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Application of cyclodextrin-mediated capillary electrophoresis to simultaneously determine the apparent binding constants and thermodynamic parameters of naphthalenesulfonate derivatives

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Abstract

Application of capillary electrophoresis (CE) to simultaneously determine the apparent binding constants and thermodynamic parameters for six positional and structural naphthalenesulfonate derivatives with β-cyclodextrin (β-CD) is presented. The change in electrophoresis mobilities was used to assess the binding constants by non-linear regression and three different linear plots methods (named double reciprocal, *x*-reciprocal and *y*-reciprocal). The substituent group(s) attached to the naphthalene ring considerably affected the inclusion behaviors of these naphthalenesulfonate derivatives. The binding constant varies over almost one order of magnitude and a highly selective sequence is obtained between these guest model compounds. Naphthalenesulfonates with the substituent(s) at the 2-position(s) displayed stronger interaction with β-CD, and gave well compatible results by these four plot methods. While at least one substituent was substituted into the 1-position of naphthalene showed the weak interaction or no interaction with β-CD. Comparison to three linear regression methods, the non-linear regression method proves to be the most suitable for these determinations. Additionally, apparent binding constants for each structural isomer with β-CD at several temperature, and thermodynamic parameters for binding were also calculated and discussed.

Keywords: Capillary electrophoresis; Cyclodextrins; Apparent binding constants; Inclusion complex; Naphthalenesulfonates; Thermodynamic parameters

1. Introduction

Currently, capillary electrophoresis (CE) has become one of the most popular analytical techniques in separation of enantiomers because CE leads to high separation power, consumes minimal organic solvent and provides rapid method development (see Ref. [1–4] and the references therein). Among the various chiral selectors, cyclodextrins (CDs) represent the major class of chiral applications to optimize the separation of two enantiomers in capillary electrophoresis (see [5–9] and the references cited therein). CDs are neutral glucose polymers with a truncated corn shape, and possess a hydrophilic exterior and a hydrophobic interior cavity, which gives to their ability to form guest–host inclusion complexes with a wide range of enantiomers in aqueous solution [10]. A simple guest–host complex model based on binding equilibria has been shown to explain excellently the effect of changing the concentration of CDs on the resolution of enantiomer separation [11–15]. Moreover, CDs can also be used to separate positional and structural achiral compounds (see [16] and the references cited therein). To understand the mechanisms of achiral separation using CDs, spectroscopic methods and calorimetric titration approaches have been used to determine the apparent binding constants of guest–CD complexation, and the thermodynamic parameters in the binding of the various positional and structural achiral compounds [17–21].

In our previous report [22], the separation and migration behavior of positional and structural naphthalenesulfonate (NS) derivatives in CD-mediated capillary electrophoresis have been systematically investigated. The data indicate that the interactions of NS derivatives with CDs are strongly affected by the position of the substituent(s) on

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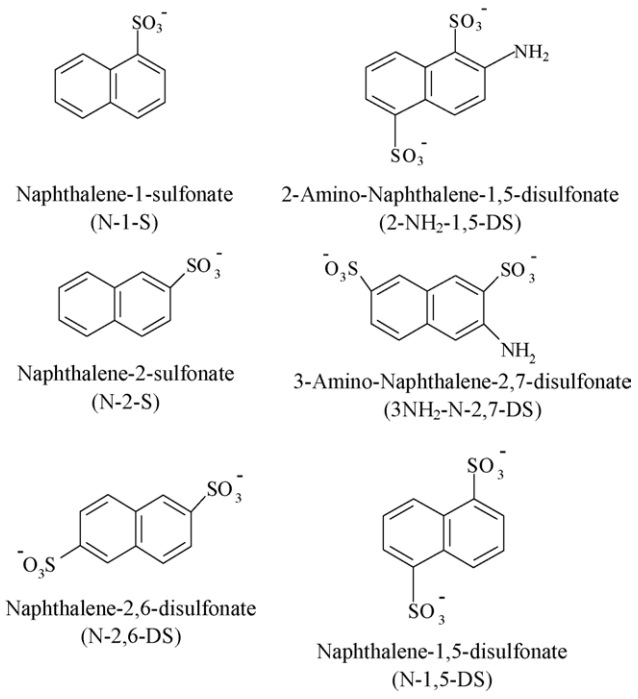

Fig. 1. Structures of naphthalenesulfonate derivatives studied in this work.

the naphthalene ring. The apparent binding constants of these guest compounds were evaluated using a simple non-linear regression model, which indicated that β-CD is found to be the better complex-forming host with NS derivatives. The quantitative analysis of the binding constant for 1:1 guest–host complex by CE technique is based on the general form of a binding isotherm and an expression for the effective electrophoretic mobilities derived for the conditions of the experiments. In this study, we attempted to examine the apparent binding constants of six model NS derivatives (as shown in Fig. 1, which can be separated into three groups: two naphthalene–monosulfonate isomers, two naphthalene–disulfonate isomers and two amino–naphthalene–disulfonate isomers) with β-CD by CE, and to compare the results obtained by non-linear regression method and three different linear plots methods, namely the double reciprocal, *y*-reciprocal and *x*-reciprocal plots. More data points were used in this study to improve the precision and accuracy. Each NS isomer was used to model positional isomers, and the compounds between each group were used to model structural compounds. NS derivatives are good model compounds for studying guest–host inclusion complexation, since they all have a rigid rectangular shape and can be used to examine the effects of position, number and type of substituents on the formation of the guest–host inclusion complex. The effects of the binding constants on the viscosity of the separation buffer by adding the native β-CD were also examined. Additionally, apparent binding constants for each guest–host pair at several temperatures, as well as the thermodynamic parameters for the inclusion complexation were calculated. The factors control the stability of complex were also discussed from the thermodynamic perspective. Furthermore, this study is also to demonstrate that CE can be used to simultaneously determine the thermodynamic parameters of a set of analytes in a mixed solution.

2. Experimental

2.1. Chemicals and reagents

Unless stated otherwise, all high purity chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Tedia (Fairfield, OH, USA) and Merck (Darmstadt, Germany), and were used without further purification. Six naphthalenesulfonate derivatives and β-cyclodextrin were purchased from Aldrich. Sodium tetraborate ($Na_2B_4O_7$) separation buffer was prepared at 20 mM in deionized water and was adjusted to pH 9.2. Stock solutions of these analytes (1000 μg/ml) were prepared in methanol. Working standard solutions were obtained by diluting the stock standard solution with deionized water to appropriate concentrations. Deionized water was further purified with a Milli-Q water purification device (Millipore, Bedford, MA, USA). To prevent capillary blockage, all solutions were filtered through 0.45 μm membrane filter (Gelman Scientific, Ann Arbor, MI, USA) prior use.

2.2. Instrumentation

All experiments were performed on a P/ACE MDQ system (Beckman-Coulter, Fullerton, CA, USA) equipped with UV–vis detector. Separations were carried out in an untreated fused-silica capillary (J&W Scientific, Folsom, CA, USA) of 75 μm i.d. and an effective length of 50 cm (total length 60 cm). All samples were hydrodynamically injected into the capillary in 5 s at 0.5 psi (1 psi = 6.9 kPa), a volume of approximately 25 nl and an applied voltage of 25 kV. The UV detector was operated at 235 nm. For temperature studies, compounds migration times were determined at the temperatures 20, 25, 30, 35, and 40 °C (the precision of each temperature was ±0.1 °C). The electrophoretic system was equilibrated at each temperature for at least 6 h prior to each experiment. The separation steps were done automatically and controlled by Beckman P/ACE System MDQ Version 2.2 software (Beckman-Coulter). Separation buffers were degassed by ultrasonication. The pH of solutions was measured by a Mettler-Toledo MP220 pH meter (Schwarzenbach, Switzerland).

2.3. Procedures

Before use, the capillary was conditioned with methanol for 10 min at 25 °C, followed by 10 min with 1N HCl, 2 min deionized water, and 10 min 1N NaOH, then rinsed capillary with deionized water for 2 min, and followed by 10 min separation buffer. Between runs, the capillary was washed with 0.1N NaOH for 2 min and deionized water for 2 min

before run. This procedure improved peak sharp and the reproducibility of migration time. The measurements were run at least in triplicate to ensure repeatability. Sample concentration was 5.0 μg/ml. Methanol was used as a marker of the electroosmotic flow (EOF). The net electrophoretic mobility of the analytes (μ_{eff}) was calculated from the migration time of the each of these analytes. Viscosity-corrected apparent mobility, μ_a, was calculated by following equation:

$$\mu_a = v\mu_{eff} = v\frac{L_d \times L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \quad (1)$$

where v is the viscosity correction factor, L_d the length of the capillary from the inlet end to the detector, L_t the total capillary length, V the applied voltage, and t_m and t_0 are the measured migration times of the analyte and EOF marker, respectively.

The corrections of the viscosity in the separation buffer may become significant when high concentrations of neutral CDs were added during the binding process [23]. Thus, the electrophoretic mobility is needed to correct in order to obtain accurate binding constants, the correction factor v for variation in viscosity may be related to the peak appearance times and can be estimated by following equation [24]:

$$v = \frac{t_1}{t_2} = \frac{\eta_1}{\eta_2} \quad (2)$$

where η_1 and η_2 are the viscosities with and without the CD, respectively, and t_1 and t_2 are the time required for viscosity correction marker (acetone, the UV detector was operated at 214 nm) migrating from the sample-inlet end of the capillary to the detector with and without the CD, respectively.

3. Results and discussion

3.1. Separation of naphthalenesulfonate derivatives

For CE separation, naphthalenesulfonate derivatives were separated into groups based on the numbers of the SO_3^- group, with migration times increasing in the order mono- <di- < tri-sulfonates in borate buffer (pH 9.0), and with little or no separation of positional isomers in each group [25,26]. The similar results were also observed in this study, as illustrated in Fig. 2a, where 20 mM borate buffer at pH 9.2 served as the separation buffer. They were separated in groups following the order naphthalene–monosulfonate isomers, amino–naphthalene–disulfonate isomers, and finally naphthalene–disulfonate isomers. With the addition of 0.5 mM β-CD, all isomers in each group were separated (Fig. 2b). The migration times of N-2-S (peak 1), 3-NH_2-N-2,7-DS (peak 3) and N-2,6-DS (peak 6) (all the substituents at the 2-position) declined markedly as β-CD concentration was increased (Fig. 2c and d), possibly indicating that these three derivatives strongly interacted with β-CD, and may completely penetrate into the CD cavity to produce more stable complexes, which reduced their electrophoretic mobility toward the anode (+) (the sample inlet of the capillary). This

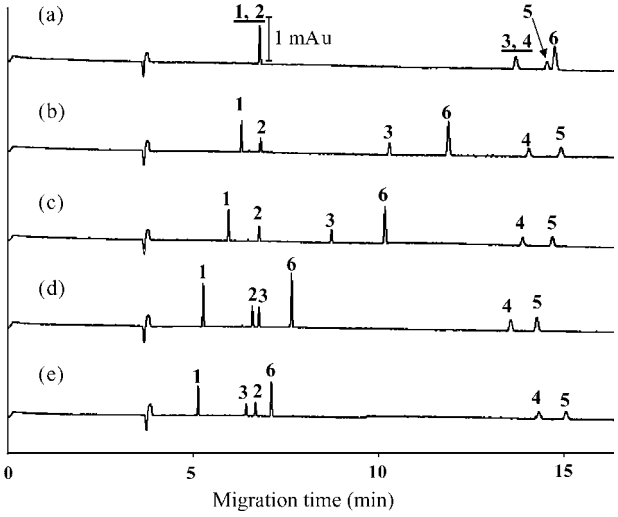

Fig. 2. Electropherograms of the separated naphthalenesulfonate derivatives and the effect of β-CDs on the separation and migration order. Electropherograms: (a) 0 mM, (b) 0.5 mM, (c) 1.0 mM, (d) 3.0 mM, and (e) 5.0 mM β-CD added in 20 mM borate buffer (pH 9.2). Standard mixture containing 5.0 μg/ml of each isomer in deionized water; separating voltage 25 kV; temperature 25 °C; detection 235 nm; hydrodynamic injection at 5 s for 0.5 psi. Peak assignment: (1) N-2-S; (2) N-1-S; (3) 3-NH_2-N-2,7-DS; (4) 2-NH_2-N-1,5-DS; (5) N-1,5-DS; (6) N-1,6-DS.

effect is expected to accelerate the migration of negatively charged complexes to the detector, and shortening the migration times. The migration times of peaks 4 and 5 are fluctuated upon the addition of β-CD maybe due to the changes of the viscosity in the separation buffer when neutral CD were added [22,26–28].

3.2. Evaluation of apparent binding constants

For a better understanding of the influence of β-CD on the separation and migration behavior of naphthalenesulfonate derivatives, the apparent binding constants of these derivatives were determined from the dependence of the apparent electrophoretic mobility of naphthalenesulfonates on the concentration of β-CD. The following model has been presented and used as a working hypothesis, for a detailed discussion of this concept, see references [11,14,29,30]. Analyte guest (A) and CD host has been proposed to form the 1:1 guest–host inclusion complexes in aqueous solution. For the equilibrium equation of the ACD complex can be written as:

$$A + CD \overset{K}{\rightleftharpoons} ACD \quad (3)$$

Here, K is the binding constant (also called inclusion complex formation constant or equilibrium constant). Under conditions of excess CD, the apparent electrophoretic mobility (μ_a) of the guest molecule and the net electrophoresis mobility ($\mu_{eff,A}$) of each species in the presence of the CD can be described by the following equation [30]:

$$\mu_a - v\mu_{eff,A} = \frac{(\mu_{eff,ACD} - v\mu_{eff,A})K[CD]}{1 + K[CD]} \quad (4)$$

where v is the viscosity correction factor, $\mu_{\text{eff, ACD}}$ is the electrophoretic mobility of the 1:1 inclusion complexes formed between the analyte and CD, and [CD] is the concentration of the free CD. Here, the values of $\mu_{\text{eff, A}}$ was measured experimentally in the absence of CD at pH 9.2, and the trial values of $\mu_{\text{eff, ACD}}$ was estimated from $\mu_{\text{eff, A}}$ according to Eq. (4), while the value of μ_a and $\mu_{\text{eff, A}}$ were calculated experimentally according to the observed migration times [31,32]. The value of $(\mu_a - v\mu_{\text{eff, A}})$ as a function of [CD] allows to determine the binding constant K by non-linear regression. The least-squares variance–covariance method was used to calculate the regressions. Moreover, this expression can be rearranged under three linear forms (Eqs. (5)–(7)) and a linear regression can be applied for each cases, as described in elsewhere [14,29,30].

$$\frac{\mu_a - v\mu_{\text{eff,A}}}{[\text{CD}]} = -K(\mu_a - v\mu_{\text{eff,A}}) + K(\mu_{\text{eff,ACD}} - v\mu_{\text{eff,A}}), \quad x\text{-reciprocal} \tag{5}$$

$$\frac{1}{\mu_a - v\mu_{\text{eff,A}}} = \frac{1}{(\mu_a - v\mu_{\text{eff,A}})K}\frac{1}{[\text{CD}]} + \frac{1}{(\mu_a - v\mu_{\text{eff,A}})}, \quad \text{double reciprocal} \tag{6}$$

$$\frac{[\text{CD}]}{\mu_a - v\mu_{\text{eff,A}}} = \frac{1}{\mu_{\text{eff,ACD}} - v\mu_{\text{eff,A}}}[\text{CD}] + \frac{1}{(\mu_{\text{eff,ACD}} - v\mu_{\text{eff,A}})K}, \quad y\text{-reciprocal} \tag{7}$$

Table 1 lists the apparent binding constants simultaneously determined by non-linear regression (Eq. (4)) and three different linearization plots methods (Eqs. (5)–(7)), and the results were adjusted and not adjusted by the viscosity correction factor. Fig. 3 shows the plots of Eqs. (4)–(7) for the binding of analytes to β-CD. Comparing the apparent binding constants of the various derivatives reveals that the substituent group(s) attached to the naphthalene ring significantly affected the inclusion behaviors. The binding constants of K values varies over almost one order of magnitude and a highly selective sequence is obtained between these guest model compounds, which can be divided into two groups, according to their inclusion characteristics. The first group, which strongly interacted with β-CD, consists of naphthalene into which substituent(s) had been substituted into the 2-position(s) (such as N-2-S, 3-NH_2-N-2,7-DS and N-2,6-DS). Their binding constants and correlation coefficients calculated from the viscosity corrected data were not different significantly from those obtained without adjusting the raw data. The K values obtained from Eqs. (5)–(7) are

Table 1
Comparison of apparent binding constants (at 25 °C) obtained by the different calculation methods and with and without the viscosity correction

Compound	Apparent binding constants (M^{-1})				Literature values
	Non-linear fitting (r^2)	Double reciprocal (r^2)	y-Reciprocal (r^2)	x-Reciprocal (r^2)	
N-2-S					
Not adjusted	390 ± 20 (0.9970)	360 ± 10 (0.9984)	380 ± 20 (0.9993)	360 ± 6 (0.9990)	380 ± 20[a], 380–450 ± 50[b] 480 ± 20[c], 240 ± 40[b]
Adjusted	420 ± 20 (0.9969)	380 ± 10 (0.9981)	410 ± 20 (0.9992)	380 ± 7 (0.9989)	
N-1-S					
Not adjusted	40 ± 9 (0.9918)	−20 ± 30 (0.9850)	30 ± 5 (0.9159)	30 ± 5 (0.8618)	30 ± 5[a],
Adjusted	50 ± 15 (0.9840)	30 ± 4 (0.9997)	40 ± 6 (0.9313)	40 ± 10 (0.8244)	
3-NH_2-N-2,7-DS					
Not adjusted	570 ± 20 (0.9975)	550 ± 10 (0.9987)	570 ± 40 (0.9997)	550 ± 3 (0.9999)	450 ± 70[a]
Adjusted	610 ± 30 (0.9972)	580 ± 20 (0.9983)	610 ± 30 (0.9997)	580 ± 10 (0.9980)	
2-NH_2-N-1,5-DS					
Not adjusted	8 (0.9091)	−4 ± 9 (0.9975)	40 ± 80 (0.1102)	−10 ± 30 (0.0601)	13 ± 3[a]
Adjusted	50 ± 20 (0.9869)	130 ± 20 (0.9967)	470 ± 850 (0.5673)	120 ± 120 (0.3464)	
N-1,5-DS					
Not adjusted	40 ± 20 (0.9896)	110 ± 10 (0.9986)	120 ± 60 (0.7600)	80 ± 30 (0.5929)	8 ± 1[a]
Adjusted	220 ± 20 (0.9347)	400 ± 40 (0.9912)	210 ± 110 (0.8264)	260 ± 200 (0.4946)	
N-2,6-DS					
Not adjusted	300 ± 40 (0.9815)	370 ± 5 (0.9997)	320 ± 10 (0.9996)	370 ± 5 (0.9992)	320 ± 30[a]
Adjusted	320 ± 50 (0.9790)	380 ± 6 (0.9996)	400 ± 20 (0.9996)	390 ± 5 (0.9992)	

[a] From Ref. [22].
[b] From Ref. [34].
[c] From Ref. [35].

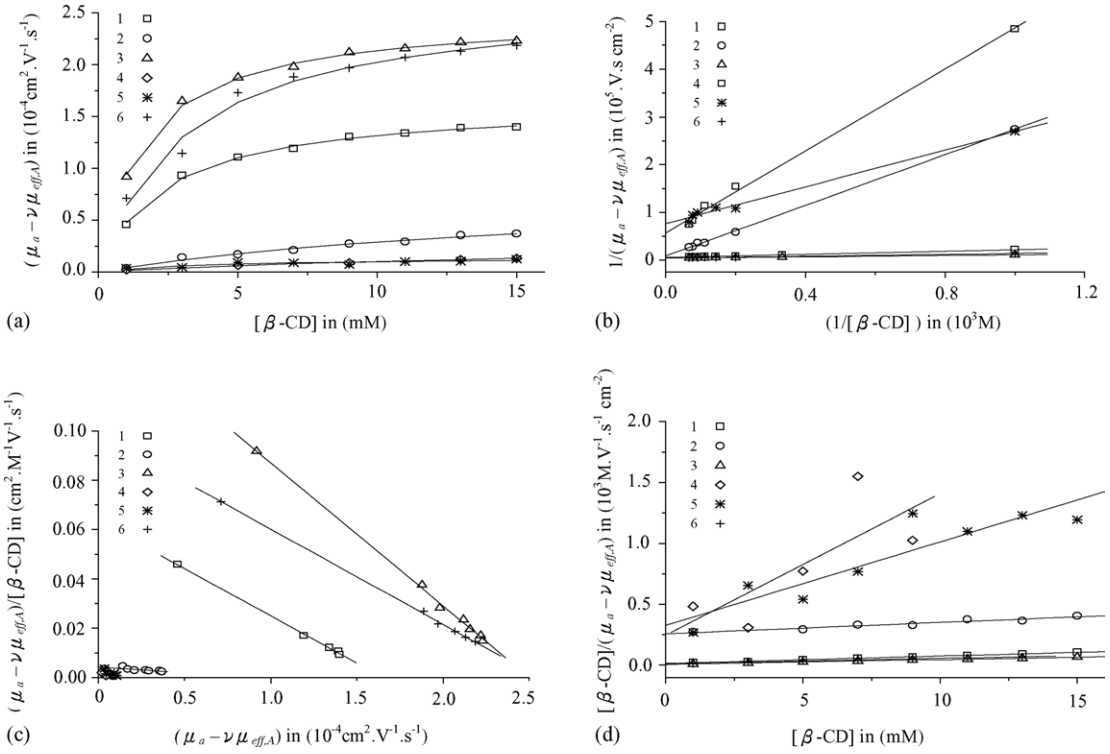

Fig. 3. Determination of the binding constants by four different plots methods: (a) non-linear regression; (b) double reciprocal method; (c) *x*-reciprocal method; (d) *y*-reciprocal method. See Section 3.2 for details.

similar with those obtained from the non-linear regression method, and all are within the range of those reported using by other methods [33–35]. Furthermore, the relative uncertainties are less than 10% in most cases. However, the second group includes naphthalenesulfonates into which at least one substituent had been substituted into the 1-position(s) (such as N-1-S, 2-NH_2-N-1,5-DS and N-1,5-DS), which interacted weakly with β-CD. Their *K* values and correlation coefficients were significantly different calculated from the viscosity corrected data and unadjusted raw data, and the relative uncertainties are high in most cases. Non-sensical values, such as negative *K* values and very low correlation coefficients for N-1-S, 2-NH_2-N-1,5-DS and N-1,5-DS (Table 1), which were calculated from linear regression methods, indicating that the apparent binding constants of these three derivatives could not be estimated from the linear regression methods because they required weighted data. When the data were not weighted, especially for the guest compound, which interacts weakly with the host compound, the linear regression procedures placed too much emphasis on the low CD concentrations, and caused the results very unreliable [15,29,36]. This phenomenon is also observed in the plots, which calculated from Eqs. (5) and (6) (see Fig. 3b and c). Moreover, the type of substituent also effects on the formation of the guest–host inclusion complex. Comparison with N-1,5-DS, low and unreliable binding constants were obtained for 2-NH_2-N-1,5-DS, indicating that the steric effect of NH_2-group may affect the formation of the complex. Since the non-linear regression fitting method was more convenient and did not need to weight the data to obtain accurate results, therefore, this method was retained for further calculations.

Table 2
Apparent binding constants at various temperatures of the complexation in 20 mM borate buffer (pH 9.2) by using a non-linear regression method and with the viscosity correction

Compound	Apparent binding constants (M^{-1})				
	20 °C	25 °C	30 °C	35 °C	40 °C
N-2-S	420 ± 5	420 ± 20	330 ± 30	220 ± 70	280 ± 10
N-1-S	18 ± 13	50 ± 15	21 ± 39	31 ± 37	13 ± 9
3NH_2-N-2,7-DS	610 ± 9	610 ± 30	440 ± 20	390 ± 20	320 ± 5
2NH_2-N-1,5-DS	0	50 ± 20	3 ± 140	0	0
N-1,5-DS	1900 ± 100	220 ± 20	24 ± 70	10 ± 110	10 ± 120
N-2,6-DS	700 ± 130	320 ± 50	300 ± 40	300 ± 40	260 ± 30

Table 3
Thermodynamic parameters for binding between naphthalenesulfonates β-CD in 20 mM borate buffer at pH 9.2

Compounds	ΔH° (kJ/mole)	ΔS° (J/mole K)	r^2	ΔG_{298} (kJ/mole)
N-2-S	−17.3 ± 3.3	−8.5 ± 11.0	0.931	−14.7 ± 0.4
N-1-S	−69.3 ± 23.7	−198 ± 77	0.895	−8.4 ± 1.3
$3NH_2$-N-2,7-DS	−26.9 ± 4.0	−38.0 ± 13.3	0.938	−15.4 ± 0.4
$2NH_2$-N-1,5-DS	−1800 ± 820	−5980 ± 2702	0.829	12.6 ± 3.3
N-1,5-DS	−210 ± 36	−655 ± 120	0.943	−11.0 ± 6.1
N-2,6-DS	−41.1 ± 20.5	−85.8 ± 67.8	0.924	−15.0 ± 0.4

r^2: correlation coefficient obtained by the least-squares method.

3.3. Temperature effect and thermodynamic study

The capillary temperature also plays an important role in determining the apparent binding constant of complex formation because it affects the viscosity of the separation buffer in the capillary, and thus, causes the migration times of the inclusion complex decreasing due to the increasing the mobilities. Table 2 lists the apparent binding constants at various temperatures for the complexation of naphthalenesulfonate derivatives and β-CD by using a non-linear regression method. The binding constants decrease as the temperature increases, perhaps because the collision between molecules increases with the temperature, reducing the possibility for stable complex formation [17,37]. However, the relative uncertainties of the binding constants are high for 2-NH_2-N-1,5-DS and N-1,5-DS, some of K values even cannot be calculated at certain temperature (Table 2). Temperature dependence of the binding constants can be described by a van't Hoff plot equation as following [23,38–40]:

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (8)$$

where ΔH° is the enthalpy change associated with inclusion complex formation, ΔS° is the corresponding entropy change, T is the temperature, and R is the gas constant. As described by Guillaume and Peyrin [39], the information of the analyte-CD inclusion complexation mechanism can be illustrated by thermodynamic data. Fig. 4 depicts the van't Hoff plots of $\ln K$ versus T^{-1} for these six naphthalenesulfonate derivatives at the temperature range from 20 to 40 °C, according to Eq. (8), to give straight lines with good correlations, except for 2-NH_2-N-1,5-DS. Table 3 lists the values of ΔH° and ΔS° calculated from the van't Hoff plots along with their correlation coefficients for the plots and Gibbs free energies ΔG° for the inclusion complex formation at pH 9.2. The negative entropy (ΔS°) values for all the analytes indicate that the complex formation with β-CD may be favored at relatively lower temperatures, when temperature increasing, the reverse reaction becomes to be favored and the apparent binding constants were reduced [38–40], as described in Table 2. The high ΔH° and ΔS° with positive ΔG° values carried large errors were obtained for 2-NH_2-N-1,5-DS, indicating that free forms of guest 2-NH_2-N-1,5-DS and host CD molecules are favored over the formation of inclusion complex at equilibrium.

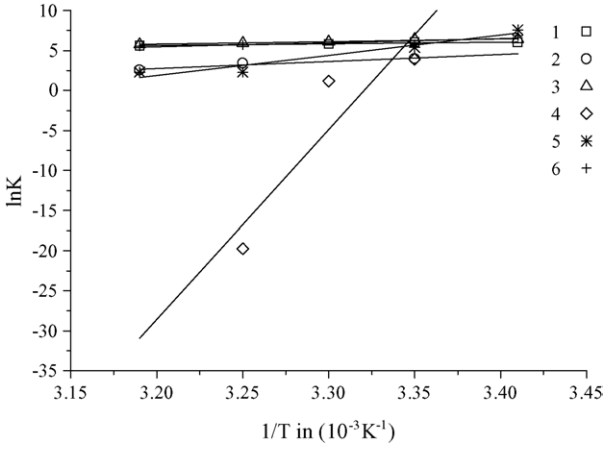

Fig. 4. van't Hoff plots of $\ln K$ vs. T^{-1} for the formation of the naphthalenesulfonate derivatives with β-CD complex.

4. Conclusion

The high efficiency of CE combined with plotting methods makes the determination of apparent binding constants and thermodynamic parameters a simple and straightforward process. Six positional and structural naphthalenesulfonate derivatives in CD-mediated capillary electrophoresis were effectively separated using a borate buffer that contained β-CD at pH 9.2. Interaction with β-CD strongly affects the selectivity and migration behavior for these derivatives. The inclusion behaviors for these derivatives were significantly affected by the position and type of substituent group(s) attached to the naphthalene ring. The thermodynamic parameters were estimated by the binding constants related to the guest–host inclusion complexation at various temperatures. Moreover, these results demonstrates that CD-mediated CE method can be used to simultaneously examine the apparent binding constants of guest–host complex and their corresponding thermodynamic parameters for a set of achiral compounds in a mixed solution.

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