-β-CD	13.9	13.6	11.8	11.9	0.8	8.6	7.0	19.8	23.8	3.3
cis-(1*S*2*R*, 1*R*2*S*)	α-CD	13.9	13.7	11.9	12.1	0.9	12.4	12.1	14.1	14.5	1.5
1-Amino-2-indanol	β-CD	14.8	14.6	11.4	11.5	0.7	11.9	11.0	14.8	15.8	2.9
	γ-CD	14.6		11.2		0.0	13.4	13.2	13.3	13.5	0.8
	DM-β-CD	11.7		13.8		0.0	8.6	7.3	20.6	23.9	3.9
	HP-β-CD	13.0	12.8	12.5	12.6	1.5	10.7	9.2	16.2	18.7	3.7
2-Amino-3-phenyl-1-propanol	β-CD	10.5	10.3	15.3	15.4	0.9	8.5	8.3	20.0	20.6	1.5
	DM-β-CD	9.0	8.9	17.2	17.4	1.0	5.9	5.7	29.3	29.9	1.3
	HP-β-CD	10.1	9.9	15.4	15.6	0.8	7.2	6.8	23.0	24.3	1.5
											
3-Amino-3-phenyl-propionic acid	β-CD	13.0		12.7		0.0	10.1	9.8	17.1	17.6	1.5
	γ-CD	12.8		12.5		0.0	9.3	9.1	18.7	19.2	1.4
	DM-β-CD	10.4	10.3	15.2	15.4	0.8	6.9	6.7	25.2	26.8	2.5
	HP-β-CD	11.5		13.8		0.0	8.7	8.7	19.6	20.3	1.6
Balcofen	β-CD	10.1		15.7		0.0	8.0	7.9	21.2	21.4	0.8
	DM-β-CD	6.4	6.2	22.6	23.0	1.1	4.9	4.8	34.2	35.2	1.5
	TM-β-CD	9.5	9.4	16.4	16.6	1.0	9.4	9.1	18.6	19.3	1.5
	HP-β-CD	8.6		17.5		0.0	6.1	6.0	26.7	27.2	1.5
p-Chlorophenylalanine	β-CD	5.8		24.4		0	4.5	4.4	35.0	35.4	0.7
	DM-β-CD	3.3		35.7		0.0	3.6	3.5	44.5	45.6	1.6
	HP-β-CD	2.7		38.1		0.0	3.9	3.8	38.9	39.3	0.7

Table 1. Continued

Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
4-Chlorophenyl-alanine ethyl ester	γ-CD	10.1	10.0	15.2	15.3	0.8	8.1	7.8	21.4	21.9	1.6
	DM-β-CD	4.8		27.8		0.0	4.7	4.6	36.0	36.3	0.8
	TM-β-CD	8.7		17.7		0.0	8.7	8.7	19.9	20.0	0.6
4-Chlorophenyl-alanine methyl ester	γ-CD	11.5		13.6		0.0	10.1	9.9	17.4	17.7	1.4
	DM-β-CD	5.7		24.6		0.0	4.9	4.8	34.3	34.8	1.0
	TM-β-CD	9.7	9.6	16.2	16.3	0.9	9.8	9.6	17.9	18.3	1.5
4-Chlorophenylalaninol	β-CD	7.8	7.7	19.4	19.7	1.1	7.0	6.9	23.8	24.2	1.5
											
Clenbuterol	HP-β-CD	7.2	6.9	20.0	20.6	1.5	6.8	6.5	24.3	25.2	2.0
											
(1*R*2*R*, 1*S*2*S*)-Diphenyl-ethylenediamine	HP-β-CD	16.2	15.9	10.3	10.5	0.8	15.7	15.4	11.4	11.6	1.3
											
Dopa	β-CD	7.5		19.9		0.0	6.2	6.1	26.6	26.8	0.7
	DM-β-CD	5.1		26.8		0.0	5.2	5.1	32.5	33.1	1.3
	HP-β-CD	5.5		24.4		0.0	5.4	5.3	29.7	29.9	0.6
Homophenylalanine	β-CD	5.6		25.2		0.0	4.6	4.6	34.0	34.2	0.9
	HP-β-CD	3.3		34.2		0.0	3.4	3.3	43.3	43.8	0.8
											
3-(1-hydroxyethyl)aniline	γ-CD	14.9		11.0		0.0	12.6	12.5	14.2	14.2	0.7
	DM-β-CD	12.6		13.0		0.0	10.6	10.6	16.8	16.9	0.6
	TM-β-CD	13.2	13.2	12.5	12.5	0.5	12.5	12.4	14.3	14.4	0.8
β-Hydroxy-phenethylamine	β-CD	13.0		12.7		0.0	9.1	9.1	18.8	18.9	0.6
	γ-CD	15.4		10.7		0.0	13.6	13.5	13.2	13.3	0.6
	HP-β-CD	10.4	10.4	14.7	14.9	0.8	6.5	6.5	24.7	25.2	1.2
α-Methylbenzylamine	γ-CD	15.5		10.6		0.0	10.6	10.5	16.6	16.8	0.8
	DM-β-CD	11.8	11.7	13.7	13.8	0.8	5.5	5.3	31.1	32.2	1.5
	HP-β-CD	14.1		11.6		0.0	7.1	6.8	23.5	24.3	1.5

(Cont.)

Table 1. Continued

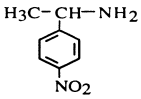
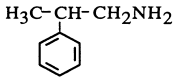
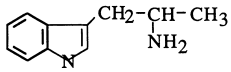
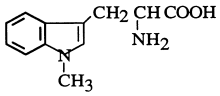
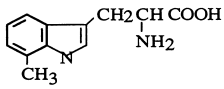
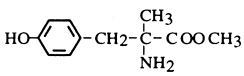
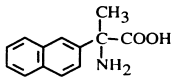
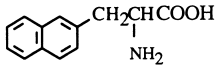
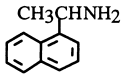
Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
α-Methyl-4-nitro-benzylamine	β-CD	12.4		13.2		0.0	7.2	7.2	23.2	23.3	0.6
	γ-CD	14.1		11.5		0.0	12.5	12.4	14.3	14.4	0.6
											
β-Methyl phenethyl-amine	β-CD	11.7		13.9		0.0	7.0	6.9	23.9	24.2	0.8
	γ-CD	14.9		11.0		0.0	10.7	10.6	16.5	16.7	0.8
	TM-β-CD	13.6		12.2		0.0	13.0	12.9	13.8	13.9	0.7
	HP-β-CD	9.3	9.3	16.4	16.4	0.0	5.4	5.4	29.4	29.7	0.7
α-Methyltryptamine	β-CD	10.4	10.3	15.4	15.5	0.7	7.3	6.9	23.1	24.1	2.2
	γ-CD	12.3	12.2	12.9	13.0	0.8	8.9	8.6	19.5	20.1	1.6
	HP-β-CD	7.4		19.7		0.0	5.8	5.6	27.7	28.9	1.8
1-Methyltryptophan	β-CD	7.7		19.7		0.0	6.7	6.7	24.7	24.8	0.6
											
7-Methyltryptophan	β-CD	7.6		19.8		0.0	6.7	6.6	24.8	25.0	0.7
	HP-β-CD	4.3		28.9		0.0	5.4	5.4	29.7	29.8	0.6
											
α-Methyl-*p*-tyrosine	α-CD	10.7		14.9		0.0	9.4	9.3	18.2	18.4	0.7
	γ-CD	10.0	10.0	15.3	15.3	0.6	9.1	8.9	19.1	19.6	1.5
	HP-β-CD	7.3		19.7		0.0	7.2	7.1	23.1	23.4	0.7
3-(1-Naphthyl)alanine	γ-CD	3.5		32.0		0.0	3.7	3.6	42.4	42.8	0.7
	DM-β-CD	3.6		33.4		0.0	4.2		39.4	39.9	1.1
	TM-β-CD	8.2		18.6		0.0	4.8	4.8	33.8	34.2	0.8
	HP-β-CD	2.6		38.9		0.0	2.8	2.8	49.3	49.6	0.6
3-(2-Naphthyl)alanine	DM-β-CD	2.4	2.3	43.0	43.4	0.7	3.1	3.0	51.1	52.0	1.4
											
1-(1-Naphthyl)ethylamine	α-CD	13.0		12.6		0.0	11.7	11.7	14.9	15.0	0.6
	β-CD	10.9		14.7		0.0	8.7	8.2	19.6	20.8	3.4
	γ-CD	12.0		13.2		0.0	7.6	7.2	22.5	23.8	2.5
	DM-β-CD	6.7		21.8		0.0	5.4	5.2	31.3	32.8	1.5
	HP-β-CD	8.5		17.6		0.0	7.0	6.4	23.8	25.5	2.9

Table 1. Continued

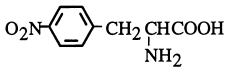
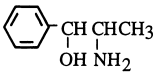
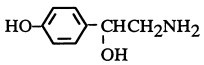
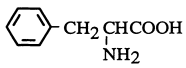
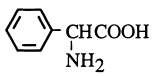
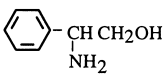
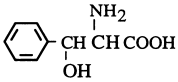
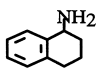
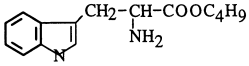
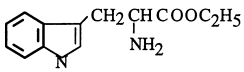
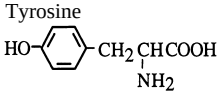
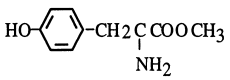
Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
p-Nitrophenylalanine	DM-β-CD	2.8		39.6		0.0	3.3	3.2	48.7	49.6	1.1
											
(1*R*2*S*, 1*S*2*R*)-norephedrine	β-CD	10.4		15.4		0.0	8.0	7.7	21.1	21.9	0.6
											
Octopamine	α-CD	13.1		12.5		0.0	11.3	11.2	15.4	15.5	0.6
	β-CD	10.8		14.9		0.0	7.8	7.7	21.7	21.9	0.6
	TM-β-CD	12.2	12.0	13.3	13.5	1.0	12.0	11.8	14.9	15.1	1.5
	HP-β-CD	8.2	8.1	18.1	18.2	0.6	5.2	5.0	30.8	31.4	1.2
Phenylalanine	β-CD	7.7		19.5		0.0	6.4	6.3	25.9	26.3	1.1
	DM-β-CD	5.3	5.3	25.8	26.0	0.6	5.4	5.3	31.6	32.3	1.3
	HP-β-CD	4.7		27.3		0.0	5.6	5.5	28.9	29.2	1.1
trans-2-Phenyl cyclopropylamine	β-CD	10.2		15.6		0.0	5.5	5.4	29.3	29.7	0.8
	γ-CD	14.5		11.2		0.0	9.8	9.5	17.9	18.4	1.3
											
2-Phenylglycine	α-CD	5.9		23.8		0.0	4.5	4.5	34.4	34.6	0.6
	β-CD	6.7		21.9		0.0	5.0	5.0	31.6	32.0	1.0
	HP-β-CD	3.2		34.8		0.0	4.2	4.2	36.4	36.7	0.8
α-Phenylglycinol	β-CD	13.5		12.3		0.0	10.0	9.9	17.4	17.5	0.6
	DM-β-CD	12.6		13.0		0.0	7.5	7.1	23.4	24.4	0.9
	HP-β-CD	12.4		12.9		0.0	8.0	8.0	21.0	21.2	0.6
DL-*threo*-3-phenylserine	β-CD	7.5		20.0		0.0	6.4	6.4	25.6	25.9	0.7
	DM-β-CD	4.6		28.6		0.0	5.2	5.1	32.8	33.4	1.4
	HP-β-CD	4.0		29.9		0.0	5.3	5.2	30.2	30.4	0.7
1,2,3,4-Tetrahydro-naphthylamine	α-CD	13.9	13.8	11.9	12.0	0.6	12.6	12.3	14.0	14.3	1.0
	β-CD	14.2	14.0	11.7	11.9	0.8	11.2	10.5	15.6	16.6	1.9
	γ-CD	14.1	14.0	11.5	11.6	0.8	7.2	6.5	23.6	26.0	2.4
	DM-β-CD	10.0	9.8	15.8	16.0	0.8	6.7	5.6	25.8	30.6	3.6
	HP-β-CD	11.5	11.2	13.8	14.1	1.1	9.4	8.1	18.3	20.8	3.0
Tryptophan butyl ester	γ-CD	6.6	6.5	21.0	21.2	0.7	5.9	5.8	28.3	28.9	1.5
	TM-β-CD	9.8		16.0		0.0	7.3	7.2	23.5	23.7	0.7
	HP-β-CD	5.0	4.8	26.2	26.6	1.0	4.9	4.8	32.1	32.8	1.3

Table 1. Continued

Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
Tryptophan ethyl ester 	γ-CD	9.9	9.8	15.4	15.6	0.9	8.4	8.2	20.7	21.1	1.5
Tyrosine 	β-CD	5.7		24.8		0	5.6	5.6	29.0	29.1	0.6
	DM-β-CD	5.0		27.0		0	5.0	4.9	33.9	34.2	0.9
Tyrosine methyl ester 	DM-β-CD	7.6		19.7		0.0	6.1	6.1	28.1	28.3	0.7
	TM-β-CD	10.5		15.1		0.0	10.8	10.7	16.4	16.5	0.6
	HP-β-CD	8.9		16.9		0.0	8.0	8.0	21.0	21.1	0.5

[a]Electrophoretic conditions: run buffer consists of CD with or without 30 m*M* 18-crown-6 in 50 m*M* sodium dihydrogenphosphate solution at pH 2.2, 15 kV were applied. The concentrations of α-CD, DM-β-CD, TM-β-CD and HP-β-CD are 30 m*M*, while β-CD and γ-CD are 20 m*M*.

[b]DM-β-CD = heptakis(2,6-di-O-methyl)-β-CD, TM-β-CD = heptakis(2,3,6-tri-O-methyl)-β-CD and HP-β-CD = 2-hydroxypropyl-β-CD.

[c]u_1 and u_2 are the effective electrophoretic mobilities ($cm^2\ kV^{-1}\ min^{-1}$) of the first- and second-eluting enantiomers.

[d]t_1 and t_2 are the migration times (min) of the first- and second-eluting enantiomers.

phenylcyclopropylamine) were best resolved with low levels of 18-crown-6 (Fig. 3). Higher concentrations caused a deterioration in the separation although the resolution was still better than that obtained in the absence of 18-crown-6. Still other compounds (aminoglutethimide, Fig. 3) show the same enhanced enantioresolution regardless of the amount of 18-crown-6 added to the run buffer.

In a second series of CE experiments the 18-crown-6 concentration was held constant at 30 m*M* while the CD concentration was varied. Typical trends are shown in Fig. 4. Naturally no enantioresolution is possible at zero CD concentration since the CD is the only chiral additive. In general, two types of behavior were observed. Either the enantioresolution increased with increasing levels of CD [i.e., 1-(naphthyl)ethylamine in Fig. 4], or there was an initial increase in enantioresolution followed by decreased resolution at higher CD concentrations (i.e., *trans*-2-phenylcyclopropylamine in Fig. 4). Even in cases where higher CD concentrations increased enantioresolution, this enhancement effect became less pronounced at the higher concentrations (i.e., diminishing returns). Also, the migration times increased with increasing CD concentration due to the greater viscosity of the solutions.

It was not possible to predict the optimum amounts of 18-crown-6 and CD for the enantioresolution of a given compound. Nor was it possible to predict the best CD or CD derivative for any given separation. However, it appeared from these results (Figs. 3 and 4) that approximately 10 m*M* CD plus approximately 10–15 m*M* 18-crown-6 were the minimum amounts of these additives needed to achieve a reasonable enantioseparation in most cases.

3.1. Mechanism

It is well known that a primary amine functional group must be protonated in order to form an inclusion complex with 18-crown-6 [30,31]. In addition it is known that potassium ion is about the same size as ammonium ion and also forms inclusion complexes with 18-crown-6 [31]. Consequently, both pH and the presence of competing potassium ion in the run buffer should affect the enantioseparations obtained with the CD/18-crown-6 system. Table 4 shows the effect of pH on the enantioresolution of four racemic analytes. Chiral recognition occurs at acidic pH values and decreases as the pH is elevated. The pH at which chiral recognition is lost varies with the nature of the compound being analyzed. It is

Table 2
CE enantioresolution data for racemic analytes in which the CD-based separations were not affected by the addition of 18-crown-6[a]

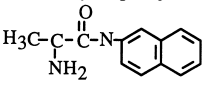
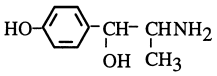
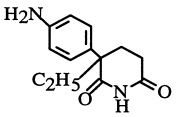
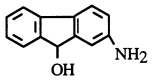
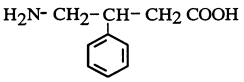
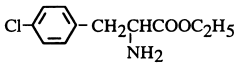
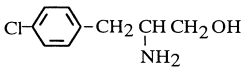
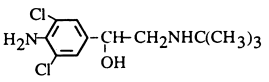
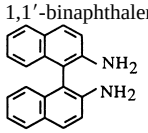
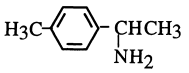
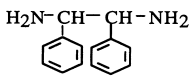
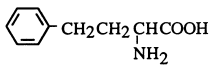
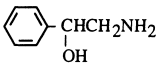
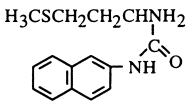
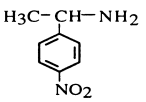
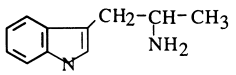
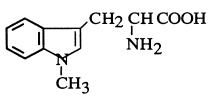
Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
Alanine-β-naphthylamide 	β-CD	5.9	5.8	24.0	24.3	1.0	5.6	5.5	9.0	29.3	1.1
	γ-CD	8.2	8.1	17.9	18.1	1.3	8.0	7.8	21.5	21.9	1.5
	HP-β-CD	4.0	4.0	29.9	30.3	1.0	4.3	4.2	35.9	36.3	1.1
(±)-α-(1-aminoethyl)-4-hydroxybenzyl alcohol 	β-CD	8.36	8.25	18.36	18.56	0.6	6.55	6.34	25.22	25.96	0.7
Aminoglutethimide 	α-CD	8.1	7.9	18.7	19.0	0.9	7.5	7.3	22.2	22.7	0.9
	TM-β-CD	10.1	9.6	15.7	16.3	1.5	9.8	9.3	18.0	18.8	1.6
2-Amino–9-hydroxy-fluorene 	α-CD	11.5	11.4	14.0	14.1	0.9	10.1	10.0	17.0	17.2	0.8
Balcofen 	α-CD	6.8	6.7	21.3	21.7	1.5	6.0	5.8	27.1	27.8	1.5
4-Chlorophenyl alanine ethyl ester 	α-CD	6.4	6.3	22.5	22.7	0.8	6.4	6.3	25.5	25.9	0.7
4-Chlorophenylalaninol 	TM-β-CD	9.6	9.5	16.4	16.5	0.8	9.6	9.5	18.3	18.5	0.9
	HP-β-CD	5.9	5.7	23.3	23.6	1.0	4.9	4.8	32.1	32.8	0.8
Clenbuterol 	β-CD	8.0	7.8	19.0	19.4	2.1	7.7	7.5	21.9	22.5	2.1
	DM-β-CD	5.5	5.1	25.1	26.7	4.9	6.7	6.2	26.1	27.8	4.9
	TM-β-CD	8.6	8.5	17.8	18.0	1.2	9.0	8.9	19.4	19.6	1.2
2,2′-Diamino-1,1′-binaphthalene 	α-CD	9.9	9.7	15.9	16.1	1.2	8.9	8.7	19.2	19.4	1.1
	β-CD	7.4	7.1	20.2	21.0	3.1	6.7	6.3	24.9	26.2	3.1
	γ-CD	7.2	7.0	19.7	20.2	1.8	6.9	6.7	24.5	25.2	1.8
	TM-β-CD	5.7	5.0	24.5	26.9	7.0	6.1	5.5	27.5	30.1	7.0
	HP-β-CD	5.2	4.7	25.2	27.0	5.1	5.5	5.0	29.5	31.5	5.1

Table 2. Continued

Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
α-4-Dimethylbenzylamine	DM-β-CD	8.4	8.3	18.2	18.3	0.6	4.7	4.7	35.6	35.8	0.6
	HP-β-CD	9.6	9.6	15.9	16.0	0.6	5.2	5.2	30.7	30.8	0.6
(1*R*2*R*, 1*S*2*S*)-diphenyl-ethylenediamine	α-CD	17.6	17.3	9.7	9.8	1.5	16.3	16.1	10.9	11.1	1.4
	β-CD	18.3	18.2	9.4	9.4	0.6	17.3	17.2	10.4	10.4	0.6
	DM-β-CD	15.2	14.7	11.1	11.3	1.7	15.6	15.2	11.7	12.0	1.5
Homephenylalanine	DM-β-CD	4.4	4.1	29.7	30.9	0.7	4.5	4.4	37.3	37.7	0.8
											
β-Hydroxy phenethyl amine	TM-β-CD	13.7	13.5	12.1	12.2	1.0	13.2	13.0	13.6	13.8	1.0
											
Methionine-β-naphthylamide	β-CD	5.7	5.6	24.7	25.1	1.2	5.4	5.3	29.9	30.4	1.2
	γ-CD	7.5	7.3	19.3	19.7	1.4	7.3	7.1	23.4	24.0	1.4
	HP-β-CD	3.9	3.8	30.8	31.3	1.2	4.1	4.0	37.4	38.0	1.2
α-Methyl-4-nitro-benzylamine	DM-β-CD	7.2	7.1	20.5	20.8	1.0	5.1	5.0	33.1	33.7	0.8
											
α-Methyltryptamine	DM-β-CD	6.1	6.0	23.3	23.6	0.9	4.9	4.8	34.2	34.8	1.0
											
1-Methyltryptophan	α-CD	7.5	7.3	19.8	20.2	1.8	6.3	6.2	25.8	26.4	2.0
	DM-β-CD	4.5	4.4	28.9	29.4	1.4	5.0	4.9	33.5	34.1	1.4
											

(Cont.)

Table 2. Continued

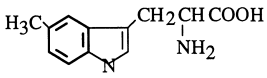
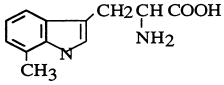
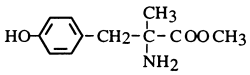
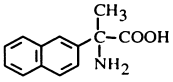
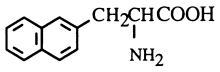
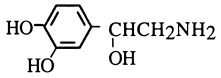
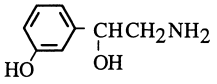
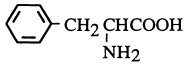
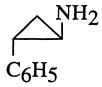
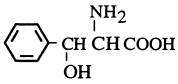
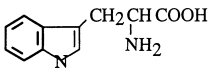
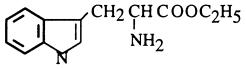
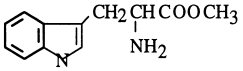
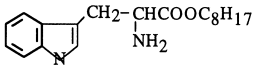
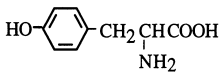
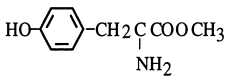
Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
5-Methyltryptophan 	α-CD	6.6	6.4	22.1	22.3	1.3	5.5	5.4	29.2	29.6	1.4
	DM-β-CD	4.1	4.0	30.9	31.3	1.3	4.6	4.5	36.5	37.0	1.4
7-Methyltryptophan 	DM-β-CD	4.4	4.3	29.5	30.0	1.1	5.0	4.9	33.7	34.2	1.3
α-Methyl-*p*-tyrosine-methyl ester 	β-CD	8.4	8.3	18.3	18.6	0.9	7.9	7.8	21.3	21.7	1.1
	DM-β-CD	6.3	6.2	22.9	23.2	0.7	5.3	5.2	32.0	32.4	0.7
3-(1-Naphthyl)alanine 	β-CD	6.5	6.5	22.3	22.4	0.8	5.5	5.5	29.3	29.6	0.9
3-(2-Naphthyl)alanine 	α-CD	6.2	6.1	22.9	23.2	1.2	5.2	5.1	30.4	30.8	1.3
Norepinephrine 	TM-β-CD	11.1	11.0	14.5	14.5	0.6	10.7	10.7	16.5	16.6	0.5
Norphenylephrine 	TM-β-CD	12.4	12.2	13.2	13.4	1.4	12.2	12.1	14.6	14.8	1.3
Phenylalanine 	α-CD	7.7	7.7	19.5	19.6	0.5	6.4	6.3	25.7	25.9	0.7
trans-2-Phenyl-cyclopropylamine 	TM-β-CD	12.7	11.9	12.9	13.6	2.7	11.8	11.0	15.1	16.1	2.7

Table 2. Continued

Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
DL-threo-3-Phenylserine 	α-CD	7.0	6.9	21.1	21.2	0.5	5.6	5.5	28.7	28.9	0.6
1,2,3,4-tetrahydro-naphthylamine 	TM-β-CD	13.1	13.0	12.6	12.7	0.7	13.1	12.8	13.7	14.0	0.8
Tryptophan 	α-CD	7.7	7.5	19.6	19.9	1.8	6.5	6.3	25.2	25.8	2.0
Tryptophan ethyl ester 	β-CD	9.0	8.7	17.4	17.8	1.5	8.7	8.5	19.7	20.1	1.5
	DM-β-CD	5.7	5.4	24.4	25.5	1.5	5.6	5.5	30.4	31.0	1.4
	TM-β-CD	9.3	9.2	16.7	16.9	0.8	9.5	9.4	18.4	18.6	0.9
	HP-β-CD	7.4	7.1	19.6	20.2	1.5	7.3	7.1	22.8	23.4	1.5
Tryptophan methyl ester 	α-CD	11.5	11.4	14.0	14.1	0.6	9.8	9.7	17.6	17.7	0.6
	β-CD	10.7	10.5	15.0	15.2	0.9	9.8	9.7	17.6	17.8	0.9
	HP-β-CD	8.6	8.4	17.5	17.8	1.4	8.4	8.3	20.2	20.5	1.4
Tryptophan octyl ester 	α-CD	3.2	3.1	36.4	36.9	0.8	3.3	3.3	43.4	44.1	0.8
	DM-β-CD	3.4	3.3	35.2	35.4	0.6	3.8	3.8	43.0	43.1	0.6
Tyrosine 	α-CD	7.7	7.7	19.4	19.5	0.6	6.2	6.2	26.1	26.3	0.7
Tyrosine methyl ester 	β-CD	10.3	10.2	15.5	15.6	0.6	8.9	8.8	19.3	19.4	0.7

[a]Electrophoretic conditions: run buffer consists of CD with or without 30 m*M* 18-crown-6 in 50 m*M* sodium dihydrogenphosphate solution at pH 2.2, 15 kV were applied. The concentrations of α-CD, DM-β-CD, TM-β-CD and HP-β-CD are 30 m*M*, while β-CD and γ-CD are 20 m*M*.

[b]DM-β-CD = heptakis(2,6-di-O-methyl)-β-CD, TM-β-CD = heptakis(2,3,6-tri-O-methyl)-β-CD and HP-β-CD = 2-hydroxypropyl-β-CD.

[c]u_1 and u_2 are the effective electrophoretic mobilities ($cm^2\ kV^{-1}\ min^{-1}$) of the first- and second-eluting enantiomers.

[d]t_1 and t_2 are the migration times (min) of the first- and second-eluting enantiomers.

Table 3
CE enantioresolution data for racemic analytes in which the CD-based separations were diminished by the addition of 18-crown-6[a]

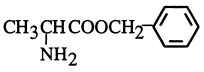
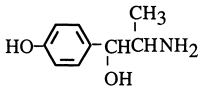
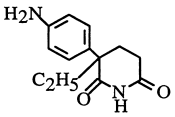
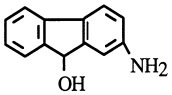
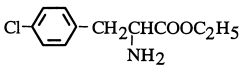
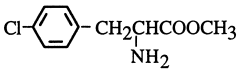
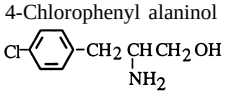
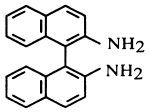
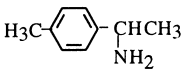
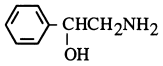
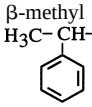
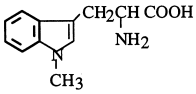
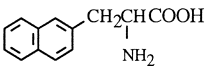
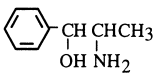
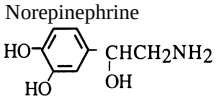
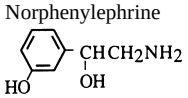
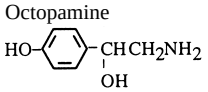
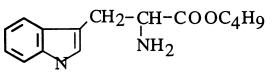
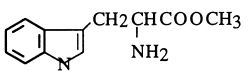
Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
Alanine benzyl ester 	β-CD	8.2	8.0	18.6	18.9	1.5	7.6	7.5	22.1	22.3	1.1
	HP-β-CD	7.2	7.1	19.9	20.2	1.3	6.9	6.9	23.9	24.1	0.9
(±)-α-(1-Aminoethyl)-4-hydroxybenzyl alcohol 	DM-β-CD	7.9	7.7	19.2	19.4	1.0	4.9		34.2		0.0
	HP-β-CD	6.5	6.4	21.6	21.9	0.9	5.8		27.8		0.0
Aminoglutethimide 	γ-CD	7.8	7.5	18.7	19.3	1.3	6.4	6.4	26.3	26.5	0.6
	DM-β-CD	4.0	3.8	31.7	32.6	1.4	4.9		34.5		0.0
2-Amino-9-hydroxy-fluorene 	DM-β-CD	5.2	5.1	26.3	26.7	1.4	5.3	5.2	32.2	32.3	0.5
4-Chlorophenyl alanine ethyl ester 	HP-β-CD	5.5	5.4	24.4	24.5	0.6	6.0		27.1		0.0
4-Chlorophenyl-alanine methyl ester 	HP-β-CD	6.5	6.4	21.5	21.7	0.7	6.4		25.5		0.0
4-Chlorophenyl alaninol 	DM-β-CD	5.0	4.9	27.0	27.3	0.7	4.7		35.6		0.0
RS-2,2′-diamino-1,1′-binaphthalene 	DM-β-CD	3.5	3.4	34.1	35.2	2.3	4.2	4.1	39.2	40.1	1.4
α-4-Dimethyl benzyl-amine 	α-CD	9.9	9.7	15.9	16.1	0.9	8.4		20.1		0.0
β-hydroxy phenethyl-amine 	DM-β-CD	10.4	10.0	15.3	15.8	1.7	5.8		29.5		0.0

Table 3. Continued

Compound and structure	Cyclodextrin[b] used	CD alone					CD + 18-crown-6				
		u_1^c	u_2^c	t_1^d	t_2^d	R_s	u_1^c	u_2^c	t_1^d	t_2^d	R_s
β-methyl phenethylamine 	DM-β-CD	8.2	8.2	18.5	18.6	0.7	4.6		36.3		0.0
1-Methyltryptophan 	HP-β-CD	4.4	4.2	28.4	29.1	1.4	5.5	5.4	29.3	29.5	0.8
3-(2-Naphthyl)alanine 	HP-β-CD	3.1	3.0	35.3	35.7	1.1	2.8		50.3		0.0
(1*R*2*S*, 1*S*2*R*)norephedrine 	DM-β-CD	7.7	7.4	19.5	20.1	2.0	5.4		31.6		0.0
Norepinephrine 	α-CD	13.2	13.1	12.5	12.5	0.7	11.5		15.2		0.0
	DM-β-CD	10.3	9.9	15.4	15.9	1.9	4.7		35.8		0.0
	HP-β-CD	8.3	8.2	17.9	18.1	1.2	5.4	5.3	29.5	29.9	0.8
Norphenylephrine 	β-CD	11.5	11.3	14.1	14.3	1.1	7.4		22.7		0.0
	DM-β-CD	8.6	8.1	17.9	18.8	2.9	4.9	4.9	34.4	34.4	0.6
	HP-β-CD	8.6	8.4	17.4	17.8	1.5	4.7	4.6	33.3	33.7	0.6
Octopamine 	DM-β-CD	8.5	8.2	18.0	18.6	1.8	5.2		32.7		0.0
trans-2-Phenyl cyclopropylamine 	DM-β-CD	6.8	6.5	21.5	22.2	1.3	4.2		39.7		0.0
	HP-β-CD	7.7	7.6	18.9	19.2	1.2	4.5		34.3		0.0
Tryptophan butyl ester 	DM-β-CD	4.3	4.2	30.1	30.6	1.3	4.4	4.4	37.4	37.9	1.0
Tryptophan methyl ester 	γ-CD	11.0	10.9	14.2	14.3	0.7	9.7		18.0		0.0
	DM-β-CD	6.8	6.7	21.5	21.8	0.9	6.4		26.9		0.0

[a] Electrophoretic conditions: run buffer consists of CD with or without 30 m*M* 18-crown-6 in 50 m*M* sodium dihydrogenphosphate solution at pH 2.2, 15 kV were applied. The concentrations of α-CD, DM-β-CD, TM-β-CD and HP-β-CD are 30 m*M*, while β-CD and γ-CD are 20 m*M*.

[b] DM-β-CD = heptakis(2,6-di-O-methyl)-β-CD, TM-β-CD = heptakis(2,3,6-tri-O-methyl)-β-CD and HP-β-CD = 2-hydroxypropyl-β-CD.

[c] u_1 and u_2 are the effective electrophoretic mobilities ($cm^2\ kV^{-1}\ min^{-1}$) of the first- and second-eluting enantiomers.

[d] t_1 and t_2 are the migration times (min) of the first- and second-eluting enantiomers.

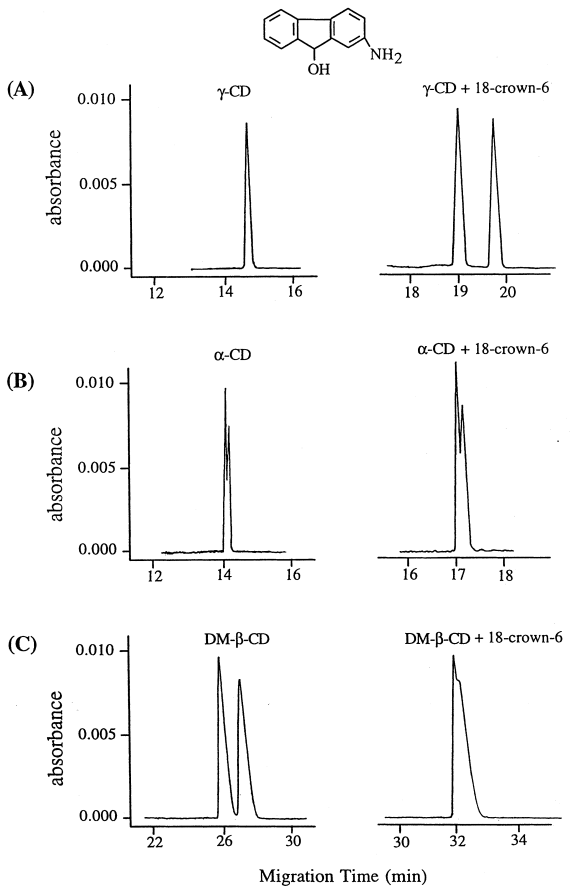


Fig. 1. Effect of 18-crown-6 on the CE enantiospearation of 2-amino-9-hydroxyfluorene with (A) γ-CD, (B) α-CD and (C) heptakis(2,6-O-dimethyl)-β-CD.

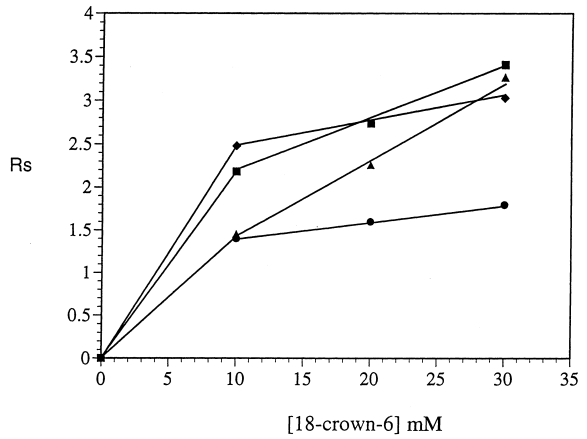


Fig. 2. Plot of resolution versus 18-crown-6 concentration for 1-aminoindan (■), *cis*-1-amino-2-indanol (●), 1-(1-naphthyl)ethylamine (▲) and 1,2,3,4-tetrahydro-naphthylamine (♦). Run buffer system: 10 m*M* Tris, 20 m*M* citric acid, pH 2.75 and 10 m*M* β-CD.

controlled by the pK_b of the compound and strength of the inclusion complex among other factors.

Table 5 shows the effect of added potassium ion on the CE enantioresolution of several racemates (using the CD/18-crown-6 system). For the compounds tested, the addition of competing K^+ either decreased or eliminated enantioresolution. The added K^+ did not appreciably change the migration times, however. Together, the results of the pH (Table 4) and the K^+ study (Table 5) indicate that an inclusion complex between the achiral 18-crown-6 and the primary amine of the chiral analyte has a significant effect on chiral recognition (provided the proper CD also is present). When the 18-crown-6/ammonium ion inclusion complex is altered or broken, the enantioselectivity decreases or is lost.

A careful consideration of the results (Tables 1–5, Figs. 1–3) sheds some light on the chiral recognition mechanism. In most cases (i.e., Tables 1 and 3) a ''three-body'' complex, consisting of the analyte + CD + 18-crown-6 is necessary to explain the observed enantioselectivity. This is true even in cases

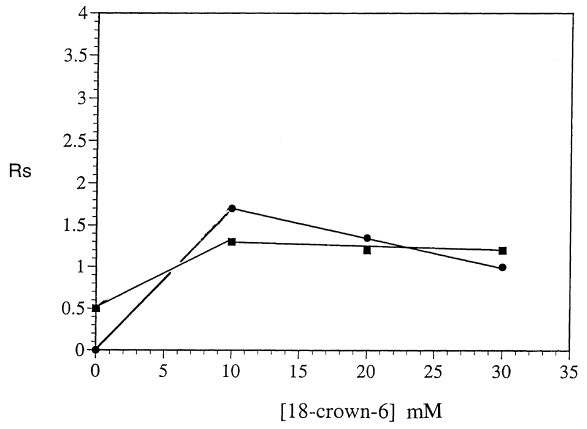


Fig. 3. Plot of resolution versus 18-crown-6 concentration for aminoglutethimide (■) and *trans*-2-phenylcyclopropylamine (●). Buffer system: 10 m*M* Tris, 20 m*M* citric acid, pH 2.75 and 10 m*M* β-CD.

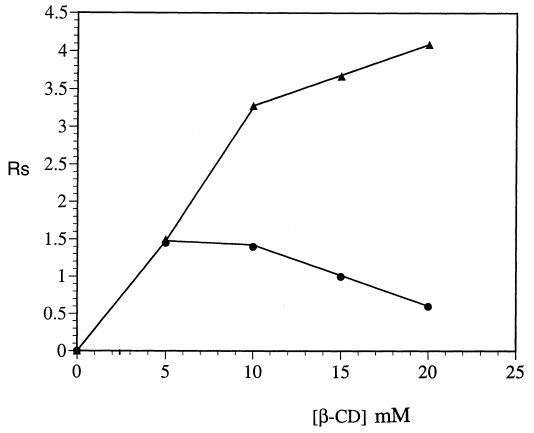


Fig. 4. Plot of resolution versus β-CD concentration for *trans*-2-phenylcyclopropylamine (●) and 1-(1-naphthyl)ethylamine (▲). Buffer system: 10 m*M* Tris, 20 m*M* citric acid, pH 2.75 and 30 m*M* 18-crown-6.

where enantioselectivity is decreased upon addition of 18-crown-6 (Table 3). Only in cases where the addition of 18-crown-6 has no effect on enantioseparation (i.e., the Table 2 compounds) does a two-body inclusion complex (between the analyte and CD) adequately explain chiral recognition. Even in this case the 18-crown-6 may be associating with the analyte and/or CD, but in such a way that it does not affect enantioselectivity.

The nature of the three-body complex is open to question. There are several possibilities. Both the analyte and the 18-crown-6 could be included in the CD as shown in Fig. 5A. In this case the role of the 18-crown-6 would be primarily to occupy space thereby creating a tighter CD inclusion complex. However, the results of the pH and K^+ studies (Tables 4 and 5) clearly show that there is an additional inclusion complex between the primary

Table 4
Effect of pH on migration times and resolution of enantiomers separated by HP-β-CD alone, and by 18-crown-6 with HP-β-CD in 50 m*M* NaH_2PO_4 buffer, pH 2.2

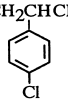
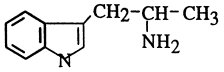
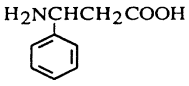
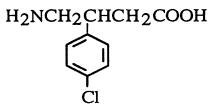
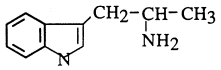
Compound	30 m*M* HP-β-CD				30 m*M* HP-β-CD +30 m*M* 18-crown-6		
	pH	t_1	t_2	R_s	t_1	t_2	R_s
3-Amino-3-phenyl-propionic acid	2.2	13.8		0	19.6	20.3	1.6
	3.0				23.0	23.8	1.5
	4.0	32.3		0	39.6		0
H_2NCHCH_2COOH	6.0	18.2		0	13.0		0
	8.0	9.1		0	8.7		0
	10.0	9.5		0	9.2		0
Baclofen	2.2	17.5		0	26.7	27.2	1.5
	3.0				27.9	28.2	0.8
$H_2NCH_2CHCH_2COOH$	4.0	29.2		0	26.5		0
	6.0	13.2		0	13.6		0
	8.0	9.1		0	8.5		0
	10.0	8.5		0	9.1		0
α-Methylbenzyl amine	2.2	12.9		0	23.5	24.3	1.5
	3.0				21.8	22.6	1.5
	4.0	12.1		0	21.8	22.6	1.4
$H_3C-CH-NH_2$	6.0	7.0		0	8.8	8.9	0.9
	8.0	6.0		0	6.5	6.5	0.7
	10.0	6.3		0	7.1		0
α-Methyl tryptamine	2.2	19.7		0	27.7	28.9	1.8
	3.0				26.0	26.9	1.5
	4.0	16.1		0	26.5	27.5	1.4
	6.0	8.1		0	9.5	9.7	1.2
	8.0	6.8		0	6.9	6.9	0.6
	10.0	6.4		0	7.0		0

Table 5
Effect of KH_2PO_4 and NaH_2PO_4 buffer concentration, pH 2.2 on migration time and resolution of enantiomers with 30 m*M* 18-crown-6 and 30 m*M* HP-β-CD used as chiral selectors

Compound and structure	0 m*M* KH_2PO_4 50 m*M* NaH_2PO_4			30 m*M* KH_2PO_4			50 m*M* KH_2PO_4			100 m*M* KH_2PO_4		
	t_1	t_2	R_s	t_1	t_2	R_s	t_1	t_2	R_s	t_1	t_2	R_s
3-Amino-3-phenyl propionic acid 	19.6	20.3	1.6	25.3	26.0	0.9	25.2	25.9	0.8	25.0	25.3	0.7
Baclofen 	26.7	27.2	1.5	36.5	37.2	1.0	38.6	39.3	0.9	39.8		0
α-Methyltryptamine 	27.7	28.9	1.9	39.3	41.5	1.8	41.1	43.1	1.6	42.3	43.5	1.4

ammonium moiety of the analyte and 18-crown-6. Hence the type of complex indicated in Fig. 5A is unlikely under the conditions of this study (although it may occur at more alkaline pH values or with other types of compounds). Given the requirement that the primary ammonium group is included in the 18-crown-6, there are at least three general modes of association with the CD. These are shown in Fig. 5B–D. Complex 5B (with the 18-crown-6/ammonium moiety buried in the CD cavity) is unlikely since it requires that the most hydrophilic part of the analyte be in the CD cavity, while its hydrophobic

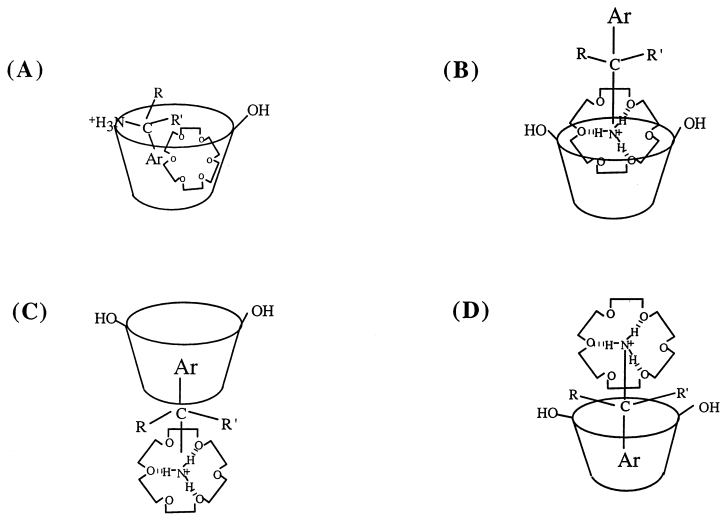


Fig. 5. Some possible ''three-body'' complexes between a hypothetical chiral amine, a cyclodextrin and 18-crown-6. ''D'' is thought to be the most important and dominant complex. ''Ar'' = aromatic moiety.

parts are projected into the bulk aqueous solution. Complex 5C requires that the aromatic or hydrophobic portion of the analyte be sufficiently small that it can penetrate the narrow end of the CD cavity. Also steric restrictions with the R and R′ groups could prevent a very deep penetration of the aromatic/hydrophobic moiety. Complex 5D is the most probable. It allows the deepest penetration of the hydrophobic-aromatic portion of the analyte into the CD cavity while simultaneously allowing the formation of the 18-crown-6/ammonium moiety inclusion complex. In addition, the orientation of this particular three-body complex allows for hydrogen bonding and steric interactions between both the 18-crown-6/ammonium moiety and the R or R′ groups with the hydroxyl groups (or other substituents) at the mouth of the CD cavity. These additional interactions are essential for chiral recognition. Also, this orientation (Fig. 5D) is most closely related to the original chiral recognition model proposed and supported for β-CD in aqueous solutions [42–44]. An additional benefit of the proposed complex (Fig. 5D) is that it also provides an explanation for the deleterious effect of 18-crown-6 on some enantioseparations (see Table 3). In these cases the CD alone provides ample chiral recognition for the analyte. One of the dominant interactions (in addition to the hydrophobic inclusion complex) undoubtedly would be a hydrogen bond between the primary amine of the analyte and the secondary hydroxy-group at the mouth of the CD. The addition of 18-crown-6 would negate this interaction when it forms an inclusion complex with the analyte's amine functional group. In these cases the result could be diminished enantioselectivity.

It should be noted that the proposed complex (Fig. 5D) is not a static species. Nor should it be considered the only complex in solution. Undoubtedly there are a wide variety of dynamic, fleeting associations. The four most important proposed equilibrium steps are shown in Fig. 6. Both the analyte's migration times and enantioresolution are a reflection of the

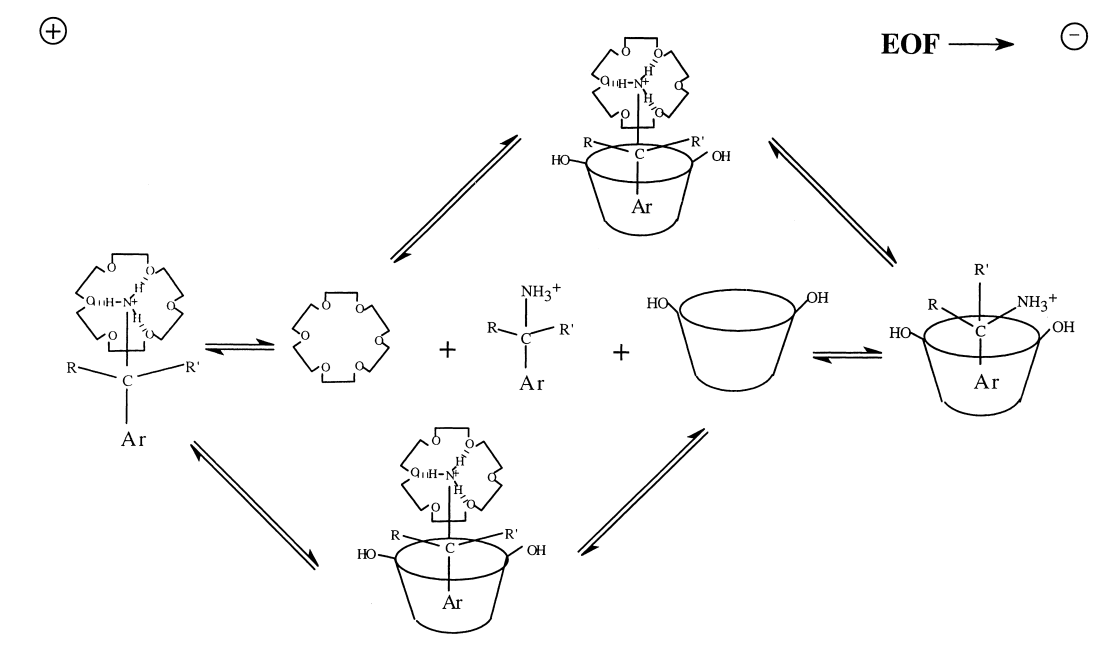


Fig. 6. Schematic showing the most important solution equilibria steps in the formation of the ''three-body'' complex which is responsible for the observed enantioselectivities in this CE system.

amount of time it spends as the monomer versus different two-body complexes versus the three-body complex in this dynamic system.

4. Conclusions

The addition of achiral 18-crown-6 to a CD-based CE enantioseparation of organic primary amine-containing compounds frequently enhances resolution. However, in a few cases, the 18-crown-6 has no effect or a deleterious effect. These results can be explained most effectively by the proposed ''three-body complex''. This consists of an inclusion complex of the aromatic portion of the analyte with the CD, and a simultaneous complex between 18-crown-6 and the analyte's primary amine moiety (which is projected away from the mouth of the CD cavity). This simultaneous three-body interaction appears to enhance more enantioseparations than it hinders. Breaking the inclusion complex between the 18-crown-6 and amine (by altering pH or adding K^+) tends to decrease or eliminate chiral recognition in many cases.

Acknowledgements

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