



Computational modeling of capillary electrophoretic behavior of primary amines using dual system of 18-crown-6 and β -cyclodextrin

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ABSTRACT

Using capillary electrophoresis (CE) three chiral primary amine compounds 1-aminoindan (AI), 1-(1-naphthyl)ethylamine (NEA) and 1,2,3,4-tetrahydro-1-naphthylamine (THAN), exhibited only partial or no separation when β -cyclodextrin (β CD) was used as chiral selector. The use of 18-crown-6 (18C6) as a second additive with β CD resulted in an enhanced separation. A molecular modeling study, using molecular mechanics and the semiempirical PM6 calculations, was used to help explaining the mechanism of the enantiodifferentiation and to predict the separation process. Optimization of the structures of the complexes by the PM6 method indicate that the poor separation obtained in the presence of the β CD chiral selector alone is due to the small binding energy differences ($\Delta\Delta E$) of 4.7, 1.1 and 1.2 kcal mol⁻¹ for AI, NEA and THAN, respectively. In the presence of 18C6 it was suggested that a sandwich compound between 18C6, amine and β CD is formed. Theoretical calculations show that a significant increase in the binding energy is obtained for the sandwich compounds indicating strong hydrophobic and van der Waals interactions that show enhanced enantiodifferentiation.

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1. Introduction

Chiral aromatic amines constitute important building blocks and intermediates in the synthesis of pharmaceuticals, agrochemicals, and fragrances and there has also been a trend towards the increased use of such amines as enantiomeric catalysts instead of the more traditional metallic compounds [1]. The determination of the enantiomeric purity of these compounds is therefore becoming increasingly important. The chiral separation of these compounds have been achieved by capillary electrophoresis using chiral crown ether [2–7], and cyclodextrins (CDs) in combination with non-chiral crown ether [8–13].

Among the various chiral selectors cyclodextrins (CDs) and their derivatives as well as macrocyclic antibiotics are the most widely used chiral selectors as mobile phase additives in liquid chromatography or as running buffer additives in capillary electrophoresis [14–18]. The mechanism of resolution by chiral stationary phases (CSP) or by chiral mobile phase additives (CMPAs) have been discussed in a number of excellent review articles recently published [19–25]. In CMPA the formation of diastereomeric inclusion complexes that possess different retention or migration characteristics are believed to result in the chiral recognition. Based on the size and

conformation of the enantiomer and the size of the chiral CD cavity one of the enantiomers will fit more strongly than the other enantiomer resulting in complexes with different binding constants [20]. The formation of these inclusion complexes involves a number of intermolecular interactions such as steric forces, electrostatic, hydrophobic, hydrogen bonding, and dipole–dipole interactions. Similarly in macrocyclic antibiotics it was reported that enantioselectivity is affected by the stereospecific interactions of the analyte with the macrocyclic cavity which act as an inclusion site [18]. Chiral recognition in macrocyclic based chiral separations was reported to be enhanced by the rigidity and bulkiness of the analytes, however no general mechanism is postulated.

The theoretical basis for the mechanism of separations involving chiral selectors added to the background electrolyte in capillary electrophoresis (CE) methods is well documented in the literature [26–30]. The major requirement for enantioselectivity in CE is believed to be the complexation between the enantiomeric analyte and the chiral selector. The mass-to-charge ratio governs the movement of the free analyte, the selector and the analyte–selector complex towards the detector. Clearly the mobilities of the free enantiomers are equal; therefore complexation between the analyte and the chiral selector must result in change in the effective mobilities of analytes. Therefore, formation of transient diastereomeric complexes of different binding constants may lead to different mobilities. The time for which the enantiomers reside in the free and complexed form is determined by the strength of intermolecular interactions between the analyte and the chiral selector.

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Furthermore different mobilities of the diastereomeric complexes may originate from differences in the fit of guests into the host resulting in differences in the shapes and net charges of these complexes [26–30].

Crown ethers are synthetic macrocyclic polyethers that form host–guest complexes with several inorganic and organic cations like alkali- and earth alkali metal ions, as well as organic compounds with an amino group [31–34]. Crown ethers are extensively applied as buffer additive in CE; the applications have been thoroughly investigated and reviewed [34–36].

It has been reported that the combination of a non-chiral crown ether, 18-crown-6 (18C6), with CDs could produce enantioseparations of racemic amines that could not be resolved or are partially resolved using only the CD [10]. The three species (analyte, CD and crown ether) form a stable complex system, where possibly a three-body complex is responsible for the enantioselectivity [10,37]. However, the mechanisms underlying the separation in the presence of CD and 18C6 as buffer additives have not been fully clarified.

In recent years, various theoretical studies have been performed to investigate CD inclusion complexes aiming at comprehending the mechanism of the complexation and correlating the experimental results with the mode of the interaction between a CD host and a guest molecule [38–45]. In this work, we are using PM6 semiempirical molecular methods for the investigation of the host–guest complexation of primary amines, namely 1,2,3,4-tetrahydro-1-naphthylamine (THAN), 1-(1-naphthyl)ethylamine (NEA) and 1-aminoindan (AI) with β CD alone and with β CD in presence of 18C6. The development of a sandwich type of compounds between the primary amine, 18C6 and the CD has been investigated as the most acceptable mechanism by which the separation is enhanced in the presence of 18C6. In this study we have also obtained the thermodynamic properties associated with the formation of β CD–amine and β CD–18C6–amine complexes using PM6 methods.

2. Experimental

2.1. Reagents

Phosphoric acid (85%, w/w), 18-crown-6 (18C6), 1,2,3,4-tetrahydro-1-naphthylamine (THAN), 1-(1-naphthyl)ethylamine (NEA) and sodium dihydrogen phosphate were purchased from Sigma–Aldrich (St Louis, MO, USA). β -Cyclodextrin (β CD), 1-aminoindan (AI) was obtained from Fluka (Buchs, Switzerland).

2.2. Buffer and sample preparation

The phosphate buffer solutions of pH 2.5 were prepared by using a 50 mM sodium dihydrogen phosphate solution adjusted to the desired pH with phosphoric acid. All samples were prepared in aqueous solution at a concentration of 0.5 mg mL⁻¹. The desired concentration of β CD, 15 mM, and that of 18C6, 30 mM, were prepared in phosphate buffer.

2.3. Instrumentation

Analytical separations were carried out on a Waters Capillary Ion Analyzer (Milford, MA, USA) which was interfaced to a Waters PC 800 Workstation using an uncoated fused-silica capillary (total length, 35 cm and internal diameter, 75 μ m; effective length, 27.5 cm). The separations were conducted at 25 °C by applying a voltage of 15 kV. Samples were injected hydrostatically for 10 s. Detection was done at 254 nm.

2.4. CE conditions

New uncoated fused-silica capillary was conditioned by flushing with 1 M NaOH for 30 min, then 0.1 M NaOH for 10 min and finally water and buffer each for 15 min. The running buffer solution was passed through 0.2 μ m cellulose nitrate membrane filters (Whatman, UK) and degassed by sonication prior to use. Prior to each analysis, the capillary column was rinsed with 0.1 M NaOH for 2 min, and then Milli-Q water for 2 min, followed by the carrier electrolyte, each for 3 min between the runs.

The selectivity factor (α) and the resolution (R_s) are calculated according to the following equations: $\alpha = t_2/t_1$ and $R_s = 2(t_2 - t_1)/(w_1 + w_2)$. Where t_1 , t_2 , w_1 , and w_2 are the migration times and peak widths at baseline for enantiomers 1 and 2, respectively.

2.5. Computational methodology

The initial geometries of AI, NEA and THAN were optimized at the PM6 level of theory using the MOPAC2009 package (www.openmopac.net) [46,47]. The β -cyclodextrin (β CD) structure was obtained from the crystallographic parameters provided by the Structural Data Base System of the Cambridge crystallographic Data center [48,49]. The inclusion complexes were constructed from the separately optimized CDs and the optimized structures of AI, NEA and THAN. The starting geometries were constructed using CS Chem 3D Ultra (Version 8.0, CambridgeSoft.com) and were fully optimized with the semiempirical method PM6. The coordinate system used to define the process of complexation is based on placing the glycosidic oxygen atoms of the CDs at the origin of the coordinate system, the longitudinal axis of the cavity being arbitrarily collinear with the z-direction of the coordinate system (Fig. 1). Two different inclusion orientations were considered. In the first orientation (orientation I) the ring bearing the amine moiety was docked into the wider rim (side containing secondary hydroxyl groups) of the CD with the bond connecting it to the benzene ring placed perpendicular to the z-axis (Fig. 1a). In the second orientation (orientation II) (Fig. 1a), the benzene ring was docked into the wider rim of the CD.

For the crown ether complexes, two possible orientations of the complex were considered where orientation I the complex face the wider rim of CD (Fig. 1b), while in orientation II the complex face the narrow rim of the CD. The relative positions of the host and the guest were measured by the position of the center of the molecule (Fig. 1a) or the center of the crown ether for the complex (Fig. 1b). The inclusion complexes were emulated by moving the guest molecule from 15 to -15 Å, at 1 Å interval. The complexation energy ΔE_{comp} is calculated from the minimum energy structures by the following equation:

$$\Delta E_{\text{comp}} = E_{\text{comp}} - E_{\text{guest}} - E_{\text{host}} \quad (1)$$

where E_{comp} , E_{guest} and E_{host} represent the total energy of the complex, the free guest molecule and the free host molecule, respectively. The magnitude of the energy change indicates the tendency towards complexation. The more negative the complexation energy change is the more thermodynamically favorable is the inclusion complex.

The two orientations adopted here for the emulation of amines into the β CD cavity are used to generate the maximum number of possible conformations. The reaction coordinates were defined by the distance between the center of mass of the amine molecule (indicated by an * in Fig. 1a) and that of the center of β CD. For the docking of amines–18C6 adducts, the reaction coordinates were defined by distance between the center of mass of the 18C6 molecule and that of β CD. Because 18C6 is a large molecule and is not expected to fit into the β CD cavity, the only part that was emulated into the cavity of β CD was the amine part of the 18C6 adduct.

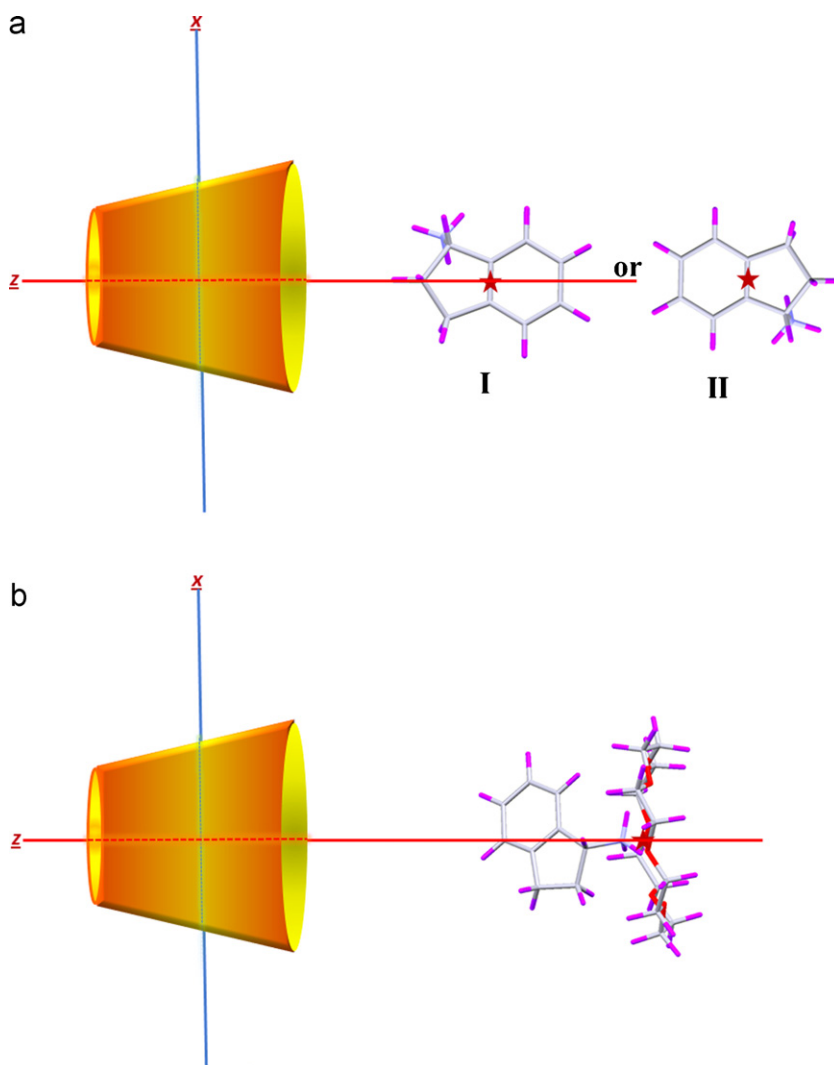


Fig. 1. Orientations of the amines and amines–18C6 penetrating the βCD cavity (a) I: orientation I for amines, II: orientation II for amines; (b) orientation I for 18C6–amine, for orientation II for 18C6–amines the adduct is docked through the narrow rim of CD.

To examine all possible conformations the amine–18C6 adduct was docked towards the wide rim as represented by orientation I (Fig. 1b) and also into the narrow rim of the βCD as represented by orientation II. Calculations provide the binding energy variations along the inclusion process from which the stability of the complexes can be inferred. This will possibly allow mapping the energy profile to delineate the association of the amines and their crown ether adducts with βCD.

18C6 is well known to be a flexible molecule and many low energy conformations at room temperature can be obtained in the gas phase as well as in the condensed phase [50]. The most important conformations are the lowest energy (*C_i*) and the highest symmetry (*D_{3d}*) conformations. In the *C_i* conformation, four oxygen atoms are directed inwards from the ether backbone and the other two are directed outward. This conformation is observed in the X-ray analysis of the crystalline 18C6 and in nonpolar solvent [51,52]. The *D_{3d}* conformation, on the other hand, is reported to be the dominant species in polar solvents [50]. In this work the starting geometry of the 18C6 was based on the *D_{3d}* conformation optimized using PM6 methods. All possible complexes of AI, NEA and THAN with 18C6 were generated and optimized fully with the semiempirical methods without any restrictions.

Using PM6 methods, harmonic frequency calculations were performed for the most stable geometries. Then using statistical

thermodynamics methods at 1 atm and 298 K we calculated the various thermodynamic properties such as the enthalpy change (ΔH), the entropy change (ΔS) and the free energy change (ΔG) using the following equations:

$$\Delta A = A_{\text{complex}} - A_{\text{amine}} - A_{\text{CD}} \quad (2)$$

$$\Delta A = A_{\text{complex}} - A_{\text{amine}} - A_{18\text{C}6} - A_{\text{CD}} \quad (3)$$

where $A = H$ or S .

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

3. Results and discussion

3.1. Electrophoretic separation of primary amines

In this work, the separations of AI, NEA, and THAN using a phosphate buffer solution of pH 2.5 containing 15 mM βCD with or without 30 mM 18C6 were investigated. In such acidic conditions the primary amino groups are protonated. Initially the chiral separation of the analytes was investigated using different concentrations of CD of 5–15 mM, higher concentration of βCD were not used because of the limited solubility specially in acidic media. An optimum value of the applied voltage of 15 kV was used as it gives a better separation in reasonable time. It was found that

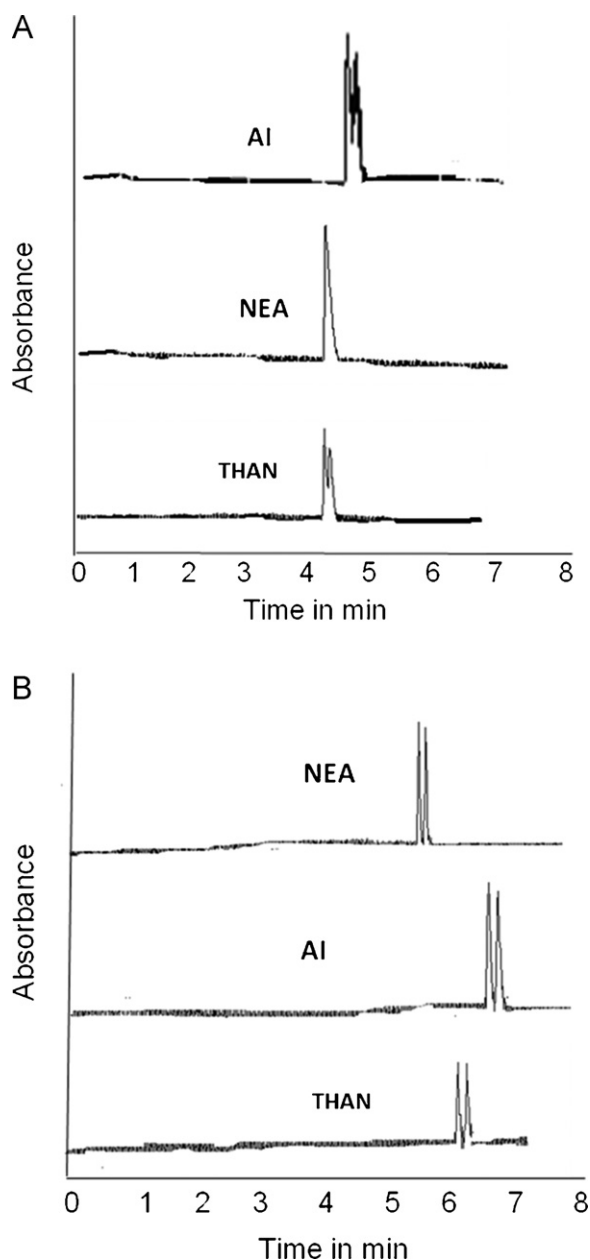
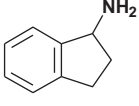
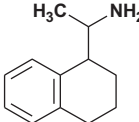
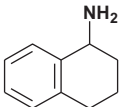


Fig. 2. Electropherograms of the primary amine compounds obtained with (A) 15 mM β -CD and (B) 15 mM β -CD + 30 mM 18-crown-6. Conditions were as follows: 50 mM sodium dihydrogen phosphate pH 2.5, injection time, 10 s; applied voltage, 15 kV; and capillary temperature, 25 °C.

only 15 mM β CD was able to partially separate the enantiomers of AI and THAN but was not able to separate the enantiomers of NEA as shown in Fig. 2a. The separation results are summarized in Table 1. From this table, clearly poor separation efficiency is observed when β CD was used as a chiral selector. The chiral center and the amino group of NEA are located on carbon atoms away from the cyclic group. Therefore probably because of this type of molecular structure the chiral separation was not achieved when β CD was used alone. When 18C6 was added to the buffer solution containing β CD the chiral recognition was achieved for all compounds investigated as shown in Fig. 2b. Furthermore, when we added 18C6 we observed that the migration times of the three analytes were increased as shown in Table 1 indicating more stable complexes are formed between the amino compound, β CD and 18C6. As it has been reported recently [11,37] a sandwich com-

Table 1
Chiral separation results of AI, NEA and THAN by β -CD alone and β -CD + 18C6.

Compound	Molecular structure	β CD alone			β CD + 18C6		
		t_1	α	R_s	t_1	α	R_s
AI		4.8	1.0	0.6	6.8	1.03	1.6
NEA		4.3	1.0	0.0	5.5	1.05	1.8
THAN		4.2	1.0	0.7	6.4	1.03	1.4

t_1 is migration times (min) of the first eluting enantiomers; α is selectivity; R_s : resolution.

plex between [18C6 + amino compound + β CD] which possesses a significant stability is responsible for such behavior.

3.2. Molecular modeling

In order to rationalize our experimental results and to further understand the mechanism of the enantiodifferentiation of amines in the presence of β CD and the role of 18C6 in the enhancement of the separation of these compounds we performed theoretical calculations using molecular mechanics and the semiempirical method PM6 [46]. The free energy profiles of the inclusion of each of the three amines and their 18C6 adduct into β CD are characterized by the presence of clear minima. For the inclusion complexes of these compounds (AI, NEA and THAN) and in both orientations (Fig. 1a) it was observed that as the molecule approaches the β CD nanocavity the binding energy decreases sharply until a minimum is obtained. As the molecule is inserted into the cavity it rotates to adjust itself in a position to maximize interactions with the inside of the β CD and to minimize steric hindrance caused by the branched groups while crossing the cavity leading to unfavorable energy changes. As the molecule move away from the cavity the binding energy starts to increase again. When the 18C6-amine adducts were docked into the β CD cavity a decrease in the energy is observed as the molecule approaches the cavity. A minimum energy is obtained when the phenyl group of the amino compound is completely inserted into the cavity while the protonated amine group of the molecule is still attached to the 18C6 molecule via strong hydrogen bonding. The optimum geometry obtained seems to stem from the fact that when the adduct approach the narrow or the wide rim of the cavity the steric hindrance with the groups at the rim repel the 18C6 back to a distance where only favorable interactions prevail resulting in the formation of a sandwich compound. When we tried to push the adducts deeper into the CD cavity we observed that the amine-18C6 adducts break apart.

In all cases investigated an almost flat minimum was observed. Several configurations from this minimum energy region were selected. Following this preliminary scrutiny procedure, the structures of the complexes obtained with the lowest energies were further optimized using the semiempirical PM6 method. The more negative is the complexation energy obtained the more stable is the complex and the more favorable is the configuration.

Table 2
Interaction energies of the amines– β CD inclusion complexes.

β CD-complex	E_{comp} (kcal mol ⁻¹)		ΔE (kcal mol ⁻¹)		$\Delta\Delta E$ (kcal mol ⁻¹)
	Orientation I	Orientation II	Orientation I	Orientation II	
R-AI	-1519.4	-1512.6	-50.3	-43.5	-4.7
S-AI	-1524.0	-1514.5	-55.0	-45.4	
R-NEA	-1531.6	-1529.0	-44.9	-42.7	-1.1
S-NEA	-1533.5	-1521.7	-46.0	-34.2	
R-THAN	-1526.5	-1524.4	-48.9	-46.7	-1.2
S-THAN	-1526.2	-1525.2	-50.1	-49.1	
R-AI-18C6	-1797.7	-1791.0	-64.9	-58.2	6.2
S-AI-18C6	-1790.0	-1791.4	-57.3	-58.7	
R-NAE-18C6	-1804.6	-1808.6	-54.2	-58.2	-5.7
S-NAE-18C6	-1815.1	-1811.5	-63.9	-60.2	
R-THAN-18C6	-1800.5	-1808.2	-59.1	-66.8	4.1
S-THAN-18C6	-1802.6	-1799.5	-62.7	-59.5	

$E_{\beta\text{CD}} = -1563.4$ kcal mol⁻¹; $E_{\text{R-AI}} = 94.2$ kcal mol⁻¹; $E_{\text{S-AI}} = 94.3$ kcal mol⁻¹; $E_{\text{R-NEA}} = 76.6$ kcal mol⁻¹; $E_{\text{S-NEA}} = 75.8$ kcal mol⁻¹; $E_{\text{R-THAN}} = 85.7$ kcal mol⁻¹; $E_{\text{S-THAN}} = 87.1$ kcal mol⁻¹; $E_{\text{crown}} = -263.7$ kcal mol⁻¹, $\Delta\Delta E = \Delta E_{\text{S}} - \Delta E_{\text{R}}$, negative sign of $\Delta\Delta E$ indicates that the R-isomer is eluted first.

3.3. β CD-amine system

The β CD-amine complexes were optimized in aqueous media and without any constraints. The results of the interaction energies of the primary amines with β CD obtained from the PM6 calculations are shown in Table 2. The most stable structures were inferred from the energies of the complexes for both orientations at the optimum locations of the amine with respect to the cyclodextrin. As shown in Table 2 the complexes of the three studied amines (AI, NEA and THAN) possess energy that is always lower than the sum of the energy of the isolated guest and host molecules. This is indicative of formation of favorable complexes for all compounds and in both orientations. Clearly, the binding energy of orientation I complex is lower than that of orientation II complexes for all molecules. As representatives of this system, the optimized guest–host structures for the binding of R-AI and RNEA with β CD are shown in Fig. 3. Interestingly, for orientation I complexes of AI and THAN the structure with the minimum energy correspond to a deep inclusion of the phenyl ring into the hydrophobic cavity of the cyclodextrin with the amine moiety protruding from the cavity via the narrow rim of the cyclodextrin. As the amine moves deeper inside the cavity the unfavorable interactions between the protonated amine group and the hydrophobic cavity of the cyclodextrin together with the steric hindrance caused by the branched groups around the amine moiety force the amine group out of the cavity. Therefore the optimum structures of AI and THAN complexes with β CD are those for which the phenyl ring remains inside the cavity and the six or five membered ring is placed just outside the β CD near the narrow rim of the CD. For R-NEA the minimum energy structures correspond to locating the molecule outside the β CD cavity and just on the exit of the cavity rim as shown in Fig. 3. The intriguing fact, for NEA, is the presence of hydrogen bonding between the protonated amine and the oxygen of the hydroxyl group at the rim. In the AI and THAN complexes hydrogen bonding is surprisingly absent. Possibly the bulky size of the NEA molecule allows its amino group to attain a position near the hydroxyl oxygen to establish strong hydrogen bonding interactions.

3.4. Amines–18C6– β CD system

In CE we have observed that addition of non-chiral crown ether, 18C6, to the buffer electrolyte that contains β CD has resulted in significantly enhanced separation efficiency for all enantiomers. This glaring characteristic has prompted us to investigate how 18C6 and cyclodextrin together resulted in a better resolution compared to β CD alone. The binding energy of the inclusion complexes of the amine–18C6 adducts with β CD is shown in Table 2. The optimized

structures of the sandwich compounds between the amine–18C6 and β CD are presented in Fig. 4 for AI. It is clear from Table 2 that the most stable structures for R-AI, S-NEA and S-THAN are obtained for orientation I complexes, whereas S-AI, R-NEA and R-THAN favor forming complexes through orientation II. As shown in Fig. 4 and in all cases the amine molecule is inserted deeper inside the cavity where the phenyl ring and all or part of the six or five membered ring of the amine is located into the cavity allowing favorable van der Waal and hydrophobic interactions to be formed. It is obvious from Table 2 that the stabilization energies of the 18C6-amine– β CD complexes are higher than those obtained

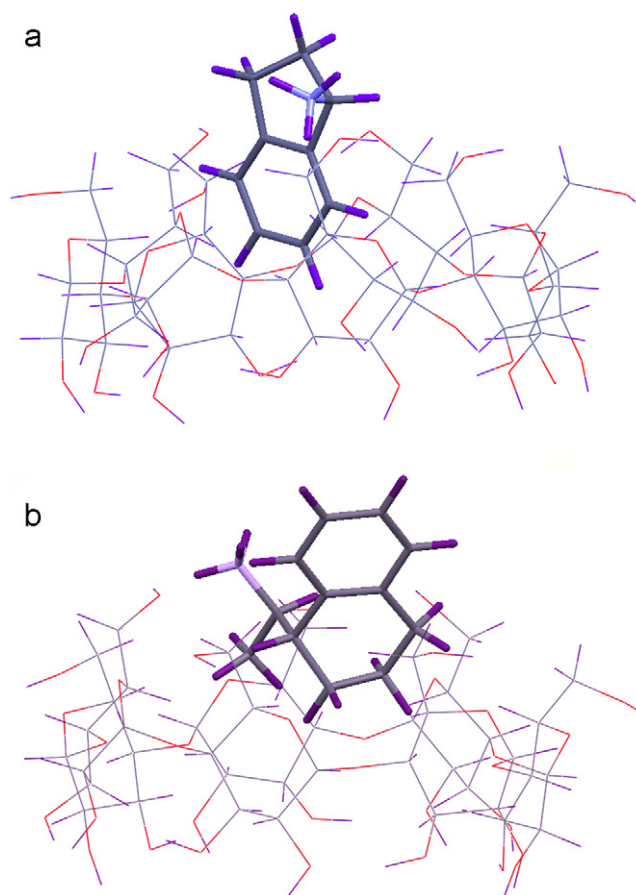


Fig. 3. Geometries of the most favorable inclusion complexes of (a) R-AI and (b) R-NEA with β CD.

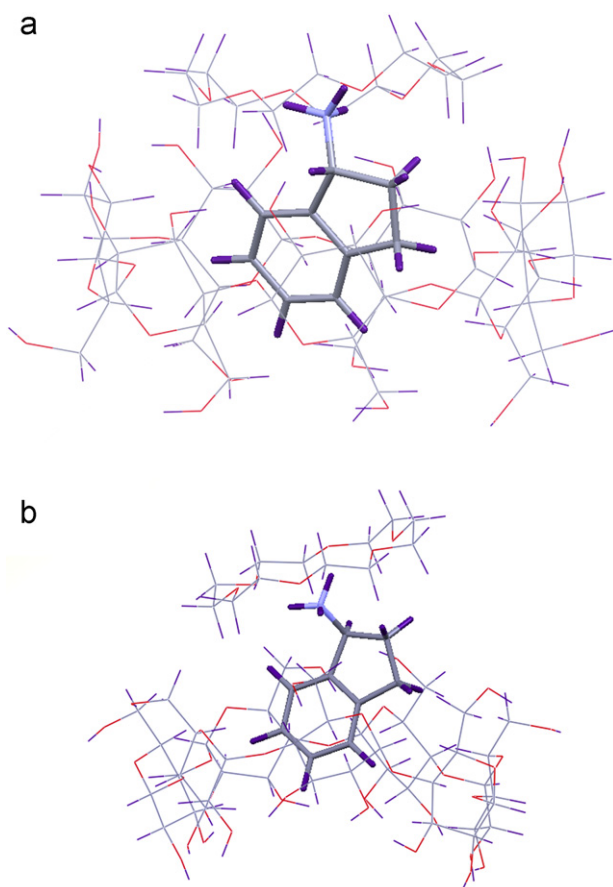


Fig. 4. Geometries of the most favorable sandwich complexes of (a) R-Al, (b) S-Al with β CD and 18C6.

for the inclusion complexes of the amines with β CD alone. For instance, the binding energy of the most stable R-THAN-18C6- β CD (orientation II) complex is $18.7 \text{ kcal mol}^{-1}$ higher than that of the most stable R-THAN- β CD complex (orientation I). This result originates from the fact that the 18C6-amine interaction facilitates the insertion of the amine deeper into the β CD nanocavity where favorable hydrophobic interactions are maximized and steric hindrances are not magnified. Additionally the stronger hydrogen bonding between the crown ether and the amine exert more stability to the system. This result in the formation of sandwich compounds as shown in Fig. 4 or Al. In most of the cases the 18C6 adopt a slightly tilted orientation to minimize the steric interactions with the groups at the rim of the cyclodextrin and to allow for better fit of the amine into the β CD cavity.

3.5. Thermodynamics of the complexes

To further investigate the thermodynamics of the binding of these amine compounds with β CD and also in the presence of 18C6, we run statistical thermodynamics calculations at 1 atm and 289.15 K in water by PM6 methods. The thermodynamic quantities of the complexation process, such as enthalpy change (ΔH), Gibb's free energy change (ΔG) and entropy change (ΔS), were calculated and presented in Table 3. From this table we can see that the complexations of the amine model compounds with β CD are exothermic as indicated by the formidably high enthalpy changes. On the other hand more negative ΔH values are observed for the amine-18C6- β CD complexes compared to the amine- β CD inclusion complexes. This suggests that the amine molecules are tightly

Table 3
Thermodynamic properties of the amines- β CD inclusion complexes.

β CD-complex	ΔH (kcal mol $^{-1}$)	ΔS (cal K $^{-1}$ mol $^{-1}$)	ΔG (kcal mol $^{-1}$)
R-Al	-48.6	-38.2	-37.2
S-Al	-53.1	-40.1	-41.2
R-NEA	-43.7	-52.2	-28.2
S-NEA	-44.4	-40.5	-32.3
R-THN	-50.3	-28.4	-43.8
S-THN	-48.4	-37.4	-37.3
R-Al-18C6	-58.6	-85.3	-33.2
S-Al-18C6	-55.4	-101.1	-25.3
R-NEA-18C6	-53.6	-89.3	-27.0
S-NEA-18C6	-60.0	-94.8	-31.7
R-THN-18C6	-62.2	-90.3	-35.3
S-THN-18C6	-56.8	-82.4	-28.9

bound to the cavity in the presence of 18C6. The main factors that contribute to the inclusion complex thermodynamics are associated with the insertion of the guest molecule into the β CD cavity, such process is usually accompanied by the dehydration of the hydrophobic part of the guest molecule as well as by the release of the bound water molecules from the cavity of the CD [53]. Charged molecules or hydrophilic groups of a molecule are expected to remain primarily exposed to the bulk of the aqueous media. From Table 3, we can observe that ΔH s of the amines-18C6- β CD inclusion complexes are higher than those of the amine- β CD complexes. Clearly, in the presence of 18C6 the hydrophobic properties of the amines increase leading to a deeper penetration of these molecules into the cavity of the cyclodextrin. This in turn results in the formation of stronger host-guest interactions. We examined carefully the presence of hydrogen bonding between the protonated amine moiety and the hydroxyl groups at the rim of the CD, but no such interactions were found. This is because the amine group is restricted by the strong interaction with the 18C6.

Determination of free energies requires an adequate sampling of the diastereomeric potential energy surfaces. A number of assumptions are usually encountered in modeling chiral separations of chromatographic techniques [30]. For example the effect of buffers and counter ions are usually not considered. Additionally, assuming that the solvation effects and entropy differences may cancel could fail especially when samples from diastereomeric surfaces are inadequate. This would lead to poor estimation of the differential free energies of the various diastereomers. Sampling a reasonable number of inclusion complexes of the chiral molecules and their host molecules can be obtained using nanosecond molecular dynamics (MD) simulations. In such techniques a large number of configurations of the guest-host systems are produced by integrating Newton's law of motion [54,55]. This approach is, however, computationally demanding and requires *a priori* knowledge of where to place the guest molecule.

The Gibbs free energies obtained from the static method adopted here should be considered cautiously and only qualitatively. The theoretically calculated entropy changes using semiempirical methods may deviate considerably from the experimental values. Adjustments of the calculated thermodynamic values are usually performed using the experimentally determined values of these parameters [56,57]. One approach is to compute these parameters in aqueous media with the assumption that the effect of water molecules on entropy changes is mainly determined by those molecules inside the cavity of CD, whereas the water molecules outside the cavity are of less importance. The thermodynamic parameters of the systems described in this paper have not been determined experimentally and therefore similar adjustment cannot be performed.

3.6. Enantiomeric separation

Enantiodifferentiation of the amines using β CD and β CD–18C6 systems were evaluated based on their binding energies. From Table 2 we can see that the binding energy of the optimum S-AI and R-AI complexes with β CD are -55.0 and -50.3 kcal mol $^{-1}$, respectively, indicating that the S-AI enantiomer– β CD complex is more stable by 4.7 kcal mol $^{-1}$. This suggests that β CD alone is capable of separating these two enantiomers. In Fig. 2 we have seen that AI racemate exhibits a partial separation in the presence of β CD. Also from binding energy (ΔE_{comp}) results in Table 2 we infer that R-AI forms less stable complex with β CD compared to S-AI therefore it is expected to elute first. The enantiodifferentiation of AI is further enhanced by the addition of 18C6. As shown in Table 2 the binding energies of both S-AI and R-AI have increased significantly in the presence of 18C6 to -64.9 and -58.7 kcal mol $^{-1}$, respectively. This extra stabilization of the complexes has also been demonstrated clearly in the electropherogram shown in Fig. 1b as an increase in the migration times. Furthermore, the $\Delta \Delta E$ value is also increased from 4.7 to 6.2 kcal mol $^{-1}$ for AI leading to noticeable enhancement in the separation efficiency of the two enantiomers with an increase in R_s from 0.6 to 1.6 (Table 1). The order of elution of the two enantiomers is opposite to that obtained when β CD alone is used.

A similar behavior is also observed for NEA and THAN. As shown in Fig. 2, for THAN a partial separation is observed as for AI, however NEA exhibited a single peak indicative of no separation. The PM6 calculated $\Delta \Delta E$ is 1.1 and 1.2 kcal mol $^{-1}$ for NEA and THAN, respectively. When 18C6 was used as another additive with β CD in the electrolyte buffer system the binding energies of the sandwich complexes of NEA and THAN increased dramatically (Table 2) accompanied by a noticeable increase in $\Delta \Delta E$ to 5.7 and 4.1 kcal mol $^{-1}$ for NEA and THAN, respectively. From Table 2 the predicted elution order for NEA is R-NEA then S-NEA, however for THAN the S-enantiomer is eluted first.

On the other hand, the theoretical calculations, using PM6 semiempirical method, indicated that the pairs of R-, S-AI, R-, S-THAN, and R, S-NEA will elute at comparable migration times in the presence of β CD as they are partially separated in the presence of β CD alone (Table 2) which correlates well with the experimental results with migration time between 4.2 and 4.8 min for all molecules. The calculated binding energy for the three amine predicted t_1 for the three compounds as AI < NEA < THAN for the 18C6–amine– β CD systems. This result, however, is different from the experimentally obtained migration times where NEA is eluted first followed by THAN and finally AI.

It is clear from Table 2 that in the presence of 18C6 a much higher difference in complexation energy is obtained, this suggests that the primary amine–18C6 adducts bind with the CD to produce diastereomeric complexes of different binding strengths and possibly of different shapes and net charges. It has been stated in the literature that two mechanisms are involved in enantioseparations in CE. The first mechanism is the based on the binding selectivity where differences in the binding constants due to the variation in the degree of complexation are responsible for differences in mobilities. In the second mechanism the differences in mobilities of diastereomeric complexes may result from difference in shapes or net charges [26–30]. The chiral recognition of AI, NEA and THAN in the presence of dimethyl- β CD and 18C6 was determined to occur primarily through the insertion of amine–18C6 into the CD cavity [11]. Furthermore, it was observed that the differences in the complexation constants were greatly increased in the presence of 18C6. By examining Table 2, it is clear that R-AI–18C6 most stable complex with β CD is obtained via orientation II, where the adduct is inserted into the CD cavity from the wide rim. On the other hand, S-AI–18C6 prefers to fit into the CD nanocavity through the nar-

row rim to form the most stable complex. The opposite behavior is observed for NEA and THAN. It is clear from these observations that the diastereomer pairs for each compound will possess different shapes due to the differences in the fits into the CD. This in turn will lead to an enhanced complex mobility selectivity resulting in better separation.

Nevertheless the PM6 methods were able to explain the mechanism of enantiodifferentiation it seems that the prediction of the migration times by this method is not trivial. The use of higher levels of theory, for example Density Functional Theory (DFT), might impart an additional accuracy for such results and a better correlation could be obtained. However, for such a large system higher theoretical methods suffer from the high computational cost.

4. Conclusions

The use of β CD as a chiral selector in capillary electrophoresis was not efficient to separate the enantiomers of three primary amines, AI, NEA and THAN. Only partial separation of AI and THAN was obtained with R_s values of 0.6 and 0.7 , respectively. For NEA β CD was unable to resolve the two enantiomers from each other. An electrolyte containing a dual system of 18C6 and β CD systems has achieved a significant improvement in the resolution of all enantiomers. Resolutions between 1.4 and 1.8 were obtained. The mechanism of enantiodifferentiation was investigated by molecular modeling using molecular mechanics and the semiempirical method PM6. From the results of the theoretical calculations it was found that the presence of 18C6– β CD system leads to the formation of stable sandwich compounds with protonated primary amines. The consequence of the formation of such compounds is the magnification of the binding energy differences ($\Delta \Delta E$) and as a result migration times of the R- and S-isomers were altered.

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