Separation and migration behavior of positional and structural naphthalenesulfonate isomers by cyclodextrin-mediated capillary electrophoresis

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Abstract

The effects of the type of buffer system, buffer pH, the polarity of electrode, and both the type and the concentration of cyclodextrins (CDs) on the separation and migration behavior of seven positional and structural naphthalenesulfonate isomers in CD-mediated capillary electrophoresis were systematically investigated. The most effective separation conditions were to use 20 mM phosphate buffer with β-CD at pH 3.0, while the polarity of the electrodes were reversed across the capillary. Under such conditions, these isomers can be separated in 10 min. The results also indicate that the interactions of naphthalenesulfonate derivatives with CDs are strongly affected by the position of the substituent(s) on the aromatic ring. The inclusion complex formation constants of these compounds were evaluated to improve our understanding of the interaction between the naphthalenesulfonate derivatives and CDs. Moreover, the formation constants of naphthalene-2-sulfonate to β-CD agreed closely with the data in the literature obtained by a spectrophotometric method and by CE methods in various pH buffers.
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1. Introduction

Cyclodextrins (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 inclusion complexes with a wide range of small molecules in aqueous solution. The commonly used CDs are α-, β- and γ-CD, which consist of six, seven and eight D-glucose units, respectively. In capillary electrophoresis (CE), CDs are widely added to as chiral selectors to optimize the separation of optical isomers, but they can also be used to separate positional and structural compounds. Luong and Nguyen have reviewed developments in this area until 1997 [1]. Their review revealed that both neutral and charged CDs could be exploited in CE to optimize the achiral separations of small molecules and various positional isomers.

Ionized naphthalenemonosulfonates and naphthalenedisulfonates and their amino derivatives are extensively used in industrial and commercial applications, especially to produce azo dyes, fluorescent whitening agents and tanning agents. These naphthalenesulfonate derivatives without long alkyl side chains have been reported to persist in aquatic environment [2–4]. They have been found in various environmental water samples, and even in tap and drinking water [5]. High-performance liquid chromatography (HPLC) [6–10], gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) [8,11–13] and liquid chromatography–mass spectrometry (LC–MS) [9,10,14–17] with various solid-phase extractions have been developed in the determination of naphthalenesulfonate residues in environmental samples. During the last few years, CE has been used to separate these ionic compounds owing to its high separating efficiency, lower solvent consumption, short analysis time and the possibility for rapid method development [18–23]. However, in pure phosphate or borate buffers, isomeric naphthalenesulfonates were only separated into groups, and the positional isomers

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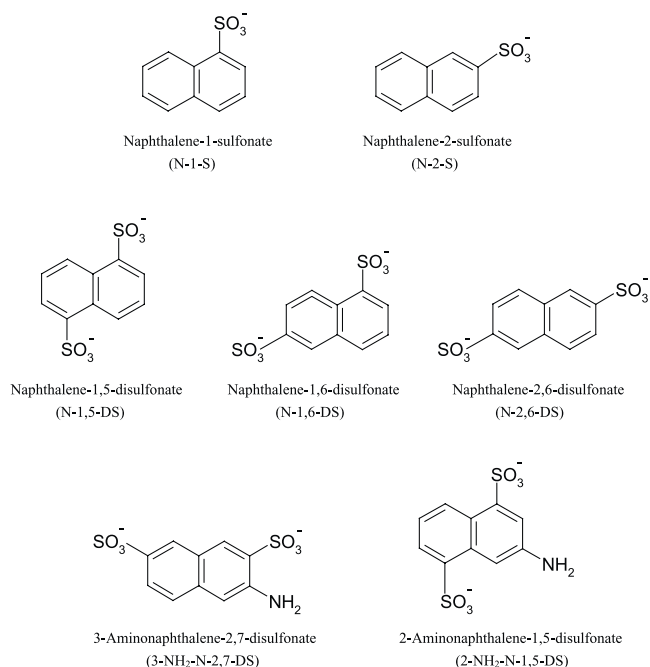*E-mail address:* wding@cc.ncu.edu.tw (W.-H. Ding).

Fig. 1. Structures of the seven naphthalenesulfonate derivatives studied and abbreviations used in the text and the figures.

showed little or no separation. When 15% (v/v) acetonitrile was added as a modifier to the borate buffer (pH 9.2), only partial separation of three isomeric naphthalenedisulfonates was observed. Recently, Fischer et al. reported that the addition of β- or γ-CD to borate buffer could significantly improve the separation of isomeric naphthalenesulfonates and their amino and hydroxy derivatives in synthetic dyes [24].

In our previous report [25], seven naphthalenesulfonate derivatives (as three groups), including two naphthalenemonosulfonate isomers, three naphthalenedisulfonate isomers and two aminonaphthalenedisulfonate isomers (see Fig. 1) were effectively separated by cyclodextrin-mediated CE with sample stacking technique at pH 9.2 in 15 mM borate buffer. In this work, the separation and migration behavior of these positional and structural compounds in cyclodextrin-mediated CE was studied in more detail. Each isomer was used to model positional isomers, and the compounds between each group were used to model structural compounds. The effects of the type and the concentration of CDs on separation and selectivity were systematically examined. The interactions of naphthalenesulfonate derivatives with CDs and the inclusion complex formation constants (also called binding constants or equilibrium constants) were evaluated. These compounds can be selected as good model compounds for studying host–guest 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 inclusion complex. Furthermore, the purpose of this work is also to demonstrate that CE can be used to simultaneously determine the

formation constants of a set of analytes in a mixed solution [26,27].

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. Seven naphthalenesulfonate derivatives, and α-, β- and γ-cyclodextrins 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, whereas sodiumdihydrogenphosphate monohydrate (NaH_2PO_4) separation buffer was prepared at 20 mM in deionized water and was adjusted to pH 3.0 for the reversed polarity separation. Stock solutions of these analytes ($1000 \, mg \, l^{-1}$) 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 Millipore 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 and separation conditions

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 capillaries (J&W Scientific, Folsom, CA, USA) of 75 μm i.d. and an effective length of 50 cm (total length 60 cm). Before use, the capillary was conditioned with methanol for 10 min at 25 °C, followed by 10 min with 1 M HCl, 2 min deionized water, and 10 min 1 M NaOH, then rinsed capillary with deionized water for 2 min, and followed by 10 min separation buffer. Between runs, the capillary was washed with 0.1 M NaOH for 2 min and deionized water for 2 min before run. This procedure improved peak sharps and the reproducibility of migration time. 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 (−25 kV for the reversed polarity separation). The UV detector was operated at 214 nm. The measurements were run at least in triplicate to ensure reproducibility. All electrophoresis runs were performed at temperature 25 °C. The on-column detection window was made by burning a small section (ca. 3 mm) of the external polyimide coating and scraping off the burned residue with methanol. The separation steps were done automatically and controlled by Beckman P/ACE MDQ system version 2.2 software (Beckman-Coulter). The

pH of solutions was measured by a Mettler-Toledo MP220 pH meter (Schwerzenbach, Switzerland).

3. Results and discussion

3.1. Cyclodextrin selections in CE separation

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 monosulfonates < disulfonates < trisulfonates in borate buffer (pH 9.0), and with little or no separation of positional isomers [22,24]. The similar results were 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 naphthalenemonosulfonate isomers, aminonaphthalenedisulfonate isomers, and finally naphthalenedisulfonate isomers. The positional isomers were not separated under these conditions. Table 1 summarizes the migration times of these seven naphthalenesulfonate derivatives in the separation buffers with various CD types and concentrations tested. With the addition of 2 or 10 mM α-CD, only aminonaphthalenedisulfonate isomers were separated, other two groups were not separated (Fig. 2b (5 mM α-CD as an example) and Table 1). Adding 1–5 mM β-CD separated almost all isomers in each group, except for two naphthalenedisulfonate isomers (N-1,5-DS (peak 5) and N-1,6-DS (peak 6)), and yielding the last two broader peaks (Fig. 2c (1 mM β-CD), Fig. 2d (2 mM β-CD) and Table 1). The migration times of N-2-S (peak 1), 3-NH₂-N-2,7-DS (peak 3) and N-2,6-DS (peak 7) (all the substituents at 2-position) declined dramatically as β-CD concentration was increased, possibly indicating that these two compounds interact strongly with β-CD, since the formation of the inclusion complexes with β-CD increases the apparent molecular masses per charge on these three negatively charged isomers, reducing their electrophoretic mobility toward the anode (+) (the sample inlet of the capillary). This effect is expected to accelerate the migration of negatively charged isomers to the detector, shortening the migration times in the separation buffers that contain β-CD. The migration times of peaks 4–6 are increased upon the
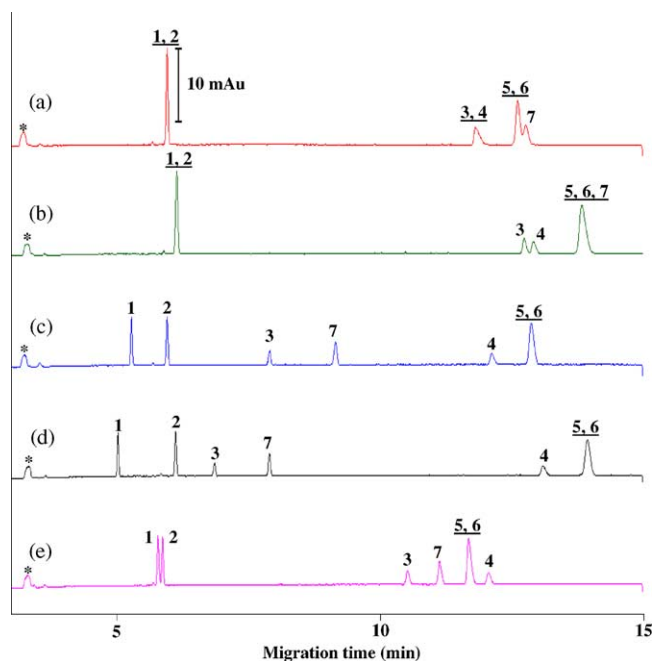
Fig. 2. Electropherograms of the separated naphthalenesulfonate derivatives and the effect of CDs on the separation and migration order. Electropherograms: (a) no CD, (b) 5 mM α-CD, (c) 1 mM β-CD, (d) 2 mM β-CD, and (e) 5 mM γ-CD added in 20 mM borate buffer (pH 9.2). Standard mixture containing 5.0 μg ml⁻¹ of each isomer in deionized water; separating voltage 25 kV; temperature 25 °C; detection 214 nm; hydrodynamic injection at 0.5 psi for 5 s. Peak assignment: (1) N-2-S; (2) N-1-S; (3) 3-NH₂-N-2,7-DS; (4) 2-NH₂-N-1,5-DS; (5) N-1,5-DS; (6) N-1,6-DS; (7) N-2,6-DS.

addition of α-CD and β-CD maybe due to the changes of viscosity of the buffer when CDs were added [24,26,27]. Adding 2–10 mM γ-CD reduces the migration times of each isomer, separates isomers of naphthalenemonosulfonates and aminonaphthalenedisulfonate, but does not separate two naphthalenedisulfonates (Fig. 2e (5 mM γ-CD as an example) and Table 1). The trends exhibited by the various migration times were similar to those obtained when β-CD was used. However, the interactions of naphthalenesulfonate derivatives with γ-CD are weaker than those with β-CD, probably because the size of these compounds makes them fit loosely into the cavity of γ-CD.

Table 1
Migration times of naphthalenesulfonate derivatives under the different conditions tested in 20 mM borate buffer (pH 9.2)

Compound	Migration time (min)									
		α-CD			β-CD			γ-CD		
	No CD	2 mM	5 mM	10 mM	1 mM	2mM	5 mM	2 mM	5 mM	10 mM
N-2-S	5.97	6.19	6.15	6.18	5.29	5.03	4.53	5.77	5.79	6.04
N-1-S	5.97	6.19	6.15	6.31	5.97	6.13	5.85	5.80	5.88	6.19
3-NH₂-N-2,7-DS	11.82	12.79	12.75	13.03	7.92	6.86	5.70	10.88	10.53	10.80
2-NH₂-N-1,5-DS	11.82	12.89	12.92	13.3	12.13	13.10	12.19	11.53	12.07	13.95
N-1,5-DS	12.62	13.86	13.84	14.37	12.88	13.95	12.89	11.72	11.69	12.43
N-1,6-DS	12.62	13.86	13.84	14.37	12.88	13.95	12.89	11.72	11.69	12.43
N-2,6-DS	12.78	14.00	13.84	14.37	9.16	7.91	6.30	11.53	11.14	11.36

3.2. CE separation by reversed polarity

Taking into account the fact that reducing the electroosmotic flow (EOF) may increase the separation selectivity of negatively charged analytes, therefore, the separation of these compounds was evaluated using a 20 mM phosphate buffer at pH 3.0 to eliminate the EOF. Furthermore, in order to prevent the migration time from increasing unacceptably due to an absence of EOF under the normal CE electrode, the polarity of the power supply was reversed. This reversed CE separation (from the cathode $(-)$ to the anode $(+)$) also separated naphthalenesulfonate derivatives into groups and the migration order was reversed as expected: naphthalenedisulfonates < aminonaphthalenedisulfonates (separated) < naphthalenemonosulfonates, as illustrated in Fig. 3a. Table 2 summarizes the migration times of these naphthalenesulfonate compounds in the phosphate buffer (pH 3.0) with various CD types and concentrations tested. With the addition of 2 or 10 mM α-CD, only aminonaphthalenedisulfonate isomers were separated, other two groups were not separated (Fig. 3b (5 mM α-CD as an example) and Table 2). When β-CD was added to the phosphate buffer, each compound, especially isomeric N-1,5-DS (peak 5) and N-1,6-DS (peak 6), was completely separated (Fig. 3c (2 mM β-CD), Fig. 3d (5 mM β-CD) and Table 2). Under these conditions, the migration times of N-2-S (peak 1), 3-NH$_2$-N-2,7-DS (peak 3) and N-2,6-DS (peak 7) increased rapidly with β-CD concentration (Fig. 3c and d and Table 2), especially for N-2-S. This phenomenon implies that N-2-S may penetrate completely into the β-CD cavity; reducing the apparent negative charge; slowing electrophoretic flow-rates toward the anode $(+)$, and cause the longer migration time in the phosphate buffers that contain β-CD. The numbers of theoretical plates per meter measured in 20 mM phosphate buffer at pH 3.0 containing 5 mM β-CD are typically in the range 200 000 (for N-2-S) to 310 000 (for N-2,6-DS). Adding 5–10 mM γ-CD reduces the migration times of each isomer, separates the isomers of naphthalenemonosulfonates and aminonaphthalenedisulfonate, but does not separate N-1,5-DS and N-1,6-DS (Fig. 3e (10 mM γ-CD as an example) and Table 2). Thus, separation of these positional and structural compounds is
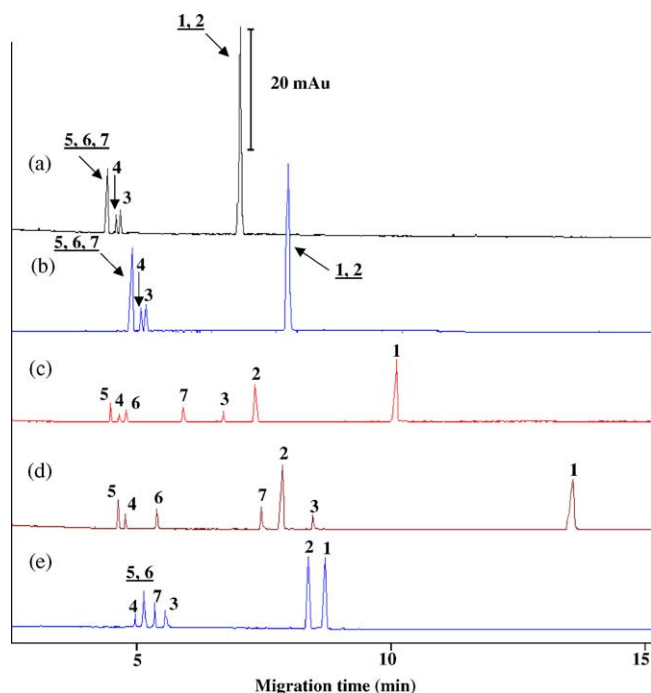

Fig. 3. Electropherograms of the separated naphthalenesulfonate derivatives and the effect of CDs on the separation and migration order while the polarity of the power supply was reversed. Electropherograms: (a) no CD, (b) 5 mM α-CD, (c) 2 mM β-CD, (d) 5 mM β-CD, and (e) 10 mM γ-CD added in 20 mM phosphate buffer (pH 3.0). Experimental conditions and peak identification as in Fig. 2, except voltage -25 kV was applied across the capillary.

promoted by reversing the polarity and using a 20 mM phosphate buffer at pH 3.0, rather than using the normal polarity and a high pH borate buffer during the addition of CDs.

3.3. Evaluation of inclusional complex formation constants

For a better understanding of the influence of CDs on the separation and migration behavior of naphthalenesulfonate derivatives, the inclusional complex formation constants of these compounds were simply evaluated based on the dependence of the effective electrophoretic mobility of these isomers on the various of CDs. Since naphthalenesulfonates are very acidic ($pK_a < -1$) and strong hydrophilic [23], all

Table 2
Migration times of naphthalenesulfonate derivatives under the reversed polarity with different conditions used in 20 mM phosphate buffer (pH 3.0)

Compound	Migration time (min)									
		α-CD			β-CD			γ-CD		
	No CD	2 mM	5 mM	10 mM	2 mM	5mM	10 mM	2 mM	5 mM	10 mM
2-NH$_2$-N-1,5-DS	4.59	4.73	4.94	5.00	4.59	4.72	4.98	4.79	4.91	5.13
3-NH$_2$-N-2,7-DS	4.67	4.81	5.03	5.10	6.83	8.42	9.92	5.13	5.54	5.86
N-2,6-DS	4.41	4.54	4.77	4.81	5.83	7.40	8.92	4.89	5.32	5.62
N-1,5-DS	4.41	4.54	4.77	4.81	4.43	4.58	4.77	4.79	5.09	5.36
N-1,6-DS	4.41	4.54	4.77	4.81	4.75	5.33	6.17	4.79	5.09	5.36
N-2-S	7.02	7.33	7.69	7.95	10.04	13.50	16.42	7.92	8.71	8.90
N-1-S	7.02	7.33	7.69	7.88	6.83	7.79	8.58	7.74	8.38	9.33

separations were performed under conditions in which only the negatively charged form of the analyte was present, even the weakest acids of aminonaphthalenedisulfonate isomers in this study (i.e., the separation at pH 3.0 was greater than 1.5 pH units above the pK_a of 3-NH$_2$-N-2,7-DS (pK_a = 1.61 [28])). Therefore, any pK_a shift due to inclusion into the CD cavity is unlikely to be sufficiently large enough to change the charge state in the separation buffer at pH 3.0. Under these conditions, the effective electrophoretic mobility of naphthalenesulfonate derivatives can be described by the following equation [29,30]:

$$\mu_{\text{eff}} = \frac{\mu_{\text{A}^-} + \mu_{\text{A}^-\text{CD}}K_{\text{A}^-\text{CD}}[\text{CD}]}{1 + K_{\text{A}^-\text{CD}}[\text{CD}]} \qquad (1)$$

where μ_{A^-} is the electrophoretic mobility of the ionic species of the analyte in the absence of CDs, $\mu_{\text{A}^-\text{CD}}$ the electrophoretic mobility of the inclusion complex formed between the ionic species of the analyte and CDs, $K_{\text{A}^-\text{CD}}$ the formation constant of the inclusion complex, and [CD] the concentration of free CDs. Here, the values of μ_{A^-} was measured experimentally in the absence of CDs at pH 9.2 and 3.0, and the trial values of $\mu_{\text{A}^-\text{CD}}$ were estimated from μ_{A^-}, according to Offord's equation (see [31–33]), while the value of μ_{eff} was calculated experimentally according to the observed migration times (t_m) [33]. The values of formation constants $K_{\text{A}^-\text{CD}}$, μ_{A^-} and $\mu_{\text{A}^-\text{CD}}$ measured in 20 mM borate buffer at pH 9.2 and in 20 mM phosphate buffer at pH 3.0 by the reversed polarity CE are listed in Table 3. Since naphthalenesulfonates are very acidic, similar formation constants were obtained from the different

pH conditions in this work. Comparing the formation constants of the various CDs reveals that β-CD is the better complex-forming host compound with naphthalenesulfonate derivatives. The substituent group(s) attached to the aromatic ring significantly affected the inclusion behaviors of these naphthalenesulfonate derivatives. The formation constant 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) (i.e., N-2-S, 3-NH$_2$-N-2,7-DS and N-2,6-DS). The second group includes naphthalene into which at least one substituent had been substituted into the 1-position(s), and which interacted weakly with CDs. In view of the structures of the inclusion complexes between N-2-S and β-CD indicates that the hydrophilic substituent group in the 2-position greatly enhances the stability of the inclusion complex, since the naphthalene nucleus can be entirely embedded in the hydrophobic host cavity in the longitudinal direction, leaving the hydrophilic group in an outer aqueous phase [36]. In contrast, N-1-S can only form a shallowly penetrating longitudinal or weakly-interacting lateral inclusion complex because of the steric hindrance by the substituent groups in the 1-position. The important point from Table 3 is that the formation constant of N-2-S with β-CD agrees closely with that determined by a spectrophotometric method and by using CE in various pH buffers [34,35]. Harata and Uedaira published the first systematic study of

Table 3
Formation constants ($K_{\text{A}^-\text{CD}}$) and mobility data of naphthalenesulfonate derivatives with CDs

Compound	Method	$K_{\text{A}^-\text{CD}}$ (M^{-1})			$\mu_{\text{A}^-\text{CD}}$ ($\times 10^{-3}$ cm^2 V^{-1} s^{-1})			μ_{A^-} ($\times 10^{-3}$ cm^2 V^{-1} s^{-1})
		α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD	
N-1-S	This work (pH 9.2)		30.2	27.9		1.0	0.9	3.4
	This work (pH 3.0)[a]	35.9 ± 3.2	31.1 ± 5.3	32.2 ± 4.9	0.9	0.8	0.8	2.9
N-2-S	This work (pH 9.2)		326	33.2		1.0	0.9	3.4
	This work (pH 3.0)[a]	36.8 ± 2.8	377.6 ± 21.4	40.0 ± 6.7	0.9	0.8	0.8	2.8
	Capillary electrophoresis [34]		380–450 ± 50					
	Capillary electrophoresis [35]		480 ± 20					
	Spectrophotometry [34]		240 ± 40					
2-NH$_2$-N-1,5-DS	This work (pH 9.2)		7.6	13.4		1.9	1.8	5.4
	This work (pH 3.0)[a]	29.4 ± 8.2	13.4 ± 2.6	17.4 ± 5.7	1.7	1.6	1.4	4.4
3-NH$_2$-N-2,7-DS	This work (pH 9.2)		431.8	23.3		1.9	1.8	5.4
	This work (pH 3.0)[a]	38.0 ± 8.8	452.6 ± 72.9	36.3 ± 6.0	1.6	1.5	1.4	4.3
N-2,6-DS	This work (pH 9.2)		293.6	32.7		1.9	1.8	5.5
	This work (pH 3.0)[a]	37.1 ± 8.1	317.0 ± 31.0	39.2 ± 4.2	1.7	1.6	1.5	4.6
N-1,6-DS	This work (pH 9.2)		66.3	23.7		1.9	1.8	5.5
	This work (pH 3.0)[a]	41.8 ± 9.7	72.7 ± 13.3	31.0 ± 4.2	1.7	1.6	1.5	4.6
N-1,5-DS	This work (pH 9.2)		8.2	23.8		1.9	1.8	5.5
	This work (pH 3.0)[a]	40.4 ± 7.1	8.4 ± 1.5	29.7 ± 3.5	1.7	1.6	1.5	4.6

[a] $n = 3$.

complex formation between naphthalene derivatives and CDs [37]. They used circular dichroism spectra to examine the structures of the inclusion complex of β-CD with positional isomers of 1- and 2-naphthylacetic acids. The spectra of these two isomers differed remarkably, indicating that the steric effect of substituents strongly affects the formation of the complex. Inclusion complex formation constants of CDs and alkylnaphthalene compounds have also been indirectly determined from spectroscopic measurements [34,38–44]. The similar trends were also indicated that substitution in the naphthalene 2-position has been led to increase the formation constants above those obtained with naphthalene with a substituent in the 1-position, and formed more efficiently with β-CD than with the smaller α-CD or larger γ-CD.

4. Conclusion

Seven positional and structural compounds of naphthalenesulfonates in CD-mediated capillary electrophoresis were effectively separated using a phosphate buffer that contained β-CD at pH 3.0, while the polarity of the power supply was reversed. Interaction with CDs strongly affects the selectivity and migration behavior of these isomers. Evaluations of formation constants of these isomers with various CDs show that β-CD is found to be the better complex-forming compound with naphthalenesulfonate derivatives. The inclusion behaviors of these isomers were significantly affected by the substituent group(s) attached to the aromatic ring. Moreover, the consistency with values obtained using other methods demonstrates that CE can be used to simultaneously determine the formation constants for a set of analytes in a mixed solution.

Acknowledgements

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