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Analysis of naphthalenesulfonate compounds by cyclodextrin-mediated capillary electrophoresis with sample stacking

Hsin-Chang Chen, Wang-Hsien Ding*
Department of Chemistry, National Central University, Chung-Li 32054, Taiwan

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Abstract

This study systematically investigates the optimal conditions for analyzing the positional isomers of multi-charged naphthalenesulfonate compounds by cyclodextrin-mediated capillary electrophoresis (CE). Specifically, this work employs large-volume sample injection with the electrode polarity switching technique. The most effective separation and sample stacking conditions were 15 m*M* borate buffer with a mixture of β- and γ-cyclodextrin (concentration ratio 3:7 m*M*) at pH 9.2, and the sample hydrodynamic injection of up to 60 s at 3 p.s.i. (around 1.8 μl, and 1 p.s.i.=6.9 kPa). Significantly selective and sensitive improvements were observed and a more than 100-fold enrichment was achieved (based on peak area). The reproducibility of migration time and quantitative results of stacking CE can be improved by using an internal standard. The quantitation limits of these naphthalenesulfonate isomers, based on a signal-to-noise ratio above 10, can be about 4 μg/l with UV detection. This method was successfully applied to determine the trace amount of naphthalenesulfonate isomers in a spiked drinking water sample.

Keywords: Sample stacking; Positional isomers; Water analysis; Large-volume injection; Naphthalenesulfonates; Cyclodextrins; Organosulfur compounds

1. Introduction

Ionized naphthalene mono- and disulfonates 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 compounds without long alkyl side chains have been reported to persist in aquatic environments [1–3]. These compounds have been found in wastewater effluents [4–7], surface waters [8–11], landfill leachates [12–14], and even in tap and drinking water [15]. High-performance liquid chromatography (HPLC) [4,8,11,13,14], gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) [11,16–18] and liquid chromatography–mass spectrometry (LC–MS) [5–7,12–14] with various solid-phase extractions have been developed in the determination of naphthalenesulfonate residues in environmental samples.

Reemtsma has extensively reviewed the relevant research completed up to 1995 [19]. This review demonstrated that ion-pair HPLC and LC–MS techniques have been successfully applied to analyze

*Corresponding author. Tel.: +886-34-227-151x5905; fax: +886-34-227-664.
E-mail address: wding@cc.ncu.edu.tw (W.-H. Ding).

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these residues in various matrices. However, long analysis times and high solvent consumption make this method inefficient. Capillary electrophoresis (CE) has recently achieved prominence as a very effective separation technique for analyzing large numbers of charged species [20,21]. Owing to its high efficiency, small sample volume requirements, lower solvent consumption than HPLC, short analysis time and the possibility for rapid method development [22,23], CE has been used to separate various naphthalenesulfonates [24–26]. However, in many cases CE appears insufficiently sensitive owing to its short optical path, which is associated with on-column detection. Large-volume sample injection with electrode polarity switching is one on-column concentration technique used to improve detection sensitivity of CE [27–32]. Briefly, this technique comprises injection of a large volume of sample dissolved in a lower conductivity buffer matrix by hydrodynamic injection mode. Then, application of voltage with electrode polarity reversed (i.e. reversed direction of electroosmotic flow, EOF) where the lower conductivity buffer matrix is pumped out from the capillary into the inlet vial, and the anionic analytes are focused upon passage through the concentration boundary. When current reaches 95% of the original values, the polarity is switched to normal condition. The separation voltage is reapplied where focused analyte zone migrates toward the detector and continues to separate. This technique has been successfully applied for on-column enrichment of various negatively charged compounds, and is termed as the reversed electrode polarity stacking mode (REPSM) [33–37]. However, this technique has not previously been applied to separate ionized naphthalene mono- and disulfonates and their amino derivative isomers.

Cyclodextrins (CDs) are neutral glucose polymers with a truncated corn shape, and possess a hydrophilic exterior and a hydrophobic interior cavity. The commonly used CDs are α-, β- and γ-CD, which consist of six, seven and eight glucose units, with internal diameters of 5.5, 6.4 and 8.3 Å, respectively. Various guest molecules can penetrate the cavity and form inclusion complexes (guest–host complex), the stabilities of which depend on the size and shape of the guest molecules, the size of the CD cavity used, and also other factors such as hydrogen-bonding, hydrophobic interactions and solvent effects. CDs are widely added to CE as chiral selectors to optimize the separation of optical isomers, but they can also be used to separate positional isomers. Luong and Nguyen have reviewed developments in this area until 1997 [38]. 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. They also investigated the addition of β- and γ-CD to borate buffer to improve the selectivity separation of isomeric naphthalenesulfonates and their amino and hydroxy derivatives in synthetic dyes [39].

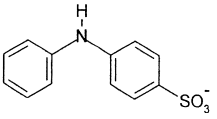
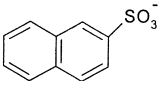
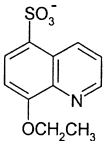
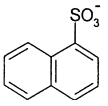
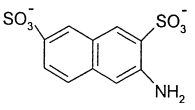
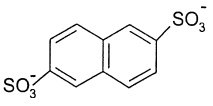
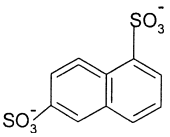
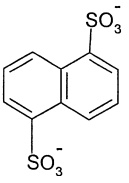
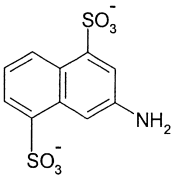
This study reports the addition of β- and γ-CD to the separation buffer to enhance the CE separation of naphthalene mono- and disulfonates and their amino derivative isomers, with simultaneous use of REPSM to increase the sensitivity. The effects of stacking and separation conditions were considered, and the influences of CE separation conditions (i.e. β- and γ-CD content and buffer concentration) were systematically investigated. The use of an internal standard to improve the peak identification and quantitation results was also reported. The results further demonstrated the effectiveness of the method in determining trace levels of positional isomers in spiked drinking water.

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. Nine naphthalenesulfonate compounds, including two naphthalenemonosulfonate isomers, three naphthalenedisulfonate isomers and two aminaphthalenedisulfonate isomers plus internal standard and surrogate (see Table 1), and α-, β-, and γ-cyclodextrins were purchased from Aldrich. Sodium tetraborate ($Na_2B_4O_7$) separation buffers were prepared at stated concentrations between 10 and 25 m*M* in deionized water and were adjusted to pH 9.2. Stock solutions of these analytes (1000 mg/l) were pre-

Table 1
Naphthalenesulfonate isomers studied with their abbreviations and peak assignment

Compound	Abbreviation	Structures	Peak assignment[a]
Diphenylamine-4-sulfonate (surrogate)	DPA-4-S		1
Naphthalene-2-sulfonate	N-2-S		2
8-Ethoxyquinoline-5-sulfonate (internal standard)	8-EQ-5-S		3
Naphthalene-1-sulfonate	N-1-S		4
3-Aminonaphthalene-2,7-disulfonate	3-NH_2-N-2,7-DS		5
Naphthalene-2,6-disulfonate	N-2,6-DS		6
Naphthalene-1,6-disulfonate	N-1,6-DS		7
Naphthalene-1,5-disulfonate	N-1,5-DS		8
2-Aminonaphthalene-1,5-disulfonate	2-NH_2-N-1,5-DS		9

[a] Peak assignment followed the migration order under the optimal REPSM conditions as shown in Fig. 1d.

pared 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 filters (Gelman Scientific, Ann Arbor, MI, USA) prior to use.

2.2. Apparatus

All experiments were performed on a P/ACE MDQ system (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV–Vis detector. Separations were carried out in untreated fused-silica capillaries (J&W Scientific, Folsom, CA, USA) of 60 cm (effective length 50 cm)×75 μm I.D. The UV detector was operated at 214 nm. All electrophoresis runs were performed at 25 °C. The on-column detection window was made by burning a small section (ca. 3 mm) of the external polymide coating and scraping off the burned residue with methanol. The pH of solutions was measured by a Mettler-Toledo MP220 pH meter (Schwerzenbach, Switzerland).

2.3. Normal electrophoresis and stacking procedures

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 the capillary was rinsed 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 each run.

Procedures for normal CE separation following the method of Fischer et al. [39] were attempted here with minor modifications. All samples were hydrodynamically injected into the capillary in 5 s at 0.5 p.s.i. (1 p.s.i.=6.9 kPa), a volume of ~25 nl, and an applied voltage of 25 kV. Procedures for REPSM sample stacking have been described elsewhere [36,37], and were used here with minor modifications. Briefly, the capillary was filled with a separation buffer, and a large volume of analytes was prepared in deionized water and hydrodynamically injected into the capillary with an injection time of 60 s at 3 p.s.i. (around 1.8 μl, and 67.9% of total column length). The small buffer plug (around 90 nl) was then injected after sample injection. At this point, the current decreased due to the lower electrical conductivity of the sample matrix (i.e. water). The ''stacking'' voltage was applied at ''reversed polarity'' (between 10 and 20 kV, 15 kV being optimal, see Results and discussion) at the sample-inlet end. When the reversed EOF pumped out the sample matrix from the capillary into the inlet vial, the current rose gradually to the initial value. The polarity was then switched to normal mode (25 kV) to allow for the analyte separation and detection. The stacking period was measured between 2 and 3 min for optimal stacking efficiency. The stacking and separation steps were done automatically and controlled by Beckman P/ACE System MDQ Ver. 2.2 software (Beckman-Coulter, Fullerton, CA, USA).

3. Results and discussion

3.1. Cyclodextrin selections

For general CE separation, naphthalenesulfonates were separated into groups based on the numbers of SO_3^- groups, with migration times increasing in the order mono<di<trisulfonates in pure borate buffer, and with little or no separation of positional isomers being observed. The same results were observed in the REPSM sample stacking studies, as illustrated in Fig. 1a, where 20 m*M* borate buffer at pH 9.2 served as the separation buffer. They were separated in groups following the order internal standard and surrogate; naphthalenemonosulfonate isomers; aminaphthalenedisulfonate isomers, and finally naphthalenedisulfonate isomers. The positional isomers were not separated under these conditions. With the addition of 10 m*M* β-CD, the isomers in each group were separated, but no separation occurred for certain homologues (Fig. 1b). Meanwhile, with the addition of 10 m*M* γ-CD, isomers of naphthalenemonosulfonates were partially separated, but no separation occurred for two naphthalenedisulfonates (Fig. 1c).

Separation selectivity improved markedly when different ratios of β- and γ-CD mixtures were added

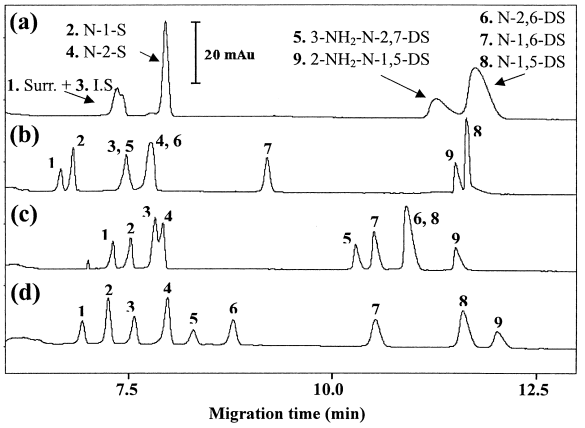


Fig. 1. Electropherograms of the separated naphthalenesulfonate isomers and the effect of CDs on the separation and migration order. Electropherograms: (a) no CD, (b) 10 m*M* β-CD, (c) 10 m*M* γ-CD and (d) 3 m*M* β-CD + 7 m*M* γ-CD added in separation buffer. Standard mixture containing 500 μg/l of each isomer in deionized water; separation buffer 15 m*M* $Na_2B_4O_7$ (pH 9.2); stacking voltage −15 kV and separating voltage 25 kV; temperature 25 °C; detection 214 nm; hydrodynamic injection at 3 p.s.i. for 60 s. See Table 1 for peak identification.

to the separation buffer. When the ratio of β- to γ-CD was 4:6, peaks 4 (N-1-S) and 5 (3-A-N-2,7-DS) were poorly separated. As β-CD decreased and γ-CD increased, short migration time was achieved for peaks 8 and 9, along with baseline separation for peaks 4 and 5. At a ratio of 3:7, perfect resolution and better peak shapes were obtained within 13 min (Fig. 1d). However, increased γ-CD led to poor separation of the naphthalenemonosulfonate compounds of peaks 2 (N-2-S) and 3 (I.S.). Adding the β- and γ-CD mixtures in separation buffer provided various sized CD cavities, and thus resulted in better separation between the groups of naphthalenesulfonates and also enhanced isomer separation in each group. Sample stacking efficiency was not influenced by the addition of CDs.

3.2. Buffer concentrations

The influence of buffer concentration on migration time and naphthalenesulfonate separation was examined in the range 10 to 25 m*M* of borate buffer at pH 9.2 with ratio of β- to γ-CD of 3:7. Migration time increased with borate concentration (results not shown). Perfect resolution and better peak shapes were obtained at 15 m*M* borate concentration. Due to the ionization forms of naphthalenesulfonate compounds over a broad pH range, their electrophoretic properties were not affected by adjusting the pH of the buffers commonly used in CE (results not shown). A buffer pH of 9.2 was used since no pH adjustment was required at this point.

3.3. Stacking periods and voltages

Several investigations have demonstrated that the sample concentration effect depends heavily on the ''stacking period'' and the voltage under reversed polarity [27,28,36]. Timely resumption of normal polarity is very important because a back-flush during the ''stacking period'' can cause the loss of analytes. Inserting a small plug of separation buffer following the sample zone can prevent the possible loss of analytes. The most significant enhancement of the peak area of individual naphthalenesulfonate isomer was observed when the voltage polarity at 15 kV for 2.3 min was reversed and the current was at −23 μA. The stacking effect decreased markedly for longer stacking periods (i.e. 3 min) or higher stacking voltages (i.e. 20 kV).

Fig. 2 displays the electropherogram of the opti-

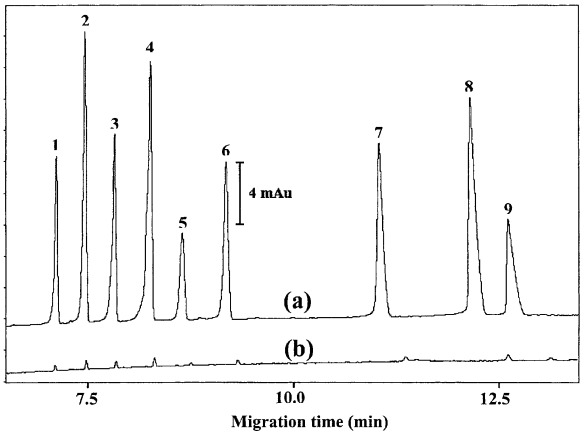


Fig. 2. Comparison of the electropherograms of naphthalenesulfonate isomers (100 μg/l of each isomer in deionized water) by (a) optimized REPSM separation, hydrodynamic injection of 60 s at 3 p.s.i. (around 1.8 μl), and (b) normal CE separation, hydrodynamic injection of 5 s at 0.5 p.s.i. (around 25 nl). Experimental conditions and peak identification as in Fig. 1.

mal CE with the REPSM technique for on-line concentration of a dilute naphthalenesulfonate mixture (100 μg/l for each isomer and I.S./surr.). Given conditions as in Fig. 2a, detector responses increased with increasing sample injection time and pressure, corresponding to an injection of ~1.9 μl (1.8 μl sample+0.09 μl buffer) into the column. An enhancement of over 100-fold was achieved compared to the normal CE, based on a comparison of the peak area of individual naphthalenesulfonate isomers. The advantage of this stacking technique is that the analysis is conducted on the sample itself, without the need for pretreatment or prior manipulation.

3.4. Validation of the method

To validate the performance of the sample stacking technique, the reproducibility (in terms of relative standard deviation; RSD) and linearity were investigated, using standard solution mixtures and under the optimal conditions described above. Table 2 summarizes the RSD of migration time, peak areas, relative migration time and relative peak areas, as well as the linearity of response in the studies of the REPSM stacking technique conducted here. The run-to-run reproducibility of the technique was tested using eight replicate injections of a mixture of naphthalenesulfonate compounds (at 100 μg/l). The RSD of the migration time and the peak areas ranged from 0.7 to 1.4% and 2.1 to 7.2%, respectively. However, these values improved when the internal standard was employed. The linearity of the curve for each naphthalenesulfonate isomer, indicated by the RSD of response factors, varied from 3.1 to 5.6%. The calibration curves were linear with coefficients of determination $r^2 \geq 0.999$. The RSD of the relative migration time varied from 0.1 to 1.0% with injections of various concentrations of standard mixtures. These measurement results verify that CE with the REPSM technique provides high reproducibility and excellent linearity. Quantitation limits, based on a signal-to-noise (S/N) ratio of above 10, were under 4 μg/l for standard mixtures spiked into deionized water, and were two times higher for drinking water samples. Fig. 3 shows the 10 μg/l level detection of naphthalenesulfonate isomers spiked into drinking water under the optimal conditions described above. Peak identities were confirmed using the relative migration times.

4. Conclusion

The analytical procedure presented herein demonstrates that cyclodextrin-mediated CE with REPSM stacking is a reliable, selective, sensitive and high-resolution technique for analyzing trace quantities of negatively multi-charged positional isomers.

Table 2
Reproducibility, linearity of response and response factors using CE with REPSM techniques

	Compound							
	DPA-4-S	N-2-S	N-1-S	3-A-N-2,7-DS	N-2,6-DS	N-1,6-DS	N-1,5-DS	2-A-N-1,5-DS
(1) Reproducibility ($n=8$, 100 μg/l of each)								
Migration time (RSD, %)	0.7	0.8	1.0	1.1	1.2	1.4	1.4	1.4
Peak area (RSD, %)	7.2	5.5	2.1	2.8	3.0	4.7	3.7	3.5
Relative migration time (I.S. 8-EQ-5-S) (RSD, %)	0.2	0.1	0.1	0.1	0.2	0.5	0.5	0.5
Relative peak area (RSD, %)	4.8	6.1	2.0	2.7	2.4	3.3	2.8	2.2
(2) Linearity of response								
Correlation coefficient (r^2)	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
Response factor (I.S. 8-EQ-5-S) (RSD, %)	3.2	3.2	3.1	3.1	4.4	4.2	5.6	5.2
Relative migration time (RSD, %) (Concentrations range from 20 to 1000 μg/l, 5-level)	0.3	0.1	0.1	0.3	0.4	0.6	0.9	1.0

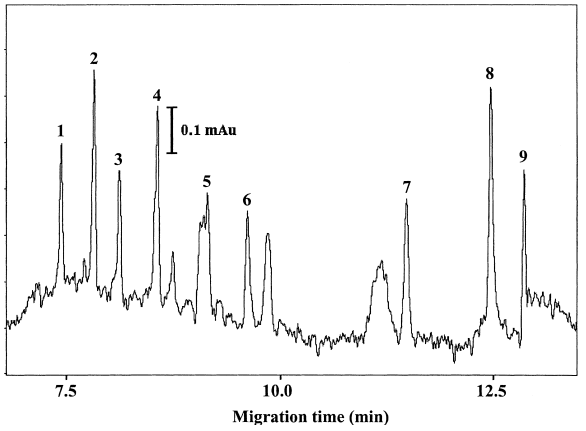


Fig. 3. Electropherogram of a drinking water sample spiked with 10 μg/l each of naphthalenesulfonate isomers under optimal REPSM conditions. Experimental conditions and peak identification as in Fig. 1.

The separation of the isomeric naphthalene mono- and disulfonates as well as the aminaphthalenesulfonates improves markedly in the presence of cyclodextrins. The size of the CD cavity significantly influences the separation. Separation of the naphthalenesulfonate isomers is optimized in borate buffer containing a mixture of β- and γ-CDs. The sensitivity improves over 100-fold compared to normal CE. The reproducibility of the migration time and the quantitative results can be enhanced using internal standards. The simplicity of this method should make it easily exportable to other achiral separation protocols that require enhanced column efficiency and reduced detection limits.

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