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Enhancement of chiral recognition by formation of a sandwiched complex in capillary electrophoresis

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Abstract

For chiral primary amino compounds not separable by cyclodextrins alone, chiral recognition was successfully achieved by the formation of a sandwiched complex of the non-chiral 18-crown-6, the chiral amine and cyclodextrin (CD) [18-crown-6+amino compound+CD]. The separation of 1-methyl-3-phenylpropylamine and 1,2,3,4-tetrahydro-1-naphthylamine racemates showed the special function of the non-chiral 18-crown-6 on chiral recognition. By formation of the sandwiched complex, the chiral center of 1-methyl-3-phenylpropylamine was successfully recognized, and resolution of 1,2,3,4-tetrahydro-1-naphthylamine dramatically increased. In these studies, the mobility differences of the enantiomers were evaluated as a function of the concentration of cyclodextrins with and without the 18-crown-6, and as a function of the concentration of the 18-crown-6. In addition, the separations by this method were compared to those by the chiral 18-crown-6 reagent.

Keywords: Chiral recognition; 18-Crown-6; Amino compounds, primary

1. Introduction

Chiral separations by capillary electrophoresis have been reported by using a variety of chiral selectors, such as optical active surfactant [1], cyclodextrins (CDs) and their derivatives [2–5], ligand-exchange [6], macrocyclic antibiotics [7], polymer additives [8], mucopolysaccharides [9], chiral crown ether [10–12] and so on. One of the most common approaches uses cyclodextrins to separate amino compounds [13,14]. Cyclodextrins alone may be effective if the chiral center and substituents of the compound have favorable interactions with rim of CD, and the bulky hydrophobic group of the compound can fit into the cavity of the CD. However, if either of these conditions are not met, no chiral separation is observed. For the compounds with a primary amino group, the separation in most situations can be achieved by using a chiral crown ether, 18-crown-6 tetracarboxylic acid [10–12]. Nevertheless, the chiral 18-crown-6 used in capillary electrophoresis is a very expensive product, thus restricting its widespread use in the chemical and pharmaceutical industries.

In our previous work, we have reported that using a non-chiral 18-crown-6 with cyclodextrins achieved the chiral recognition of primary amino compounds. A variety of the amino compounds with different substituents were successfully separated by this method [15–18]. Armstrong et al. did some further studies on the method. Their results indicated that in most cases the addition of the non-chiral 18-crown-6 in buffer solution was significantly enhanced enantioselectivity of these separations. However, in some cases the addition of 18-crown-6 was detrimental to the separations [19]. This paper focuses on the effect

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of the non-chiral 18-crown-6 on chiral separations of 1-methyl-3-phenylpropylamine and 1,2,3,4-tetrahydro-1-naphthylamine. The mobility differences of these enantiomers as a function of the concentration of cyclodextrins with and without the 18-crown-6, and as a function of the concentration of the 18-crown-6 with a fixed CD concentration are discussed. In addition, the separations by this method are compared to those by the chiral 18-crown-6 method.

2. Experimental

2.1. Chemicals and reagents

Sodium phosphate monobasic, phosphoric acid, 1,2,3,4-tetrahydro-1-naphthylamine (THNA), [18-crown-6]-2,3,11,12-tetracarboxylic acid (chiral 18-crown-6), citric acid and 1-methyl-3-phenylpropylamine (MPPA), were obtained from Aldrich (Milwaukee, WI, USA). 1,4,7,10,13,16-Hexaoxacyclooctadecane (non-chiral 18-crown-6), tris-[hydroxymethyl]aminomethane, and 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD) were obtained from Sigma (St. Louis, MO, USA). γ-Cyclodextrin (γ-CD) were obtained from Advanced Separation Technologies (Whippany, NJ, USA).

2.2. Buffer and sample preparations

The phosphate buffer solutions were prepared from sodium phosphate monobasic and adjusted with phosphoric acid to pH 2.0. The concentrations of DM-β-CD, and γ-CD in the buffer solutions were between 1 m*M* and 60 m*M* without or with 10 m*M* 18-crown-6. The concentration of 18-crown-6 was between 1 m*M* and 60 m*M* with 15 m*M* γ-CD and with 10 m*M* DM-β-CD for MPPA and THNA, respectively. The Tris–citrate buffer solution was prepared from tris[hydroxymethyl]aminomethane and adjusted with citric acid to pH 2.0. The concentration of the chiral 18-crown-6 in the buffer solution was 10 m*M*. The samples prepared in aqueous solutions were approximately 0.5 mg/ml.

2.3. Capillary electrophoresis

Capillary electrophoresis was performed on Beckman P/ACE 5000 instrument with a fused-silica capillary tube (67 cm×50 μm I.D.) which was obtained from Polymicro Technologies (Phoenix, AZ, USA), The capillary was pretreated with 0.1 *M* NaOH for 20 min (by high pressure) and rinsed with deionized water for 20 min. The detector window was located 7 cm from the end of the capillary. The slit aperture in the capillary holder was 800 μm×100 μm. The UV detection wavelength was 214 nm. The electric field applied at a constant strength was 30 kV. The injection time for samples was 5.0 s by pressure and the separation temperature was 23°C.

3. Results and discussion

The non-chiral 18-crown-6 is a polyether ring compound, in which the oxygen can form a selective complex with suitable metal or organic cation [20]. The 18-crown-6 was employed in this work as a molecular modifier to alter the molecular structure of the chiral molecule, when either the chiral center or the substituents of the analyte had an unfavorable interaction or improper distance with the rim of the cyclodextrin. In this method, the amino group of the compound is protonated in the lower pH buffer solution. The protonated amino group of the compound forms a host–guest complex [21] with the 18-crown-6. The host–guest complex [18-crown-6+amino compound] can further interact with cyclodextrin to form a secondary complex. Hence, the amino compound is sandwiched between the 18-crown-6 and cyclodextrin [18-crown-6+amino compound+CD]. We have postulated that the formation of this sandwiched complex results in a more rigid molecular structure around the chiral center and creates more selective molecular interaction between the amino compound and cyclodextrin [15,16]. Although the sandwiched complex is metastable in the buffer solution, the chiral recognition is dependent on its formation. When one of the enantiomers forms relatively stronger molecular interactions in the sandwiched complex than that of its mirror image, the chiral recognition can be achieved.

In the following discussions, the mobility differ-

ence ($\Delta\mu$) is used rather than resolution. The mobility difference ($\Delta\mu$) is one of the main separation characteristics and has a simple mathematical formula which does not contain diffusion, and other factors such as injection volume and detector length and so on. The mobility (μ) is calculated from $\mu=(L_tL_d)/(Vt_m)$, where L_t and L_d are the total length of the capillary and the length of the capillary to detector, respectively, V is applied voltage and t_m is the migration time of analyte. In this method, the electroosmatic flow is neglected due to the buffer solution at pH 2.0. The mobility difference ($\Delta\mu$) is calculated from $\Delta\mu=\mu_2-\mu_1$, where μ_1 and μ_2 represent the mobility of enantiomers with the longer and shorter migration times, respectively. The enantiomer which forms the stronger molecular interactions in the sandwiched complex will have the longer migration time.

3.1. Enantiomeric separation of MPPA

The chiral center and amino group of MPPA are located three-carbon atoms away from the aromatic group. Due to this kind of the molecular structure,

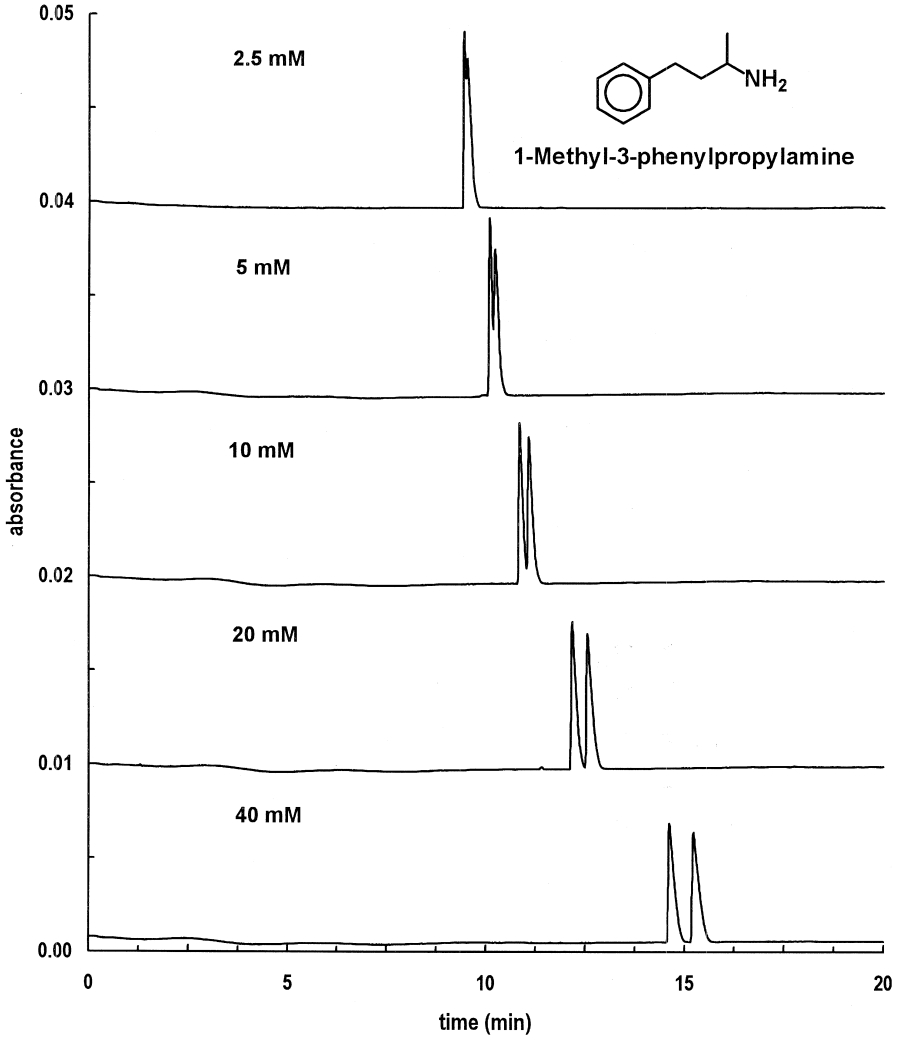

Fig. 1. Effect of non-chiral 18-crown-6 on chiral separation of MPPA. Buffer solutions consisting of 50 m*M* phosphate (pH 2.0) and 10 m*M* 18-crown-6 with different concentrations of γ-CD.

the chiral separation of MPPA was not achieved by using six different types of cyclodextrins, α-CD, β-CD, DM-β-CD, TM-β-CD, HP-β-CD and γ-CD [17,18].

To achieve the chiral separation, the non-chiral 18-crown-6 was used in the buffer solution. The purpose of using the non-chiral 18-crown-6 is to modify the molecular structure of MPPA. When the amino group of MPPA was locked into the 18-crown-6 ring, the flexibility of the chiral center and enantioselective interaction sites of MPPA were changed. As a result of the modification, the chiral molecules in the complex [18-crown-6 + MPPA] can be effectively recognized by γ-CD. No separation was observed by other types of CDs with 18-crown-6, probably due to the unfavorable molecular interaction, or improper interaction distance among the CD, MPPA, and the 18-crown-6. Fig. 1 shows the separation of MPPA by 18-crown-6 with different concentrations of γ-CD. When γ-CD concentration was used from 2.5 m*M* to 60 m*M* without 18-crown-6, no separation of MPPA was observed. Only when 10 m*M* 18-crown-6 was added to the buffer solution, the chiral recognition was achieved. With 20 m*M* γ-CD and 10 m*M* 18-crown-6 in the buffer solution, baseline resolution was observed.

As we expected, the migration time of MPPA increased when 18-crown-6 is present in the buffer solution. The change of the migration time (lower mobility) supports the hypothesis for the formation of the sandwiched complex [18-crown-6 + amino compound + γ-CD]. To further elucidate the effect of the non-chiral 18-crown-6 on the chiral separation, the mobility difference of the enantiomers was plotted as a function of the concentrations of γ-CD with and without 18-crown-6. Two different curves were obtained as shown in Fig. 2. When the separation buffer contained γ-CD alone, no mobility difference between the enantiomers was found. The mobility difference was observed only when the

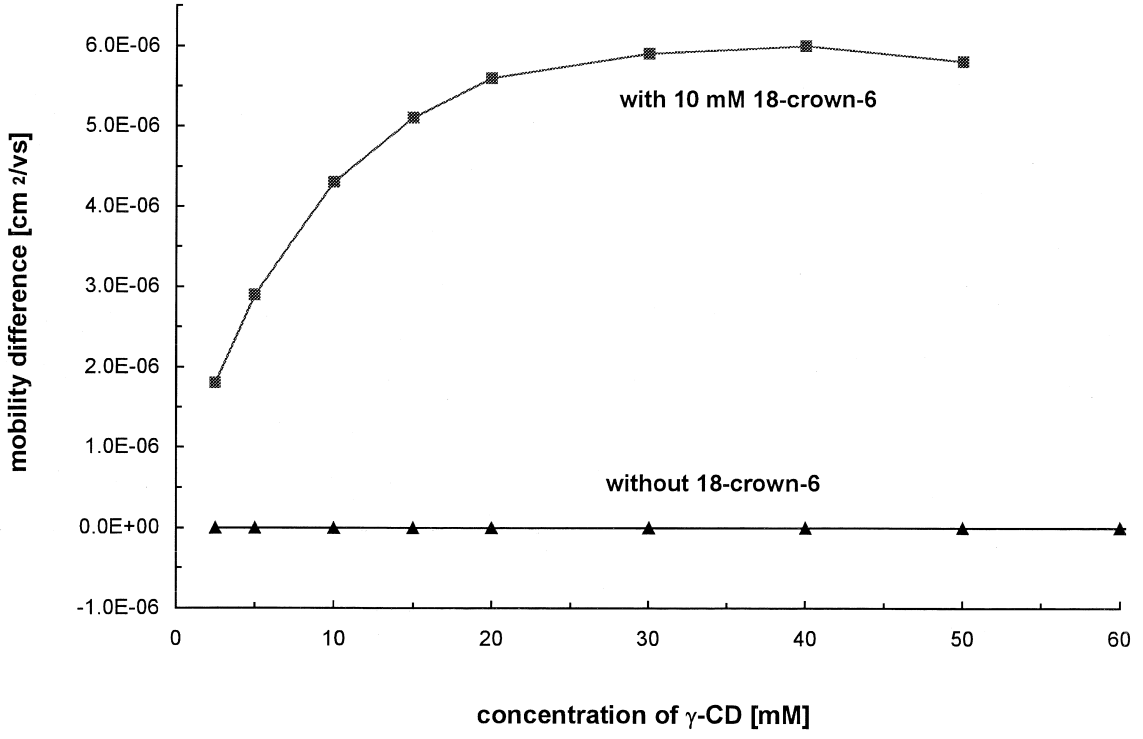

Fig. 2. The mobility differences of MPPA as a function of the concentrations of γ-CD with and without the 18-crown-6.

18-crown-6 was included in the buffer solutions. With 10 m*M* 18-crown-6, the maximum mobility difference was obtained at 40 m*M* γ-CD. In the curve, the point of 60 m*M* γ-CD is absent because the buffer solution became cloudy at that concentration with presence of 10 m*M* 18-crown-6. Fig. 3a shows the mobility difference of the enantiomers as a function of the concentration of 18-crown-6 with 15 m*M* γ-CD. This curve clearly shows that the effect of the concentration of 18-crown-6 on the chiral separation. With the increasing concentration of 18-crown-6, the mobility difference significantly increased up to 40 m*M* 18-crown-6. The maximum of mobility difference was found at 40 m*M* 18-crown-6 with 15 m*M* γ-CD. Above 40 m*M* 18-crown-6, the mobility difference decreased. These two plots obviously reveal that the mobility difference of the enantiomers can be changed by altering the ratio of the concentrations of γ-CD and 18-crown-6 in the buffer solution.

It should be noted that the separation of MPPA by 18-crown-6 with γ-CD perhaps has a different separation mechanism than that by CD alone. In general, chiral compounds like MPPA with single aromatic ring and single substituent on the ring are expected to be separated by a small size cavity CD such as α-CD, β-CD or their derivatives, because one of the three-point chiral selection interactions is based on the cavity inclusion [22]. If the cavity of CD used is much larger than the size of the aromatic ring, the inclusion interaction may become ineffective. In our method, the chiral recognition of the sandwiched complex appears to be less dependent on the cavity size. In the sandwiched complex, the three-point molecular interactions may be established among the secondary hydroxyl groups of the CD, the substituents of MPPA, and the surface of 18-crown-6.

Fig. 4 shows the separation results of MPPA by 10 m*M* non-chiral 18-crown-6 with 15 m*M* γ-CD and by 10 m*M* chiral 18-crown-6. Under the same CE conditions, the enantiomeric separation is superior with the non-chiral 18-crown-6 sandwiched complex. The resolution of MPPA with non-chiral 18-crown-6

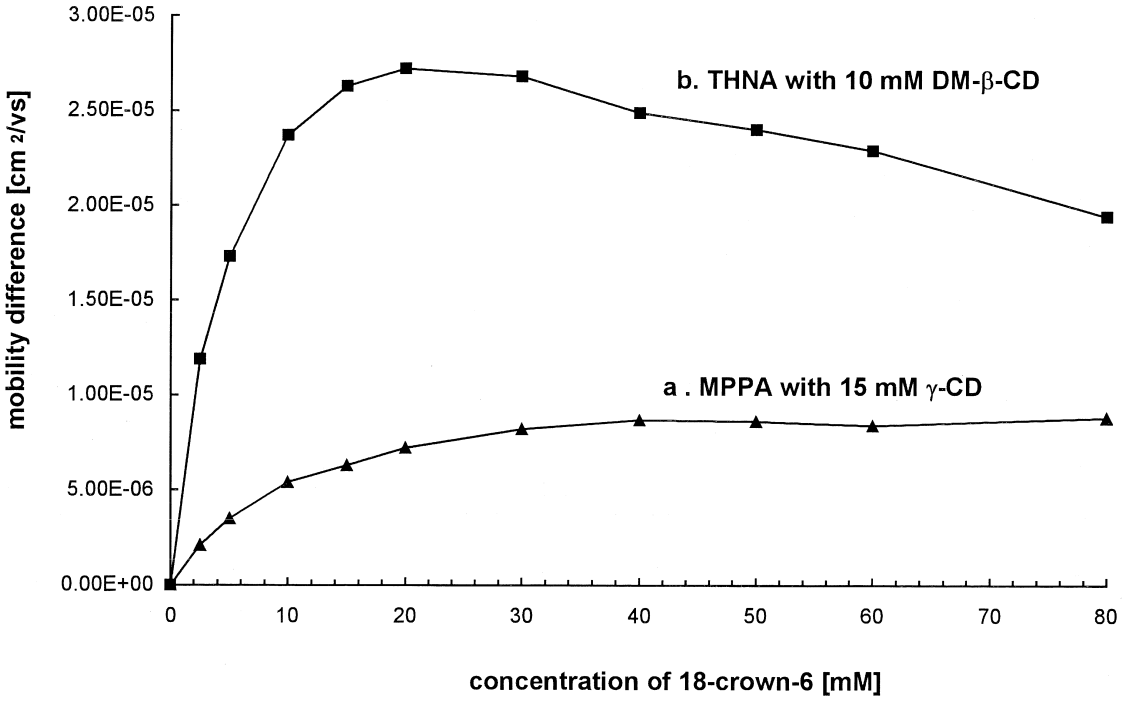

Fig. 3. The mobility differences of MPPA and THNA as a function of the concentrations of the 18-crown-6.

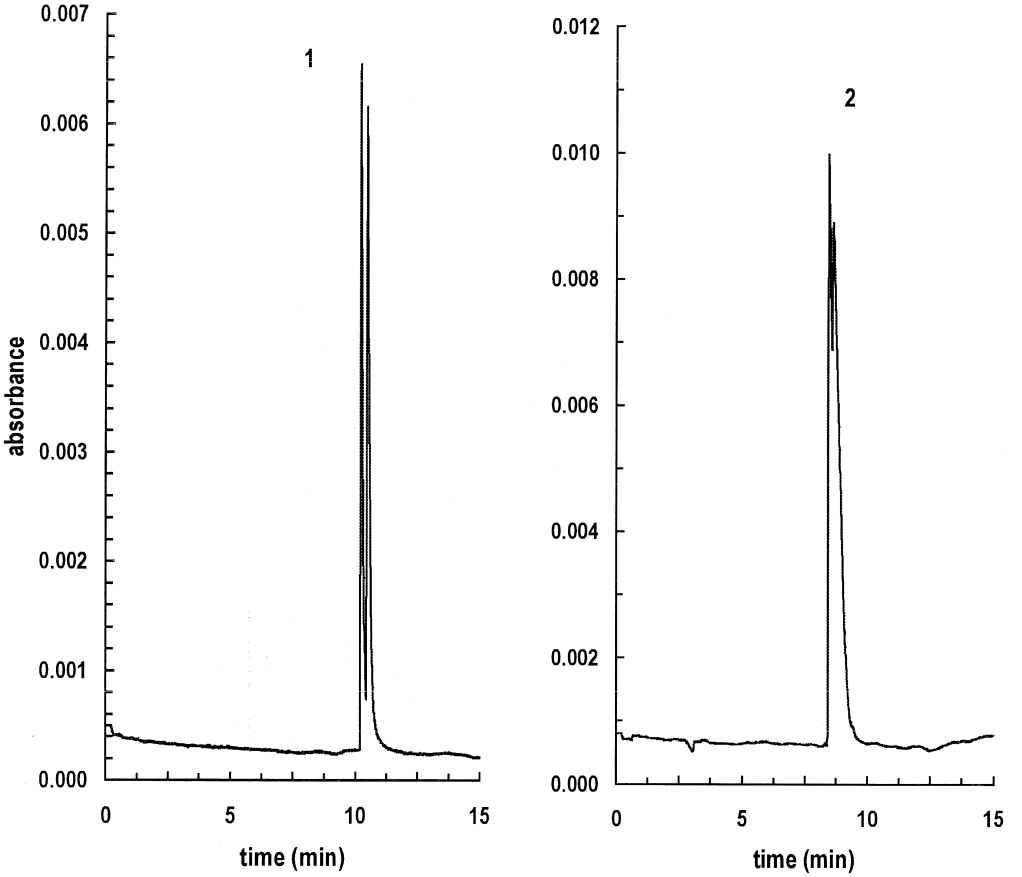

Fig. 4. Chiral separation of MPPA by: (1) 50 m*M* phosphate (pH 2.0), 10 m*M* non-chiral 18-crown-6 and 10 m*M* γ-CD; (2) 30 m*M* Tris–citrate (pH 2.0) and 10 m*M* chiral 18-crown-6.

and with chiral 18-crown-6 is 1.3 and 0.3, respectively. The migration time in both of the methods is approximately the same.

3.2. Enantiomeric separation of THNA

When marginal resolution can be achieved with CDs alone, the addition of 18-crown-6 can dramatically improve chiral resolution for some amino compounds. One of the typical examples is the separation of THNA racemate. THNA has a bulky cyclic group on its chiral center, and is recognized by HP-β-CD, DM-β-CD or γ-CD alone. The separation by DM-β-CD is shown in Fig. 5. When DM-β-CD was used at low concentration, no separation was observed. Using 18-crown-6 in DM-β-CD solution showed a dramatic increase in resolution as shown in Fig. 6. In comparison to using DM-β-CD alone, the migration time of THNA with 18-crown-6 increased a few minutes, but resolution increased several times.

Fig. 7 shows that the mobility difference of the enantiomers was plotted as a function of the concentration of DM-β-CD with and without 10 m*M* 18-crown-6. Two distinct curves are observed. The lower curve without 18-crown-6 shows very small mobility difference after the concentration of DM-β-CD increased above 10 m*M*. However, the upper

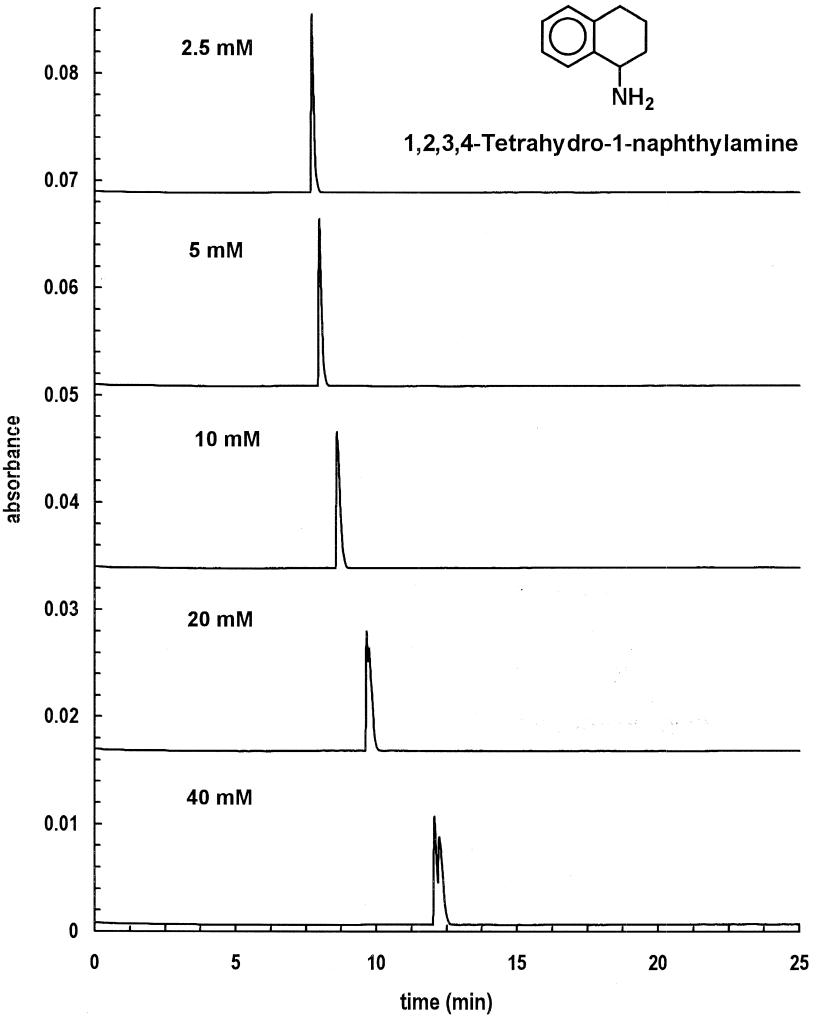

Fig. 5. Chiral separation of THNA. Buffer solutions consisting of 50 m*M* phosphate (pH 2.0) with different concentrations of DM-β-CD.

curve with 10 m*M* 18-crown-6 shows large mobility difference even at 2.5 m*M* DM-β-CD. The mobility difference of the enantiomers at 20 m*M* DM-β-CD with 10 m*M* 18-crown-6 is approximately 9 times as large as that at 20 m*M* DM-β-CD without 18-crown-6. When the mobility difference was plotted as a function of the concentration of 18-crown-6 with 10 m*M* DM-β-CD, the mobility difference also yields an optimum concentration as shown in Fig. 3b. The optimum condition for the separation was found at 15–30 m*M* 18-crown-6 with 10 m*M* DM-β-CD. By comparison of these curves, the effect of the concentration of 18-crown-6 on the separation was also very significant, although the compound was able to be marginally separated by DM-β-CD alone.

The separation result of THNA by 10 m*M* non-chiral 18-crown-6 with 10 m*M* DM-β-CD was also compared with that by 10 m*M* chiral 18-crown-6. Under the same capillary electrophoresis conditions, The enantiomeric separation of THNA in both of the methods is approximately the same. The resolution of these two methods are larger than 3.5. The

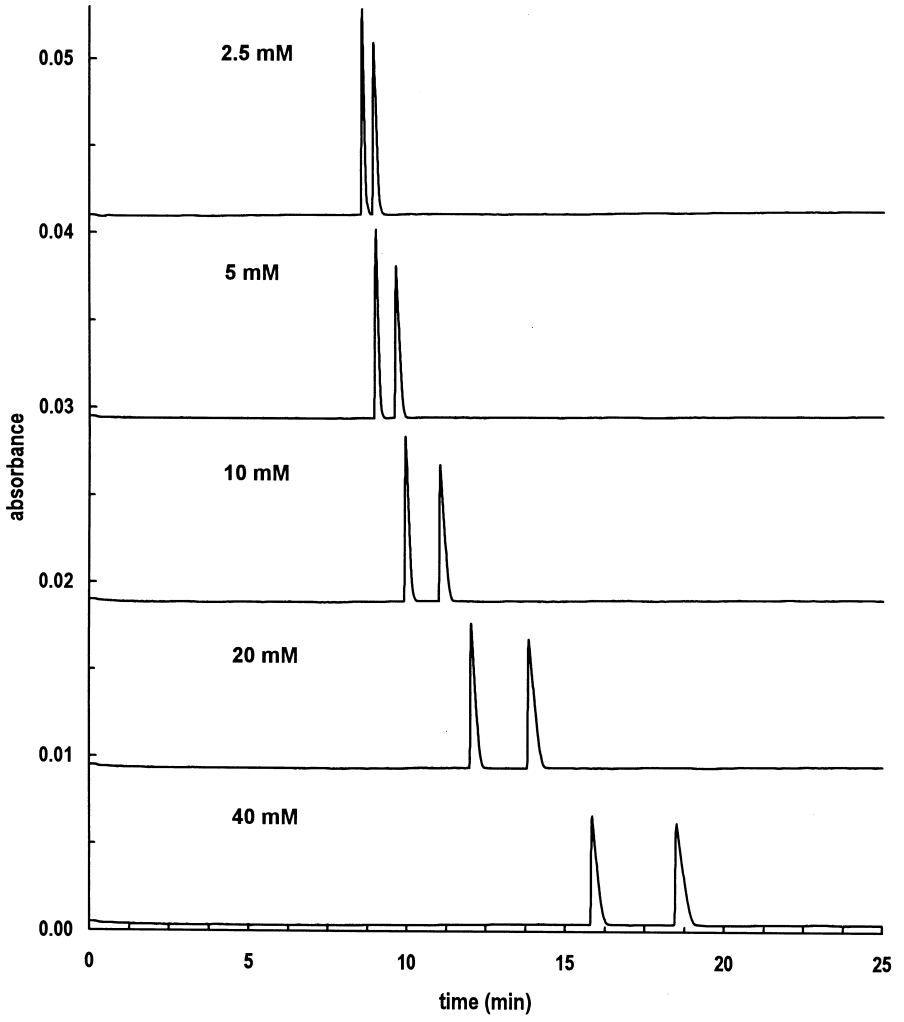

Fig. 6. Effect of non-chiral 18-crown-6 on chiral separation of THNA. Buffer solutions consisting of 50 m*M* phosphate (pH 2.0) and 10 m*M* 18-crown-6 with different concentrations of DM-β-CD.

migration times of THNA with non-chiral 18-crown-6 and with chiral 18-crown-6 are 11.3 min and 7.8 min, respectively.

4. Conclusion

The chiral separations of MPPA and THNA described above illustrate the effect of the non-chiral 18-crown-6 on chiral recognition in capillary electrophoresis. By forming the sandwich complex [18-crown-6 + amino compound + CD], the chiral center of primary amino compounds can be effectively recognized. The chiral separation of the amino compounds is dependent on both of the molecular modifier and chiral selector. The mobility difference of the enantiomers in the sandwich complex method show dramatic increases compared with the use of CD alone. In addition, the optimum conditions for these separations can be found by adjusting the ratio of the concentrations of 18-crown-6 and the CD.

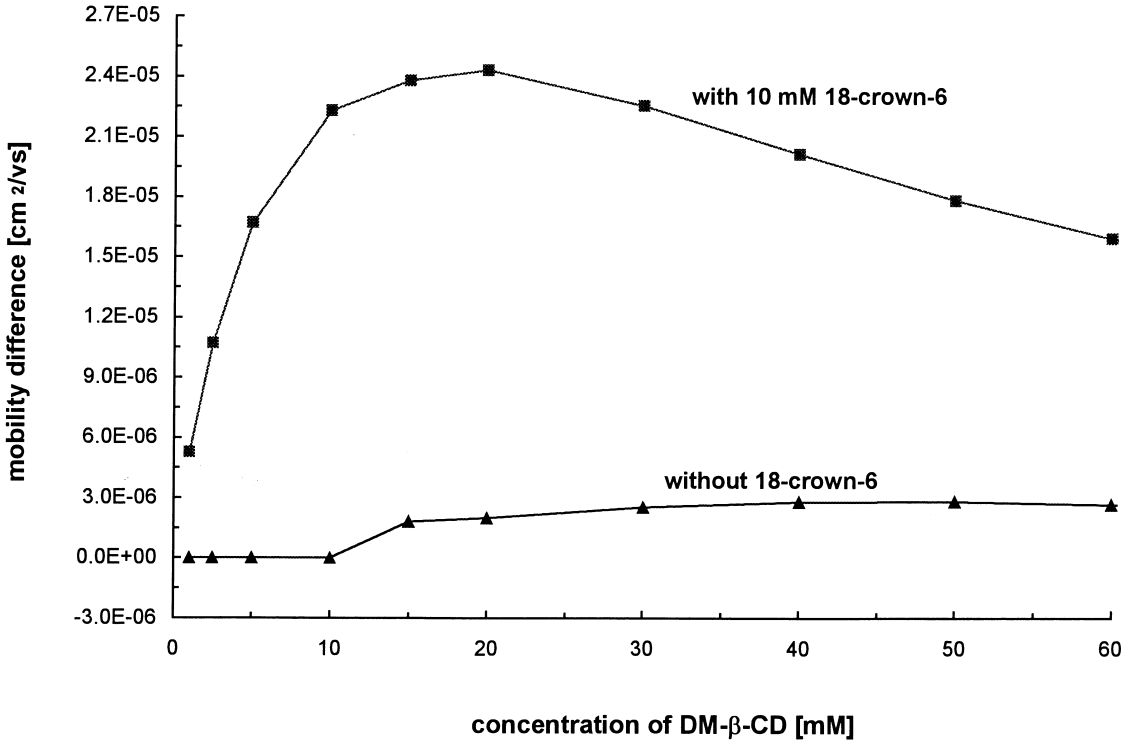

Fig. 7. The mobility differences of THNA as a function of the concentrations of γ-CD with and without the 18-crown-6.

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