Chiral separation of primary amino compounds using a non-chiral crown ether with β-cyclodextrin by capillary electrophoresis

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Abstract

A non-chiral crown ether (18-crown-6) along with β-cyclodextrin (β-CD) was used to achieve enantioselective separations of primary amino compounds in capillary electrophoresis. In this new method, the amino group of these compounds is protonated in a low pH separation buffer and forms a selective host–guest complex with the crown ether (amino compound + 18-crown-6). The hydrophobic portion of the host–guest complex is then incorporated into the cavity of the β-cyclodextrin. The amino compound is sandwiched between the crown ether and the cyclodextrin (18-crown-6 + amino compound + β-CD) and thus determines or enhances the enantioselective recognition. It is postulated that the formation of this sandwich results in a more selective chiral interaction between the molecule and β-cyclodextrin. The chiral recognition is dependent upon the formation of this sandwich complex. This method has been used to achieve enantioselectivity of primary amino compounds with a wide variety of substitutions.

Keywords: Enantiomer separation; Amino compounds, primary; Crown ether, non-chiral ; Cyclodextrins

1. Introduction

The determination of optical purity of starting materials and intermediates used in the synthesis of drug substances are critical issues which ultimately effect the purity of chiral pharmaceutical drug substances. A review of chiral separations by capillary electrophoresis [1,2] has shown success using mobile phase modifiers such as, optically active micelles [3,4], cyclodextrins [5–8], modified cyclodextrins [9,10], ligand-exchange [11], macrocyclic antibiotics [12,13], polymer additives [14], mucopolysaccharides [15], and a chiral crown ether [16–21]. The most common approach is to use cyclodextrins to separate the enantiomers [22–27]. This approach may be effective only when the chiral center and substituents of the compound have a favorable

distance from the rim of the cyclodextrins, and when the hydrophobic group of the compound can fit into the cavity of the cyclodextrins. However, when both of these conditions cannot be met, chiral separation of primary amino compounds have been achieved using a chiral crown ether [16–21] and a chiral crown ether combined with cyclodextrins [18,28]. Kuhn et al. [17,18] were the first to report the use of a chiral 18-crown-6 tetracarboxylic acid, shown in Fig. 1a, as a chiral recognition agent to separate the enantiomers of a variety of primary amino compounds. In the method of Kuhn et al. [16], two compounds, 1-phenylethylamine and phenylglycine, could not be separated due to a steric hindrance of the chiral crown ether [16]. The chiral 18-crown-6 tetracarboxylic acid, used by Kuhn et al., is an expensive product, thus restricting its widespread use.

This work demonstrates the use of a non-chiral

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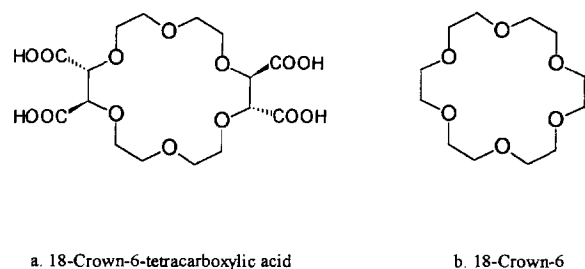a. 18-Crown-6-tetracarboxylic acid   b. 18-Crown-6

Fig. 1. Molecular structures of crown ethers; (a) the chiral crown ether and (b) the non-chiral crown ether.

crown ether (18-crown-6), 1,4,7,10,13,16-hexaoxacyclooctadecane, shown in Fig. 1b, used in conjunction with β-cyclodextrin to achieve or enhance chiral separation in capillary electrophoresis. The 18-crown-6 ether was introduced in 1967 and is a synthetic polyether compound [29,30] in which the six oxygen atoms form a hydrophilic plane around the lower inside of the crown cavity and the $-CH_2-$ groups form a hydrophobic plane at the upper outside ring of the crown. Oxygen as an electron-donor in the polyether ring can form a selective complex with suitable metal or organic cations. The 18-crown-6 ether was used to achieve or enhance the resolution of primary amino compounds when the enantiomers could not be separated or had poor resolution by β-cyclodextrin alone. In this method, the amino group of the compounds studied are protonated, and hence, carry a positive charge in a low pH buffer solution. The primary amino group forms a selective host–guest complex [31] with the non-chiral 18-crown-6 ether in the buffer solution. The hydrophobic portion of the host–guest complex is then incorporated into the cavity of the β-cyclodextrin. The amino compound is sandwiched between the crown ether and the cyclodextrin [18-crown-6+amino compound+β-CD] and thus determines or enhances the enantioselective recognition. It is postulated that the formation of this sandwich results in a more selective chiral interaction between the molecule and β-cyclodextrin. The chiral recognition is dependent upon the formation of this sandwich complex in which additional interactions, such as dipole–dipole, hydrogen bonding and steric repulsion, occur between hydroxyl groups of the β-CD and the functional groups of the amino compound, or between hydroxyl groups of β-CD and the surface of
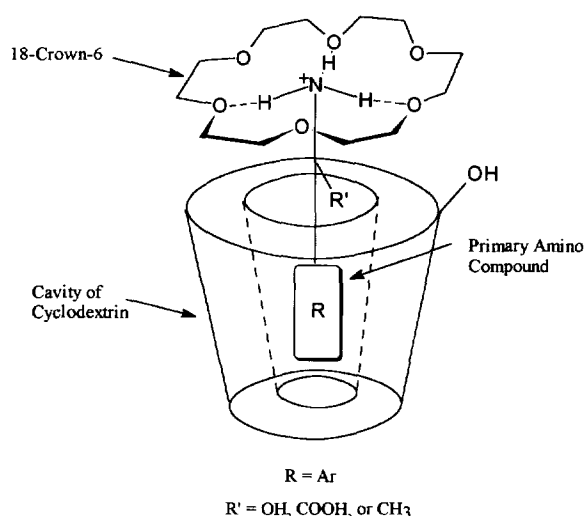

R = Ar
R' = OH, COOH, or CH₃

Fig. 2. Schematic molecular model of the complex consisting of the non-chiral crown ether, the protonated primary amino compound and the β-cyclodextrin.

18-crown-6. Fig. 2 illustrates a possible schematic model of the sandwich complex. In this paper, several amino alcohols, amino acids and the other amino compounds were investigated.

2. Experimental

2.1. Reagents and chemicals

Sodium phosphate monobasic and phosphoric acid were obtained from Aldrich (Milwaukee, WI, USA). Tyrosine, 2,6-di-O-methyl-β-cyclodextrin (DM-β-CD), 1-phenylethylamine, phenylalanine, 1-(1-naphthyl)ethylamine, phenylgycinol and 18-crown-6 were obtained from Sigma (St. Louis, MO, USA). 1-Aminoindan, 2-amino-3-phenyl-propanol and 3-fluorophenylalanine were obtained from Fluka (Ronkonkoma, NY, USA). 3-(p-Chlorophenxoy)-2-hydroxypropylamine was obtained from Sandoz Pharmaceuticals.

All of the samples were prepared in a 50 mM phosphate buffer solution which was made from sodium phosphate monobasic and adjusted with phosphoric acid to pH 2.0. The concentration of these samples was approximately 0.3 mg ml⁻¹. The concentration of DM-β-CD in the buffer solution

was 5 mM for the non-polar compounds, 10 mM for amino alcohols and 20 mM for amino acids. The concentration of the 18-crown-6 was 5 mM for the non-polar amino compounds, 10 mM for the amino alcohols and 10 mM for amino acids.

2.2. Capillary electrophoresis

Capillary electrophoresis was performed on Beckman P/ACE 5000 instrument with a fused-silica capillary tube (67 cm×50 μm I.D.). The capillary was obtained from Polymicro Technologies (Phoenix, AZ, USA). The detector window was located 7 cm from the end of the capillary. The slit aperture in the capillary holder was 100 μm×800 μm. The UV detection wavelength was 214 nm. The electric field applied at a constant strength was 400 V cm^{-1}. The injection time for samples was 4.0 s at low pressure and the separation temperature was 25°C.

3. Results and discussion

The separation results of non-polar amino compounds, amino alcohols and amino acids which were initially analyzed by DM-β-CD alone and then by 18-crown-6 with the DM-β-CD are summarized in Table 1. In Table 1, t_1 represents the migration time of the faster migrating enantiomer, α represents the separation factor (t_2/t_1), and R_s represents the resolution calculated using $R_s=2(t_2-t_1)/(w_2+w_1)$, where t represents the migration time of each enantiomer and w is the peak width at baseline.

3.1. Separation of non-polar amine compound

The enantiomers of 1-phenylethylamine, 1-(1-naphthyl)ethylamine and 1-aminoindan could not be separated using 5 mM DM-β-CD in 50 mM phosphate buffer solution even when a 20 mM concentration of DM-β-CD was used as shown in Table 1. However, the chiral separation was achieved when

Table 1
The chiral separation data of the amino compounds by capillary zone electrophoresis

| STRUCTURES	NAME	CYCLODEXTRIN			18-CROWN-6		
		t_1(min)	α	R_s	t_1(min)	α	R_s
	1-Phenylethylamine	8.66	1.00	0.00	13.5	1.03	1.10
	1-(1-Naphthyl)ethylamine	9.05	1.00	0.00	14.1	1.07	7.78
	1-Aminoindan	8.81	1.00	0.00	12.5	1.15	5.37
	Phenylglycinol	9.58	1.00	0.00	11.8	1.02	0.78
	2-Amino-3-phenyl-propanol	10.6	1.01	0.44	14.7	1.05	1.46
	3-(p-Chlorophenoxy)-2-hydroxypropylamine	16.5	1.01	0.50	20.4	1.03	1.35
	Phenylalanine	20.7	1.00	0.00	23.9	1.02	1.26
	3-Fluorophenylalanine	18.9	1.00	0.00	23.5	1.02	1.13
	Tyrosine	25.8	1.00	0.00	33.4	1.02	1.21

CE conditions refer to the paper.
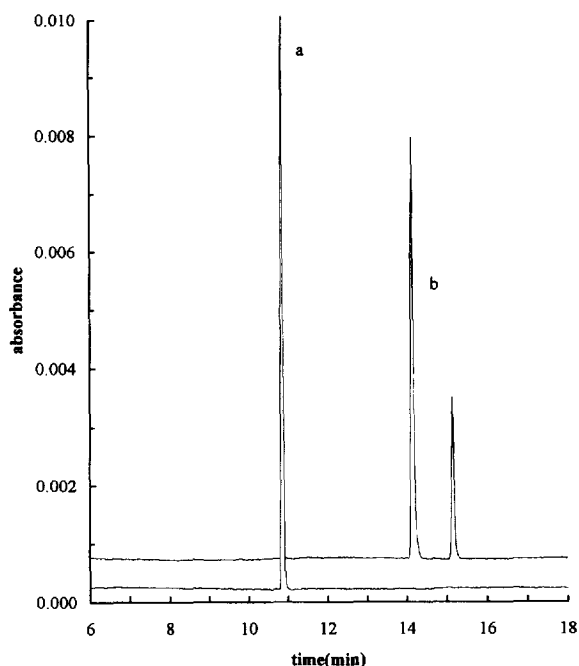
Fig. 3. Chiral separation of 1-(1-naphthyl)ethylamine by CZE. A buffer solution consisting of 50 mM phosphate (pH 2.0), 5 mM DM-β-CD and (a) no the modifier; (b) 5 mM non-chiral 18-crown-6.

the non-chiral 18-crown-6 was added into the buffer solution, as shown in the electropherogram of Fig. 3b. As expected, the migration time increased in the presence of the 18-crown-6. The different peak heights for the isomers of 1-(1-naphthyl)ethylamine are due to different concentrations of the isomers as shown in Fig. 3b. The migration times of 1-phenyl-ethylamine, 1-(1-naphthyl)ethylamine and 1-amino-indan shifted from 8.7, 9.1 and 8.8 min using DM-β-CD alone to 13.5, 14.1 and 12.5 min with 18-crown-6, respectively. Since the concentration of 18-crown-6 was 5 mM (approximate 0.1%), the increase in migration time was due to a change in electrophoretic mobility of the sandwich complex neglecting any change of viscosity in the buffer solution. The increase of the migration time indicates that a selective interaction occurred in the sandwich complex [18-crown-6+amino compound+β-CD] compared with the use of DM-β-CD alone. The chiral resolution of these compounds was dramatically enhanced from 0 to 1.02 for 1-phenylethylamine,

from 0 to 7.78 for 1-(1-naphthyl)ethylamine and from 0 to 5.37 for 1-aminoindan.

3.2. Separation of amino alcohols

Phenylglycinol, 2-amino-3-phenylpropanol, and 3-(p-chlorophenoxy)-2-hydroxypropylamine were also investigated. These compounds have similar hydrophobic groups but differ in chain length between the chiral center and the aromatic ring as shown in Table 1. The enantiomers of phenylglycinol could not be separated by DM-β-CD alone as shown in Fig. 4a. Even though the chiral center in phenylglycinol is adjacent to an aromatic ring, there is still an improper distance between the functional groups of the compound and the secondary hydroxyl groups of DM-β-CD or there exists steric hindrance. When, 10 mM 18-crown-6 was added to the buffer solution, the resolution increased from 0 to 0.78 and the
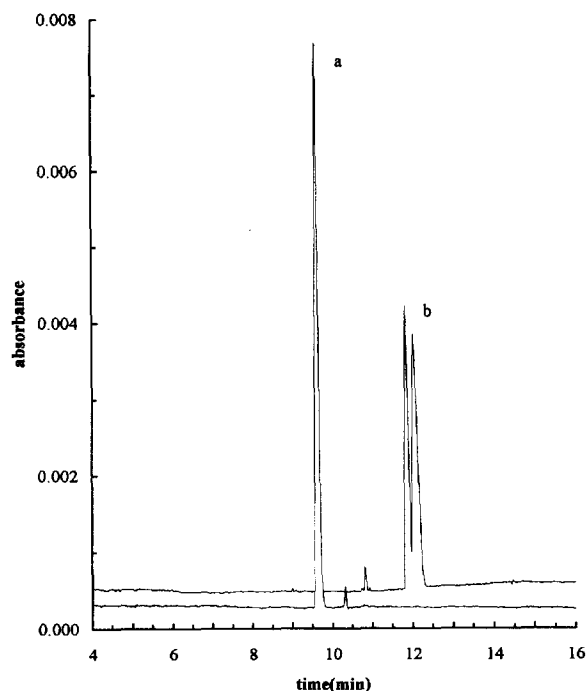

Fig. 4. Chiral separation of phenylglycinol by CZE. A buffer solution consisting of 50 mM phosphate (pH 2.0), 10 mM DM-β-CD and (a) no modifier; (b) 10 mM non-chiral 18-crown-6.

migration time of phenylglycinol increased by 2.2 min as shown in Table 1 and Fig. 4.

The chiral centers of 2-amino-3-phenylpropanol and 3-(p-chlorophenoxy)-2-hydroxypropylamine are located two and three bonds away from the aromatic ring, respectively. The chiral center of these two amino alcohols are more flexible in contrast to that of phenylglycinol. Alternatively, the interaction distances between the functional groups of analytes and the hydroxyl groups of DM-β-CD seem favorable because the enantiomers of these two compounds begin to exhibit some separation using DM-β-CD alone as shown in Table 1. An increase in the concentration of DM-β-CD or addition of organic modifiers, such as methanol or acetonitrile to the buffer did not improve the resolution of these two compounds. However, when 18-crown-6 was used in the separation buffer, the resolution of these compounds was easily increased as shown in Table 1. The resolutions were significantly enhanced from 0.44 to 1.46 for 2-amino-3-phenylpropanol, and from 0.50 to 1.35 for 3-(p-chlorophenoxy)-2-hydroxy-propylamine. The migration time of these compounds increased from 10.6 to 14.7 min for 2-amino-3-phenylpropanol and from 16.5 to 20.4 min for 3-(p-chlorophenoxy)-2-hydroxypropylamine.

3.3. Separation of amino acids

Amino acids, such as phenylalanine, 3-fluoro-phenylalanine and tyrosine, could not be separated by DM-β-CD alone. The separation of these amino acids was still not observed when the concentration of DM-β-CD was increased from 20 to 40 mM in the buffer solution. After addition of 18-crown-6 in the buffer solution, the chiral separations of these three compounds were achieved as shown in Table 1. Fig. 5a and b show the electropherograms of the enantiomers of 3-fluorophenylalanine with and without the 18-crown-6, respectively. The resolution of these compounds was enhanced from 0 to 1.26 for phenyl-alanine, from 0 to 1.13 for 3-fluorophenylalanine and from 0 to 1.21 for tyrosine as shown in Table 1. The individual migration time of phenylalanine, 3-fluoro-phenylalanine and tyrosine increased approximately 3.2, 4.6 and 7.6 min by the addition of the 18-crown-6, respectively.
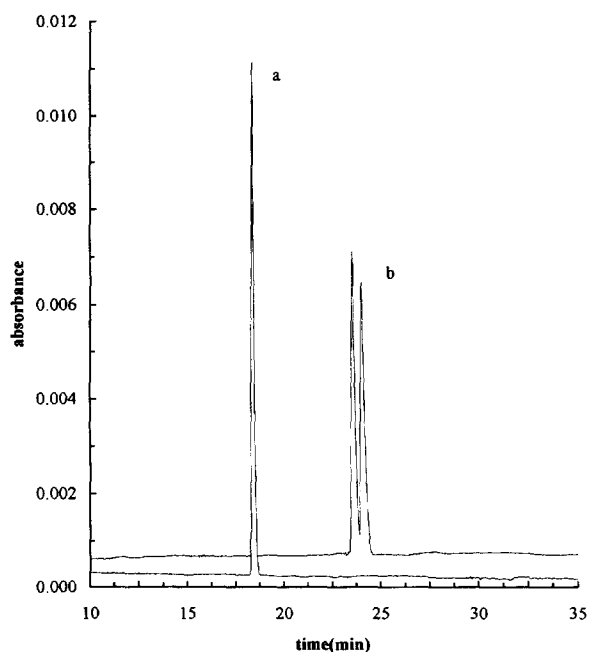

Fig. 5. Chiral separation of 3-fluorophenylalanine by CZE. A buffer solution consisting of 50 mM phosphate (pH 2.0), 20 mM DM-β-CD and (a) no modifier; (b) 10 mM non-chiral 18-crown-6.

4. Conclusion

This paper described a new chiral separation method which employed a non-chiral crown ether (18-crown-6) along with β-cyclodextrin (β-CD) to achieve enantioselective separations of primary amines, such as amino alcohols, amino acids and non-polar amino compounds. The method was used when the enantiomers of the amino compounds could not be resolved by the DM-β-CD alone under the conditions tested. Although the mechanism of the separation is not clear, we postulate that the proton-ated amino group of these compounds forms a selective host–guest complex with the crown ether [amino compound + 18-crown-6]. The hydrophobic portion of the host–guest complex is then incorporated into the cavity of the β-cyclodextrin. The amino compound is sandwiched between the crown ether and the cyclodextrin [18-crown-6 + amino compound + β-CD] and thus determines or enhances the enantioselective recognition. The formation of

this sandwich results in a more selective chiral interaction.

This paper demonstrated the special modification function of the non-chiral 18-crown-6 in DM-β-CD system compared with using DM-β-CD alone. The enantioselectivity for these compounds was significantly improved after the 18-crown-6 ether was added to the separation buffer. The resolution of the separation was dramatically increased while the migration time of these compounds increased by only several minutes.

In addition, the non-chiral 18-crown-6 can also be used with different types of the cyclodextrins, although only DM-β-CD was selected in this paper.

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