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Achievement of enantioselectivity of non-polar primary amines by a non-chiral crown ether

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Abstract

A non-chiral crown ether (18-crown-6), as a molecular modifier, along with cyclodextrin (CD) was used to achieve or enhance enantioselective separations of non-polar primary amines in capillary electrophoresis. Chiral separations of the primary amines are rarely achieved using cyclodextrins alone in capillary electrophoresis, because the three-point intermolecular interaction between the chiral amine and the cyclodextrin is not selective enough to yield resolution of the enantiomers of interest. To resolve these types of compounds, the non-chiral crown ether (18-crown-6) has been used with cyclodextrins (CDs) in the buffer. In the method, the amino group of the compounds is protonated using a low pH buffer solution and locked into 18-crown-6 ring forming a host–guest complex (amine+18-crown-6). The hydrophobic portion of the complex is then incorporated into the cavity of the cyclodextrin forming a secondary sandwich complex (18-crown-6+amine+CD), in which the chiral center of the amine molecule is recognized. It is postulated that the sandwich complex results in a more rigid structure around the chiral center and more selective interaction between the chiral amine and cyclodextrin. This method can be used to separate a variety of non-polar primary amines, even when the chiral center is three-carbon atoms or more from the hydrophobic group. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Buffer composition; Amines; Crown ethers; Cyclodextrins

1. Introduction

Chiral separation of enantiomers by capillary electrophoresis (CE) has become a more and more important technique in chemical and pharmaceutical analysis, because of its high resolution, high efficiency and low cost. Chiral separations by CE have been successful using mobile phase modifiers such as optically active micelles [1], cyclodextrins (CDs) [2–5], derivative CDs [6,7], ligand-exchange [8], macrocyclic antibiotics [9,10], polymer additives [11], mucopolysaccharides [12] and chiral crown ethers [13–18]. One of the common approaches is to use CDs to separate the amine compounds [19–23]. This approach may be effective only when the chiral center and substituents of the compound have a favorable distance from the rim of the CD, and the hydrophobic group of the compound can fit into the cavity of the CD. However, when both of these conditions cannot be met, chiral separation of primary amines has been achieved using a chiral crown ether [13–18] or a chiral crown ether combined with β-cyclodextrin (β-CD) [24]. The chiral 18-crown-6 tetracarboxylic acid used in CE is an expensive product[1], thus restricting its wide spread use in chemical and pharmaceutical industries.

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[1]The price of the chiral 18-crown-6 tetracarboxylic acid is US$81.65/100 mg in the Aldrich catalog.

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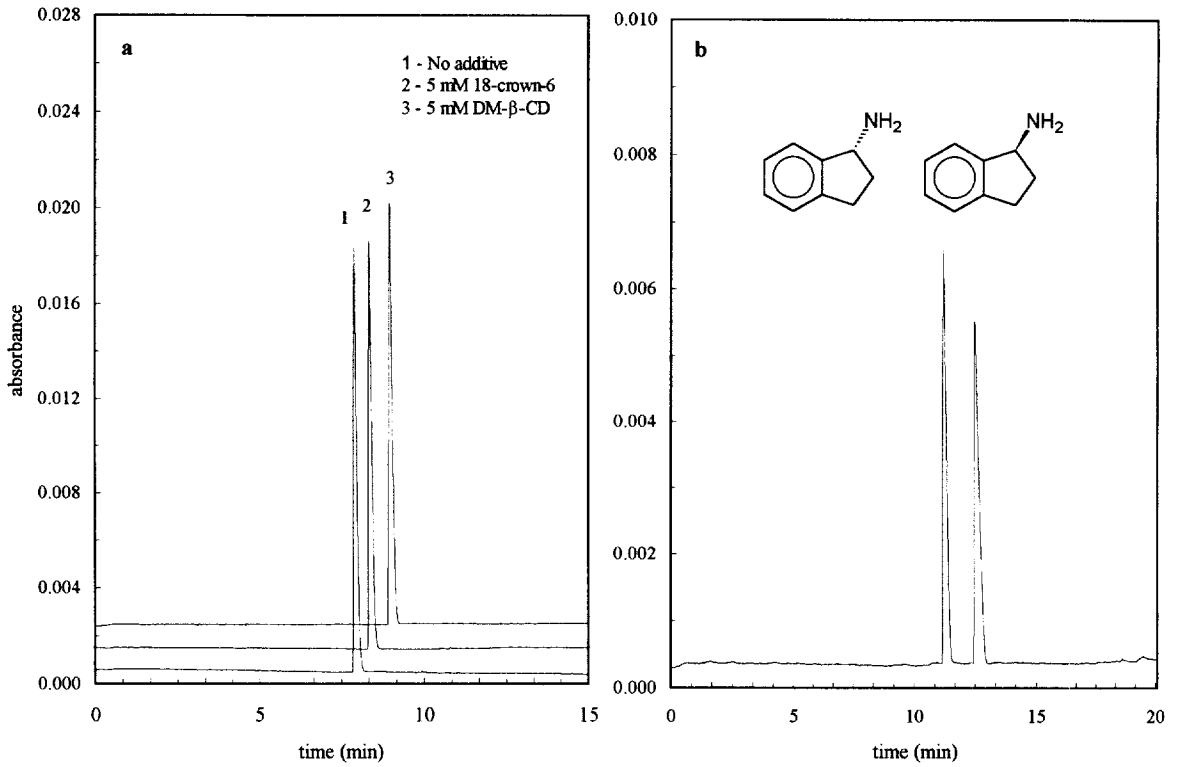

Fig. 1. Effect of non-chiral 18-crown-6 on chiral separation of AI. Buffer solution consisting of 50 m*M* phosphate (pH 2.0) and (a) no additive, 18-crown-6, and DM-β-CD; (b) 5 m*M* 18-crown-6 along with 5 m*M* DM-β-CD.

In our previous paper [25], we reported the use of a non-chiral 18-crown-6, 1,4,7,10,13,16-hexaoxacyclooctadecane[2], with 2,6-di-O-methyl-β-cyclodextrin in a buffer solution to achieve enantioselectivity of primary amines when these compounds could not be separated by the β-CD alone. This work further demonstrates the use of the non-chiral 18-crown-6 with different types of CDs to separate the enantiomers of non-polar primary amines in CE.

2. Experimental

2.1. Materials

Monobasic sodium phosphate, phosphoric acid, 1,2,3,4-tetrahydro-1-naphthylamine (THAN), 1-methyl-3-phenylpropylamine (MPPA), 2-phenyl-1-propylamine (PPA), 2-*sec.*-butylaniline (2BA) and 4-*sec.*-butylaniline (4BA) were obtained from Aldrich (Milwaukee, WI, USA). 1,4,7,10,13,16-Hexaoxacyclooctadecane (18-crown-6), 2,6-di-O-methyl-β-cyclodextrin (DM-β-CD) and 2,4,6-tri-O-methyl-β-cyclodextrin (TM-β-CD) were obtained from Sigma (St. Louis, MO, USA). 1-Aminoindan (AI) was obtained from Fluka (Ronkonkoma, NY, USA). Hydroxylpropyl-β-cyclodextrin (HP-β-CD), hydroxylethyl-β-cyclodextrin (HE-β-CD), γ-cyclodextrin (γ-CD) and α-cyclodextrin (α-CD) were obtained from Advanced Separation Technologies (Whippany, NJ, USA).

2.2. Buffer and sample preparations

The phosphate buffer solutions were prepared from monobasic sodium phosphate and adjusted with phosphoric acid to pH 2.0. All samples were pre-

[2]The price of the non-chiral 18-crown-6 is US$0.27/100 mg in the Aldrich catalog.

pared in aqueous solution at a concentration of approximately 0.5 mg/ml. The concentrations of DM-β-CD, TM-β-CD, HE-β-CD, HP-β-CD, γ-CD and α-CD in the buffer solutions were between 5 m*M* and 40 m*M* without 18-crown-6 present, and between 5 m*M* and 20 m*M* with 18-crown-6. The concentration of 18-crown-6 was between 5 m*M* and 20 m*M* in the buffer solutions.

2.3. Capillary electrophoresis

CE was performed on a 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. The injection time for samples was 3.0 s by pressure and the separation temperature was 23°C.

3. Results and discussion

The non-chiral 18-crown-6 is a polyether compound [26] in which oxygen as an electron-donor in the polyether ring can form a selective complex with suitable metal or organic cations. The 18-crown-6 was used here as a molecular modifier to alter the molecular structure of the enantiomers in which either their chiral center or functional groups had an unfavorable interaction distance with the rim of CDs. In this method, the amino group of the compound is protonated, and carries a positive charge in a low pH buffer solution. The protonated amino group of the compound can form a host–guest complex [27] with the non-chiral 18-crown-6 in the buffer solution. The stability of the host–guest complex in the buffer solution depends on the molecular structure around the amino group of the guest molecule and molecular structure of the crown ether, the host molecule. The hydrophobic portion of the host–guest complex is then incorporated into the cavity of the CD. Hence, the amino compound is sandwiched between the non-chiral crown ether and the CD (18-crown-6+

Table 1
The chiral separation results of non-polar primary amines by cyclodextrins alone and by 18-crown-6 with cyclodextrins

Enantiomers	18-Crown-6	5 m*M* DM-β-CD (50 m*M* phosphate)			10 m*M* HE-β-CD (100 m*M* phosphate)			10 m*M* γ-CD (100 m*M* phosphate)		
		t_1	α	R_s	t_1	α	R_s	t_1	α	R_s
1-Methyl-3-phenylpropylamine	w/o[a]	13.6	1.00	0.00	11.9	1.00	0.0	9.96	1.00	0.0
	w	18.1	1.00	0.0	15.8	1.00	0.0	11.5	1.02	1.2
2-Phenyl-1-propylamine	w/o	9.68	1.00	0.0	91.0	1.00	0.0	8.73	1.00	0.0
	w	16.5	1.02	0.2	12.2	1.02	0.8	10.1	1.00	0.0
4-*sec*.-Butylaniline	w/o	19.9	1.00	0.0	19.2	1.00	0.0	11.5	1.00	0.0
	w	24.5	1.00	0.0	25.7	1.00	0.0	15.1	1.03	0.4
2-*sec*.-Butylaniline	w/o	10.5	1.01	0.0	9.58	1.00	0.0	10.1	1.00	0.0
	w	11.2	1.01	0.5	11.2	1.00	0.0	11.3	1.00	0.0
1-Aminoindan	w/o	8.95	1.00	0.0	8.17	1.00	0.0	8.70	1.00	0.0
	w	11.2	1.13	3.4	10.1	1.10	4.7	12.5	1.06	3.1
1,2,3,4-Tetrahydro-1-naphthylamine	w/o	9.27	1.01	0.0	8.07	1.01	0.3	9.37	1.00	0.0
	w	10.5	1.09	3.2	10.5	1.05	2.7	13.4	1.12	5.0

[a] w/o: without 18-crown-6; w: with 18-crown-6, 5 m*M* 18-crown-6 with 5 m*M* DM-β-CD, 10 m*M* 18-crown-6 with 10 m*M* HE-β-CE and 10 m*M* 18-crown-6 with 10 m*M* γ-CD.

amine+CD) in which the chiral center of the amine is recognized [25]. Fig. 1 shows the electropherograms of AI with different additives. The chiral separation of AI was only successful in the 18-crown-6 with DM-β-CD solution. We have postulated that the formation of this sandwich complex results in a more rigid molecular structure around the chiral center and more selective molecular interaction between the amine and CD. The chiral recognition is dependent on the formation of this sandwich complex in which additional interactions such as dipole–dipole, hydrogen bonding and steric repulsion, occur between the hydroxyl groups of the CD and the functional groups of the amine, or between the hydroxyl group of CD and the surface of 18-crown-6. When one of the enantiomers forms relatively stronger three-point molecular interactions in the sandwich complex than its mirror image, the separation of these enantiomers becomes possible.

The separation results of the non-polar amines, which were initially analyzed by CDs alone and then by 18-crown-6 with CDs, 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) [28] 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. Achievement of enantioselectivity

The chiral center and amino group of MPPA are located three-carbon atoms away from the aromatic group. This distance results in a more flexible chiral center. As a result of the flexibility and distance from the aromatic ring, chiral separation of MPPA was not achieved even though six different types of CDs were used in the buffer solutions as shown in Fig. 2.

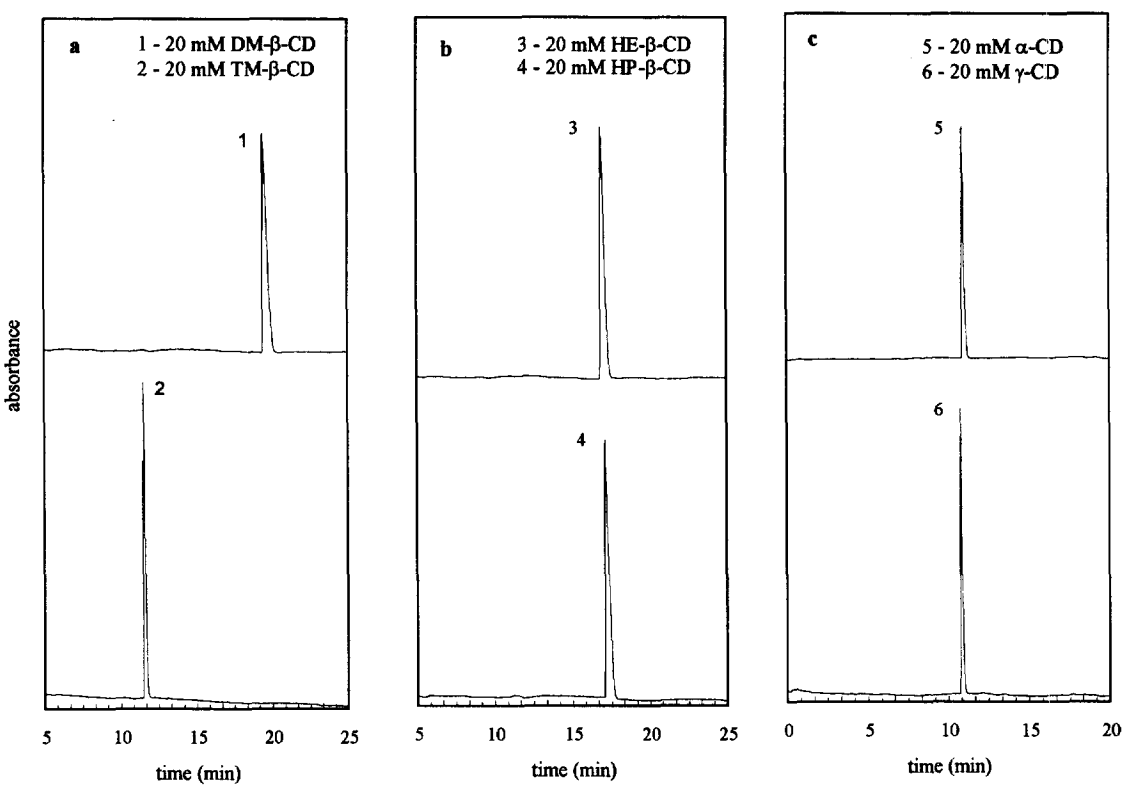

Fig. 2. Chiral separatin of MPPA. Buffer solution consisting of 100 m*M* phosphate (pH 2.0) and (a) different selectivity of the cavities; (b) different depth of the cavities; (c) different size of the cavities.

When the γ-CD concentration was increased from 10 m*M* to 40 m*M*, chiral separation of MPPA was still not achieved as shown in Fig. 3a. Only when 20 m*M* 18-crown-6, the molecular modifier, was added to the 20 m*M* γ-CD buffer, chiral separation of MPPA was successfully achieved, as shown in Fig. 3b. As expected, the migration times of MPPA increased from 10.9 min without 18-crown-6 to 15.1 min with 18-crown-6 present. The migration time increased in the presence of the 18-crown-6, due to the lower mobility of the sandwich complex (18-crown-6+amine+γ-CD). As a result of more selective interaction, the resolution of MPPA dramatically increased from 0 to 2.8.

The chiral center of PPA is adjacent to the aromatic group while the amino group is located two atoms away from the aromatic ring. The chiral center is more rigid due to the close proximity to the aromatic ring. These conditions should allow some selective interactions with the CDs alone. However, in the four CDs studied, chiral separation was not achieved with either DM-β-CD, HP-β-CD, HE-β-CD, or γ-CD as shown in Table 1 and Fig. 4a. When 18-crown-6 was added to each CD solution, chiral separation occurred with DM-β-CD, HE-β-CD and HP-β-CD buffer solution but not with γ-CD, as shown in Table 1 and Fig. 4b. The resolution was from 0 without 18-crown-6 to 0.2 and 0.8 for DM-β-CD and HE-β-CD with the 18-crown-6, respectively. When 20 m*M* 18-crown-6 was used in 20 m*M* HP-β-CD buffer solution, the resolution increased from 0 to 1.3 and the migration time increased by 2 min compared with 20 m*M* HP-β-CD alone, as shown in Fig. 4b.

The chiral center of 4-*sec.*-butylaniline is located in the *para*-position on aniline. In the three different CDs studied, the chiral separation of 4BA was not achieved with either DM-β-CD, HE-β-CD and γ-

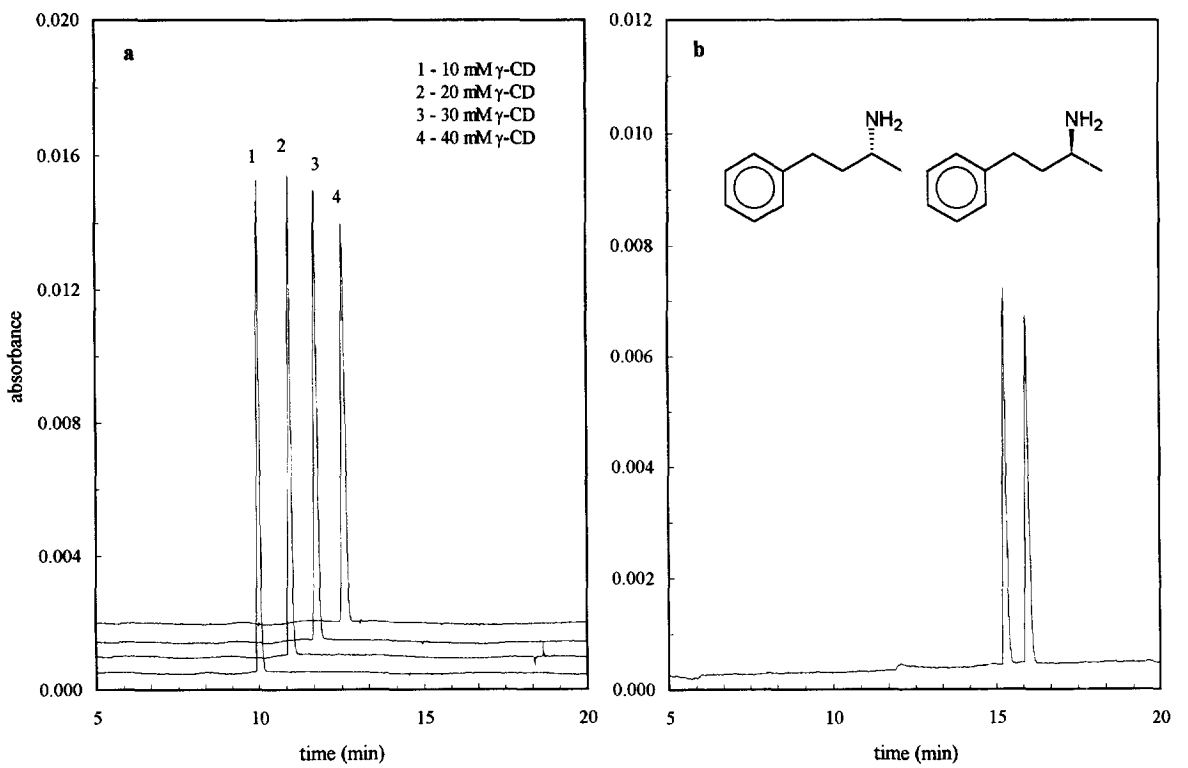

Fig. 3. Chiral separation of MPPA. Buffer solution consisting of 100 m*M* phosphate (pH 2.0) and (a) different concentrations of γ-CD; (b) 20 m*M* 18-crown-6 with 20 m*M* γ-CD.

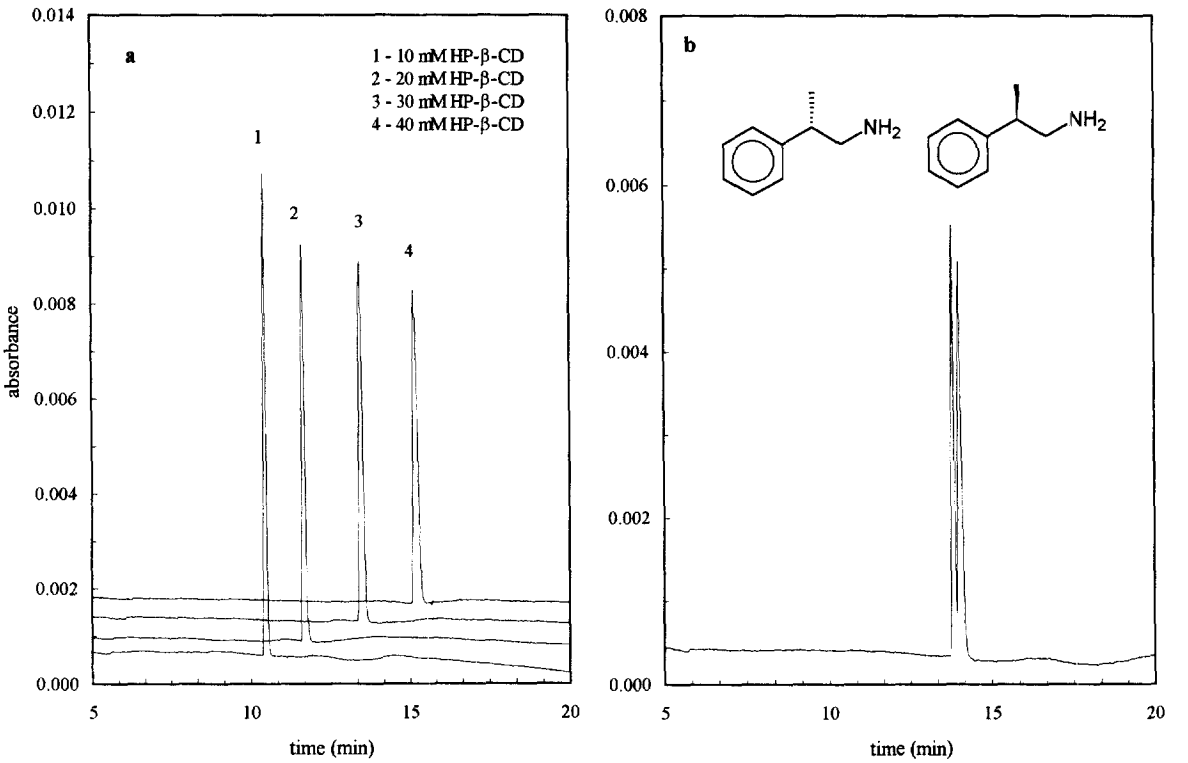

Fig. 4. Chiral separation of PPA. Buffer solution consisting of 100 m*M* phosphate (pH 2.0) and (a) different concentrations of HP-β-CD; (b) 20 m*M* 18-crown-6 with 20 m*M* HP-β-CD.

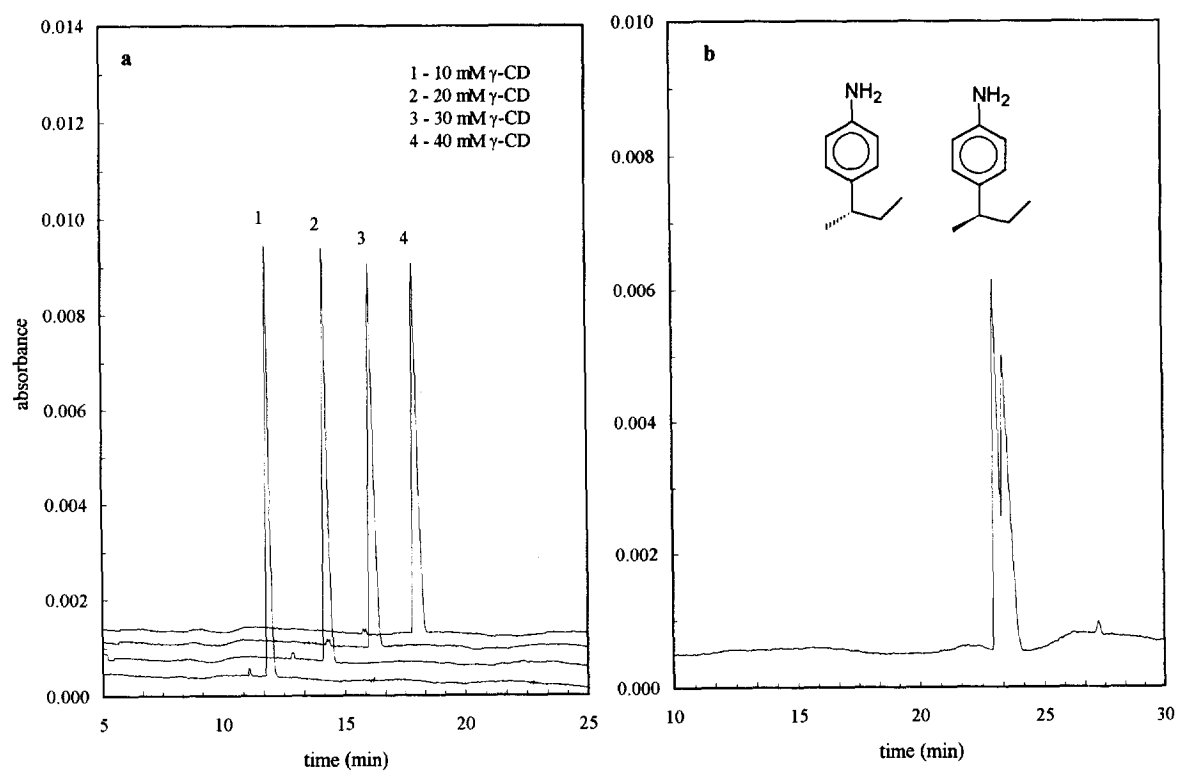

Fig. 5. Chiral separation of 4BA. Buffer solution consisting of 100 m*M* phosphate (pH 2.0) and (a) different concentrations of γ-CD; (b) 20 m*M* 18-crown-6 with 20 m*M* γ-CD.

CD, as shown in Table 1. The chiral separation was also unsuccessful when the γ-CD concentration was increased from 10 m*M* to 40 m*M* as shown in Fig. 5a. However, when the 18-crown-6 was added to each CD solution, chiral separation occurred only with γ-CD as shown in Table 1 and Fig. 5b. In this case, the resolution increased from 0 to 0.7.

3.2. Enhancement of separation resolution

2BA, an isomer of 4BA, has a chiral center located at the *ortho*-position on aniline and the chiral center is adjacent to the amino group. The chiral separation of 2BA, unlike 4BA, can be achieved by DM-β-CD at 10 m*M* and above. The chiral resolution and migration time increased with increasing the DM-β-CD concentration from 10 m*M* to 30 m*M*. When the DM-β-CD concentration was increased to 40 m*M*, the resolution did not continuously increase as shown in Fig. 6a. To enhance the resolution, 10 m*M* 18-crown-6 was added to the 10 m*M* DM-β-CD buffer solution. As a result, the resolution of the separation was further improved as shown in Fig. 6b.

THNA has a bulky cyclic group on its chiral center which was recognized by DM-β-CD alone. The resolution and migration time increased with increasing the DM-β-CD concentration from 10 m*M* to 40 m*M* as shown in Fig. 7a. When the concentration of DM-β-CD was increased to 40 m*M*, the resolution reached 1.2 and the migration time was 14.4 min. The addition of 18-crown-6 to the buffer showed a dramatic increase in the resolution as shown in Fig. 7b. In comparison to 40 m*M* DM-β-CD alone, the migration time of THNA with 18-crown-6 was reduced by 3.8 min and the resolution increased 2.6-fold as shown in Fig. 7b.

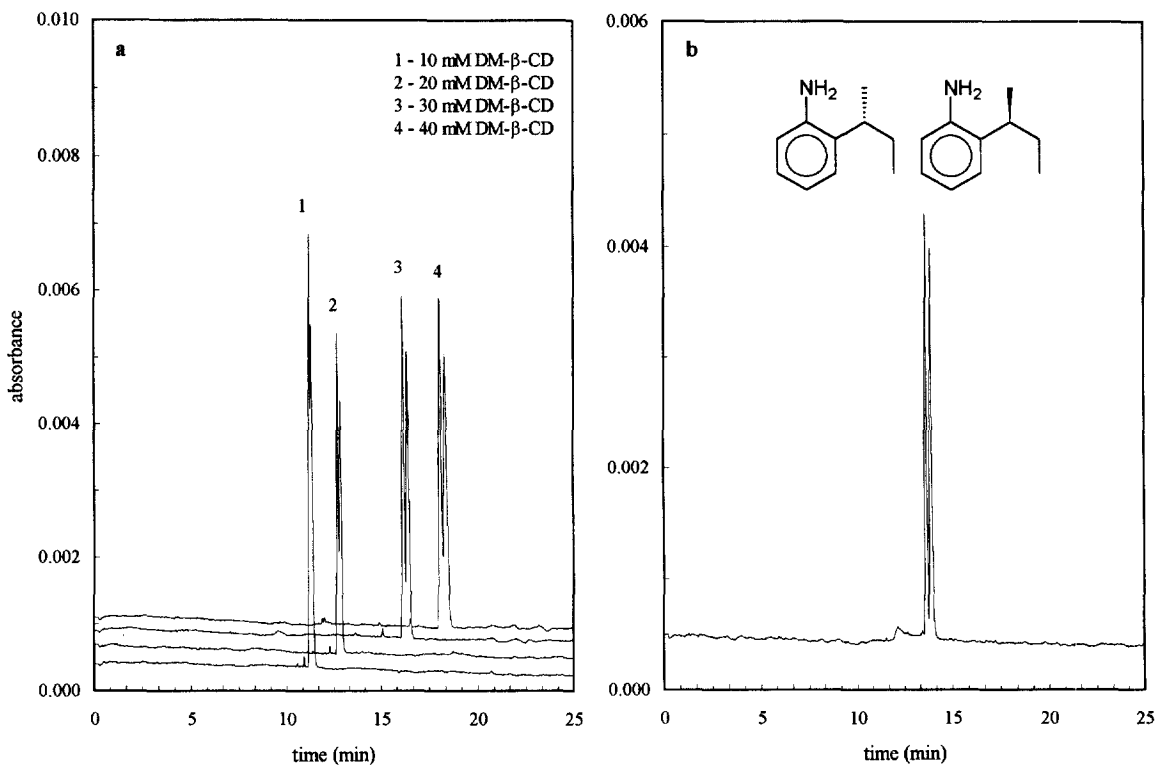

Fig. 6. Chiral separation of 2BA. Buffer solution consisting of 50 m*M* phosphate (pH 2.0) and (a) different concentrations of DM-β-CD; (b) 10 m*M* 18-crown-6 with 10 m*M* DM-β-CD.

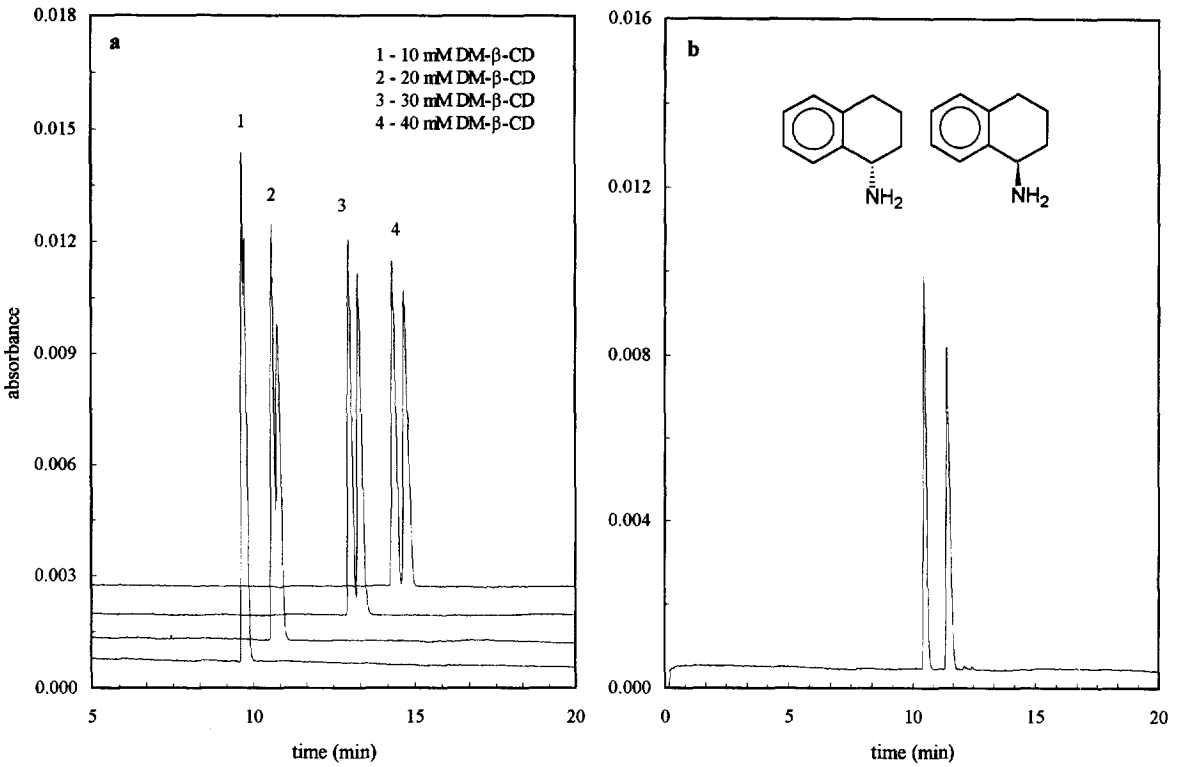

Fig. 7. Chiral separation of THNA. Buffer solution consisting of 50 m*M* phosphate (pH 2.0) and (a) different concentrations of DM-β-CD; (b) 5 m*M* 18-crown-6 with 5 m*M* DM-β-CD.

4. Conclusions

This paper further extends the new chiral separation method which employs a non-chiral crown ether (18-crown-6) along with CDs to achieve or enhance enantioselective separations of non-polar primary chiral amines. We have postulated that the amine is sandwiched between the crown ether and the CD (18-crown-6+amine+CD) and the formation of this sandwich complex results in more selective chiral interaction. From the chiral separations studied in this paper, the best resolution is achieved when the amino and aromatic group are both attached directly to the chiral center.

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