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Effects of the working electrolyte (cyclodextrin type and pH) on the separation of aromatic sulphonic acids by capillary zone electrophoresis

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Abstract

The effects of the composition of the working electrolyte on the capillary electrophoretic separation of various naphthalenesulphonic acids used as intermediates in production of synthetic dyes were investigated. Borate buffers should be preferred to phosphate buffers and the working electrolytes should contain cyclodextrins for a successful separation. α-, β-, and γ-cyclodextrin were compared as the additives to the working electrolyte. Best separation of strong unsubstituted isomeric naphthalene sulphonic acids can be achieved in electrolytes containing a mixture of β- and γ-cyclodextrin. β-cyclodextrin additive is also necessary for separation of the aminonaphthalenesulphonic acids present in technological samples of 6-amino-1-hydroxynaphtalene-3-sulphonic acid and 1-amino-8-hydroxynaphtalene-3,6-disulphonic acid (I and H acids). The pH of the borate buffer used as the working electrolyte affects the ionisation of these weak acids which is enhanced at higher pH. Optimum separation is obtained in borate buffers with β-cyclodextrin at pH=8.5 or 9. To speed up the elution, elevated pressure can be applied across the capillary. Under these conditions, quantitative analysis of technological samples can be obtained in 15–25 min.

Keywords: Buffer composition; Sulfonic acids; Naphthalenesulfonic acids

1. Introduction

Aromatic sulphonic acids and their amino and hydroxy derivatives are extensively used as intermediates in the production of synthetic dyes, optical brighteners and fluorescent whitening agents. They are strong acids, completely dissociated over a broad pH range, except for aminosulphonic acids, whose acidobasic properties are similar to those of carboxylic acids. High-performance liquid chromatography (HPLC) and more recently capillary zone electrophoresis (CZE) have been used for their analytical separations.

Anion-exchange chromatography is occasionally used for this purpose [1–5], but this method usually lacks selectivity to allow separation of isomeric compounds. In reversed-phase systems with pure aqueous–organic mobile phases sulphonic acids are usually eluted close to the column dead volume with little separation and often with a strongly asymmetrical peak shape. To increase the retention and to achieve successful separations, it is necessary to add ionic compounds to the mobile phase. Ion-pair reversed-phase chromatography with mobile phases containing tetraalkylammonium salts in concentrations 10^{-3}–10^{-4} mol/l [6–9] can be used for this purpose. Better separation of some isomeric acids can be achieved by reversed-phase HPLC with mobile phases containing strong electrolytes (salts) in concentrations 0.1–1 mol/l, where ionic interactions

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of the acids with unreacted silanol groups in the stationary phase are suppressed, retention is increased and separation selectivity enhanced [10,11]. The retention can be controlled by adjusting the concentrations of either the salt or the organic solvent in mixed mobile phases. Using this technique, more than ten isomeric naphthalene mono- to tetra-sulphonic acids could be completely separated [12,13]. The acids are eluted in the order of decreasing number of sulphonic groups. Various substituted amino- and hydroxynaphthalenesulphonic acids [11,14], or isomers of 4,4′-diaminostilbene-2,2′-disulphonic acid [15] can also be separated. Aqueous–organic mobile phases were used to separate sulphonic acid derivatives of phenol on a β-cyclodextrin bonded phase [16].

The interest in CZE for the analysis of ionic compounds has increased rapidly during the past few years because of high efficiency and peak capacity inherent to this technique and improved possibilities of quantitation using sophisticated instrumentation. This technique has been used for separation and determination of dyes and artificial food colourants [17–24], for separation of several substituted aromatic sulphonic acids [25–28] or of isomeric 4,4′-dinitrostilbene-2,2′-disulphonic acid [15].

Micellar electrokinetic chromatography has been reported as a useful method for separation of naphthalenesulphonates [27] or acidic azo dyes and aromatic sulphonic acids in borate buffers containing cholic acid [29].

Complexation by 1,3-bis[tris(hydroxymethyl)-methylamino]propane (bis-tris propane) and interaction with linear polymers (polyvinylpyrrolidone) added to the working electrolyte and acting as pseudo-phase has been used for CZE separation of textile dyes [20].

Cyclodextrin additives to working electrolytes are widely used as chiral selectors for separation of optical isomers, but they can also be used for separation of positional isomers [30]. Addition of β-cyclodextrin to the working electrolyte was suggested to improve selectivity of separation of isomeric sulphonic acid dyes used as artificial food colourants [21–23] or for separation of naphthalenesulphonic acids and their amino and hydroxy derivatives [26,27].

In our previous work [26] we reported the significant effect of the addition of β-cyclodextrin to the working electrolyte on the separation selectivity of various unsubstituted and amino- or hydroxy-substituted naphthalenesulphonic acids. The objective of the present work was to study in more detail the effects of the composition of the working electrolyte on the separation selectivity of complex mixtures of unsubstituted naphthalene mono- to tetrasulphonic acids and of hydroxy- and aminonaphthalenesulphonic acids occurring in technological samples in production of synthetic dyes to improve the separation. As aminonaphthalenesulphonic acids are weak acids and their ionisation can be controlled by adjusting the pH of the working electrolyte, the effect of pH on the separation selectivity of aminonaphthalenesulphonic acids occurring in technological mixtures of 6-amino-1-hydroxynaphtalene-3-sulphonic acid (I-acid) and 1-amino-8-hydroxynaphtalene-3,6-disulphonic acid (H-acid) was investigated. pH of the electrolyte does not significantly affect the migration and separation of unsubstituted naphthalene sulphonic acids as these acids are completely ionized over broad pH range of the working electrolyte. Departing from our earlier results demonstrating the utility of the addition of β-cyclodextrin for this type of separation, possibilities of using different types of cyclodextrins as additives to the working electrolytes were studied here to further improve the separation.

2. Experimental

2.1. Chemicals

Pure standards and technical samples of sulphonic acids were obtained from Synthesia, Pardubice-Semtín, Czech Republic. Their structures are given in Fig. 1. Individual standards, synthetic mixtures and real samples were dissolved at appropriate concentrations in the working electrolytes used.

Sodium tetraborate, boric acid, sodium diphosphate, disodium monophosphate and sodium hydroxide (all analytical grade) were obtained from Lachema (Brno, Czech Republic). α-, β- and γ-cyclodextrins (analytical grade) were obtained from Fluka (Buchs, Switzerland). Water, deionized, was double distilled in glass with addition of potassium per-

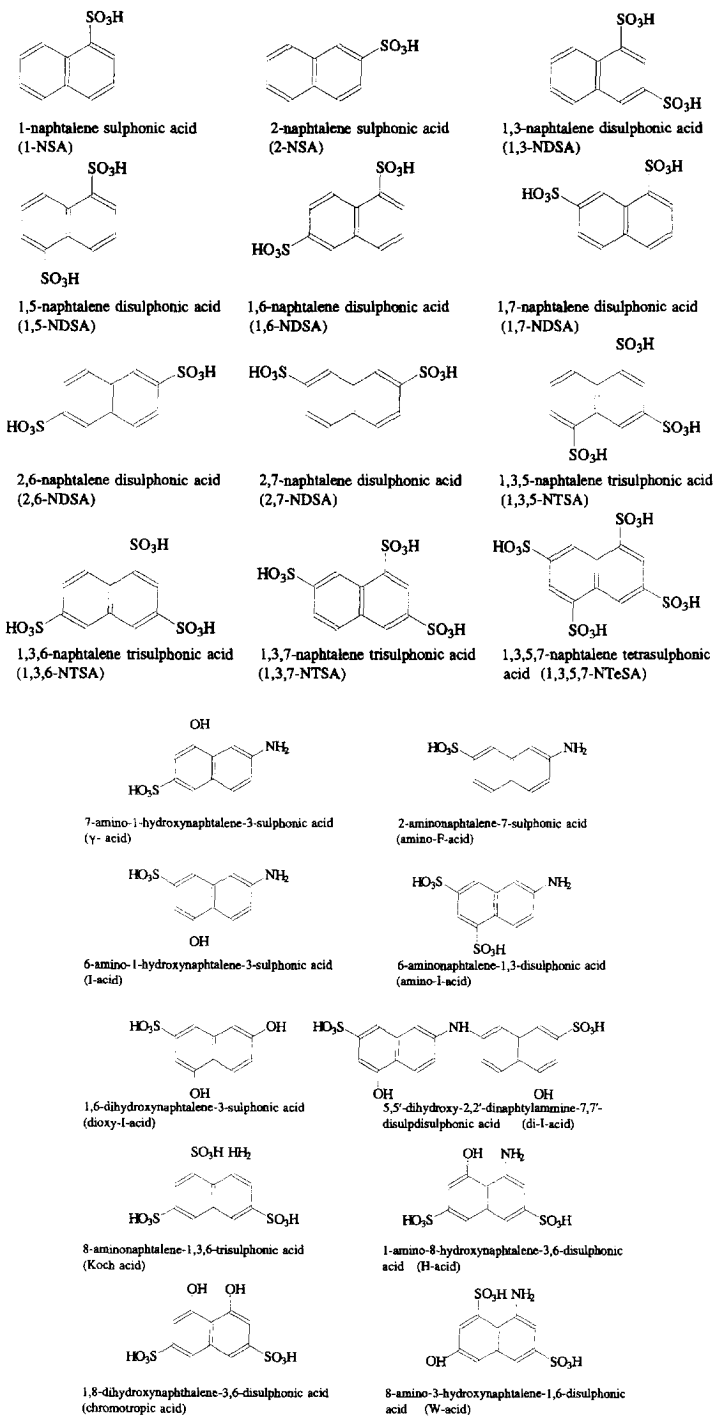


Fig. 1. Compounds analysed, structure and abbreviations used in the text and the figures.

manganate. Working electrolytes for CZE were prepared by dissolving the buffer components in water. The pH was adjusted by mixing of buffers components in appropriate ratios using an OP 208 pH meter (Radelkis, Budapest, Hungary). All electrolytes were filtered using a Millipore 0.45 μm filter and degassed by ultrasonication before the use.

2.2. Apparatus

A Crystal 310 capillary zone electrophoresis system (ATI Unicam, Cambridge, UK) equipped with a variable wavelength detector was used. Silica capillaries, 75 cm (60 cm effective length to the detector)×50 μm I.D.; J&W, Folsom, USA) were subsequently washed with 0.1 mol/l NaOH (10 min), water (10 min) and working electrolyte (until a stabilised baseline was obtained, ca. 20 min) before use. The temperature of the capillary was set at 35°C and the samples were introduced into the capillary using pressurized injection (0.1–0.5 min at 2500 Pa). The separation was performed at a potential of +25 kV applied across the capillary. The detection wavelength was set at 230 nm. Some experiments were run using parallel application of pressure (2500 Pa) and electric field applied across the capillary to speed-up the separation.

3. Results and discussion

3.1. Cyclodextrin selectivity study

Because the electroosmotic migration velocity is higher than the electrophoretic migration velocity of the acids tested, a positive potential should be applied across the capillary and the acids migrate from the source to the detector at a velocity lower than that of the electroosmotic flow. The selectivity of separation does not depend significantly on the applied potential, but the migration times decrease as the potential is increased. To keep the time of analysis short, the separations were run at 25 kV. Due to complete ionisation of naphthalenesulphonic acids over a broad pH range it is not possible to affect their electrophoretic properties by adjusting the pH of the buffers commonly used in CZE as working electrolytes. In pure phosphate or borate buffers, unsubstituted naphthalenesulphonic acids are only separated into groups according to the numbers of the SO_3H groups, with migration times increasing in the order mono-<di-<tri-<tetrasulphonic acids, with little or no separation of positional isomers, as it is shown in Fig. 2, where 0.025 mol/l borate buffer at pH=9 is used as the working electrolyte. To keep the time of analysis acceptable, it is necessary to apply elevate pressure 2500 Pa across the capillary during the electrophoresis. This agrees with the results reported earlier by Kok et al. [27]. They were able to separate 1- and 2-naphthalene sulphonic acids in borate buffers after addition of sodium dodecyl sulphate (SDS), but 1,5-, 2,6- and 2,7-naphthalenedisulphonic acids were not separated under these conditions. In borate buffers with 15% (v/v) acetonitrile, they achieved partial separation of three isomeric naphthalenedisulphonic acids, but little separation of monosulphonic acids.

The addition of β-cyclodextrin to a phosphate buffer used as the working electrolyte made possible separation of 1- and 2-naphthalene sulphonic acids

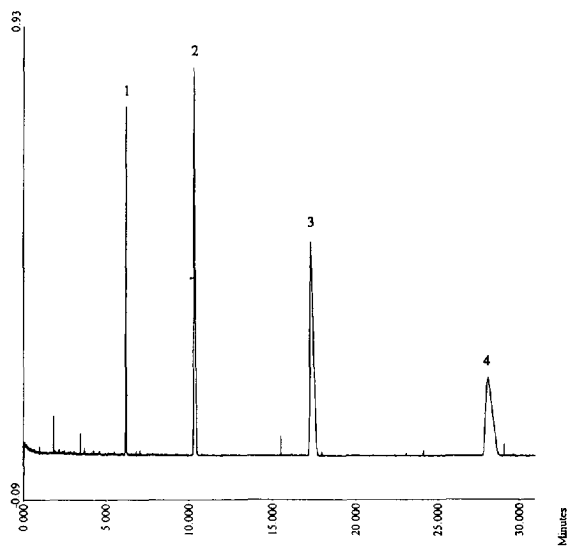


Fig. 2. Separation of a mixture of naphthalenesulphonic acids without cyclodextrin. Monosubstituted (1), disubstituted (2), trisubstituted (3) and tetrasubstituted (4) naphthalenesulphonic acids, respectively. Capillary, 75 cm (60 cm to detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l (pH=9); voltage +25 kV; injection at 25 mbar, 0.1 min. Detection UV, 230 nm; capillary temperature 35°C; overpressure 2500 Pa applied across the capillary.

and of four isomeric naphthalenedisulphonic acids, as reported earlier [26]. Cyclodextrins are neutral polymers of glucose with a shape of a truncated cone. The size and shape of the cavity in their molecules is a function of the number of glucose units; the common cyclodextrins used are α-, β- and γ-cyclodextrin consisting of 6, 7 and 8 glucose units. The diameters of inner cavities are 5.5 Å, 6.4 Å and 8.3 Å for α-, β- and γ-cyclodextrins, respectively. Polar -OH groups are placed outside the cavity and its interior is more hydrophobic than the working electrolyte. A variety of guest molecules can penetrate into the cavity and form inclusion complexes, whose stabilities depend on the size and shape of sample molecules, on the size of the cavity of the cyclodextrin used and on other factors such as hydrogen bonding, hydrophobic interactions and solvent effects.

As the sample ions are in dynamic equilibrium between the free solution and the inclusion complexes in the presence of cyclodextrin, they migrate for a part of time as free ions and for the remaining time they move together with cyclodextrin, i.e., at the velocity of the electroosmotic flow. This effect is expected to accelerate the migration of negatively charged solutes to the detector when positive polarity of high voltage is connected to the sample end of the capillary. However, as shown in Fig. 3, addition of 0.01 mol/l α-cyclodextrin to the borate buffer resulted in increased migration times of naphthalene mono- and di-sulphonic acids and unacceptably long

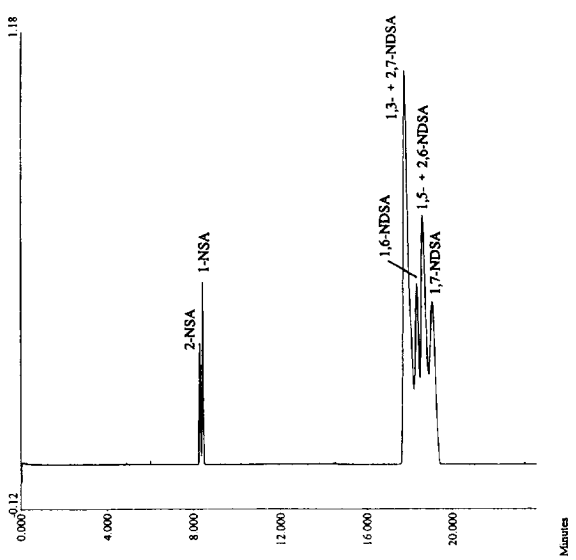
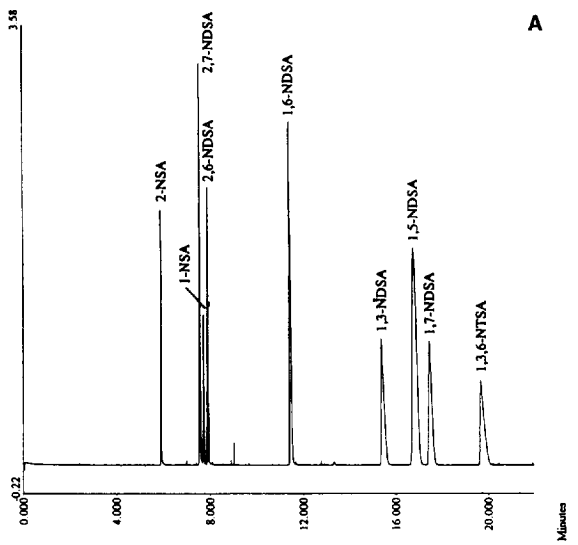


Fig. 3. Separation of a mixture of naphthalenesulphonic acids with α-cyclodextrin. Borate buffer, 0.025 mol/l (pH=9) +0.01 mol/l α-cyclodextrin; for other conditions, see Fig. 2. Compounds as in Fig. 1.



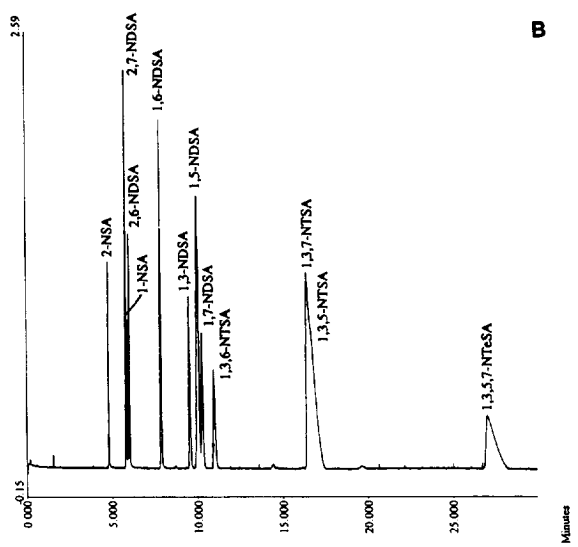


Fig. 4. Separation of a mixture of naphthalenesulphonic acids with β-cyclodextrin without overpressure (A) and with overpressure 2500 Pa applied across the capillary (B). Borate buffer, 0.025 mol/l (pH=9) +0.01 mol/l β-cyclodextrin; for other conditions, see Fig. 2. Compounds as in Fig. 1.

migration times of naphthalenetri- and -tetrasulphonic acids even when overpressure of 2500 Pa was applied across the capillary during the analysis. It is obvious that the phenomena affecting the separation are more complex than the simple scheme outlined above. The electroosmotic flow is approximately at 20% lower in comparison with pure borate buffer (0.025 mol/l, pH=9). The sample molecules can be further retarded by formation of inclusion complexes with adsorbed cyclodextrin molecules. The addition of α-cyclodextrin to the working electrolyte made possible separation of 1- and 2-naphthalenesulphonic acids, but the separation of naphthalene disulphonic acids was still not satisfactory (Fig. 3).

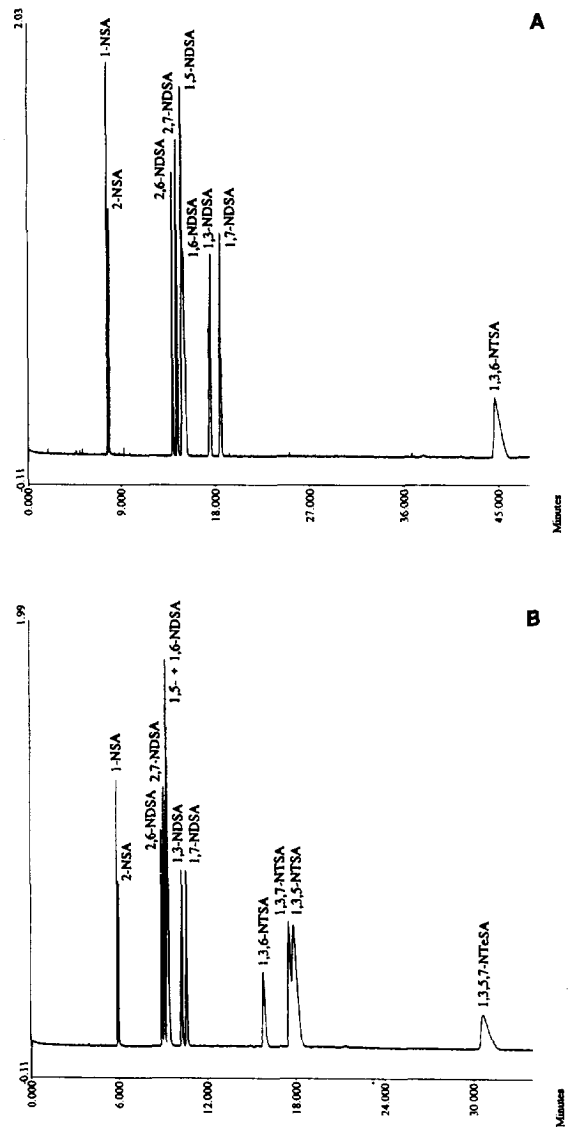


Fig. 5. Separation of a mixture of naphthalenesulphonic acids with γ-cyclodextrin without overpressure (A) and with overpressure 2500 Pa applied across the capillary (B). Borate buffer, 0.025 mol/l (pH=9) +0.01 mol/l γ-cyclodextrin; for other conditions, see Fig. 2. Compounds as in Fig. 1.

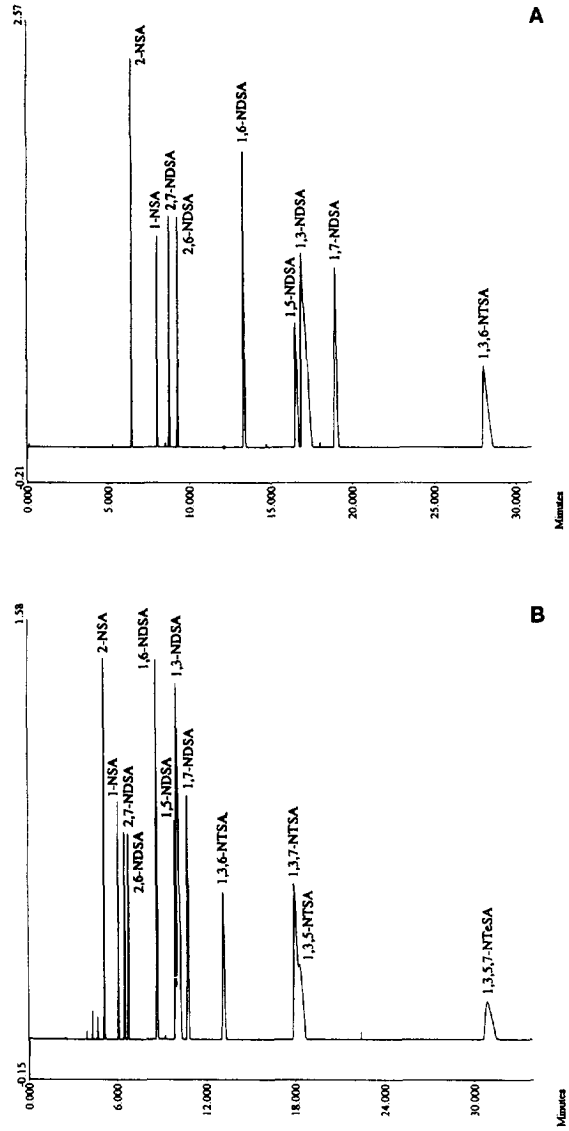


Fig. 6. Separation of a mixture of naphthalenesulphonic acids with mixed β- and γ-cyclodextrin without overpressure (A) and with overpressure 2500 Pa applied across the capillary (B). Borate buffer, 0.025 mol/l (pH=9) +0.005 mol/l β- and γ-cyclodextrin each; for other conditions, see Fig. 2. Compounds as in Fig. 1.

The separation selectivity improved significantly when β-cyclodextrin was added to the working electrolyte (0.025 mol/l borate buffer, pH=9) instead of α-cyclodextrin (Fig. 4). The size and shape of naphthalenesulphonic acids probably better fits the cavity of β-cyclodextrin than that of α-cyclodextrin, which is beneficial for the selectivity of separation of naphthalenemono- and -disulphonic acids (Fig. 4A). β-Cyclodextrin is obviously less strongly adsorbed on the capillary walls than α-cyclodextrin, as the

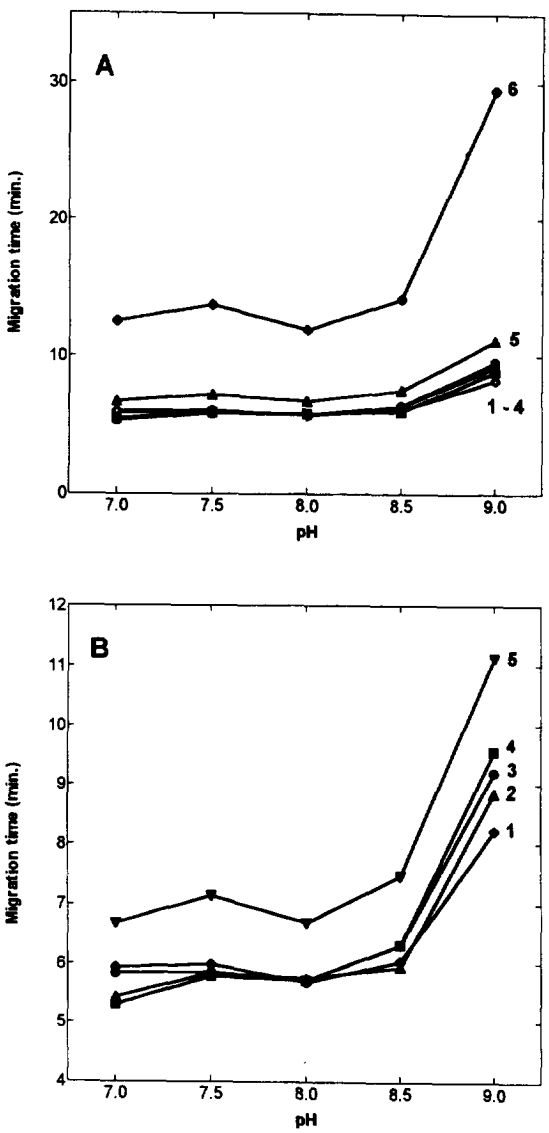


Fig. 7. Dependence of migration times of a mixture of aminonaphthalenesulphonic acids occurring in technological samples of I-acid on the pH of working electrolyte. Amino-F-acid (1), I-acid (2), γ-acid (3), dioxy-I-acid (4), di-I-acid (5) and amino-I-acid (6). Capillary: 75 cm (60 cm to detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l +0.01 mol/l β-cyclodextrin; voltage +25 kV; capillary temperature 35°C.

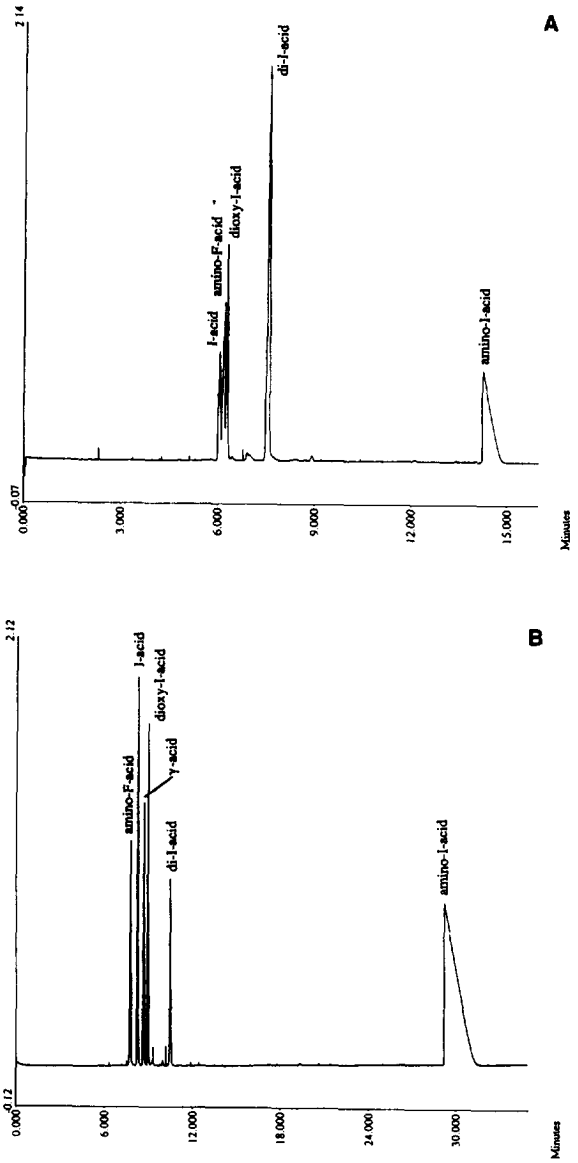


Fig. 8. Separation of a mixture of aminonaphthalenesulphonic acids occurring in a technological sample of I-acid in working electrolyte with pH=8.5 (A) and pH=9 (B). Borate buffer, 0.025 mol/l (pH=8.5, A, and pH=9, B) +0.01 mol/l β-cyclodextrin; without overpressure; for other conditions, see Fig. 2. Compounds as in Fig. 1.

electroosmotic flow was only approximately at 4% lower than in the buffer without β-cyclodextrin and the time of analysis is significantly reduced in comparison with working electrolyte with α-cyclodextrin and was comparable with the separation in pure borate buffer (approximately 30 min at 2500 Pa applied across the capillary). If elevated pressure is applied across the capillary to speed up the analysis, all 12 naphthalenemono- to -tetrasulphonic acids are well separated, except for the pair of 1,3,5- and 1,3,7-naphthalenetrisulphonic acids (Fig. 4B).

Increasing size of cavity of γ-cyclodextrin (8.3 Å with respect to 6.4 Å for the cavity of β-cyclodextrin) resulted in better separation between the groups of naphthalenemono- and -disulphonic acids (Fig. 5A) and improved the separation of 1,3,5- and 1,3,7-naphthalenetrisulphonic acids with respect to β-cyclodextrin (Fig. 5B). The order of migration of naphthalene monosulphonic acids was reversed (1-, 2- with γ-cyclodextrin and 2-, 1- with β-cyclodextrin) and the migration window of naphthalenedisulphonic acids was much narrower than in the buffer with β-cyclodextrin and the pair of 1,5-+1,6-naphthalenedisulphonic acids was not resolved. This behaviour was similar to peak reversal of enantiomers using different cyclodextrin concentrations [31–33]. The electroosmotic flow was similar as in the buffer with β-cyclodextrin, at 6% lower than in the pure borate buffer and the time of analysis was similar as in the buffer with β-cyclodextrin under overpressurised conditions.

Finally, the possibility of further improvement of the separation by the simultaneous addition of β- and γ-cyclodextrin (0.005 mol/l each) to the working electrolyte (0.025 mol/l borate, pH=9) was studied. As shown by the electrophoregram of a mixture of 12 naphthalenesulphonic acids in Fig. 6A, the separation selectivity of 1-, 2-, 1,6-, 1,5-, 1,7-, 2,6- and 2,7-naphthalenesulphonic acids is better than in other working electrolytes tested (Fig. 4A and Fig. 5A). If elevated pressure is applied across the capillary (Fig. 6B), 1,5- and 1,3-naphthalenedisulphonic acids are not resolved to the baseline and the separation of 1,3,5- and 1,3,7-naphthalenetrisulphonic acids is poorer than in the working electrolyte with γ-cyclodextrin.

Table 1
Migration times of naphthalenesulphonic acids under the different conditions used

Compound	Migration time (min)					
	Borate buffer[a] without CD	Borate buffer[a] 0.01 mol/l α-CD	Borate buffer[a] 0.01 mol/l β-CD	Borate buffer[a] 0.01 mol/l γ-CD	Borate buffer[a] 0.005 mol/l β- +γ-CD	Phosphate buffer[b] 0.01 mol/l β-CD
1-NSA	6.3	8.5	5.9	5.9	6.1	6.2
2-NSA	6.3	8.3	4.8	6.0	5.2	5.0
1,3-NDSA	10.3	17.8	9.5	10.3	10.1	9.7
1,5-NDSA	10.3	18.6	10.0	9.3	10.0	10.2
1,6-NDSA	10.3	18.4	7.9	9.3	8.7	8.1
1,7-NDSA	10.3	19.0	10.2	10.6	10.8	10.2
2,6-NDSA	10.3	18.6	6.0	9.0	6.8	6.3
2,7-NDSA	10.3	17.8	5.8	9.1	6.5	6.3
1,3,5-NTSA	17.3	N/A	16.4	17.9	18.2	16.6
1,3,6-NTSA	17.3	N/A	10.9	15.8	13.2	11.3
1,3,7-NTSA	17.3	N/A	16.4	17.6	18.0	16.6
1,3,5,7-NTeSA	28.1	N/A	27.0	30.7	31.0	27.7
t_0(A)	N/A	N/A	4.56	4.66	4.60	N/A
t_0(B)	3.73	4.45	3.87	3.95	3.89	4.10

t_0, electroosmotic break-through time without overpressure (A) and with pressure 2500 Pa (B) applied across the capillary, respectively. Capillary, 75 cm (60 cm to detector)×50 μm I.D.; voltage, +25 kV; temperature, 35°C.

[a] 0.025 mol/l borate buffer, pH=9.0.

[b] 0.025 mol/l phosphate buffer, pH=9.0.

Table 2
Migration times of amino-substituted naphtalene sulphonic acids for the different conditions used

Overpressure conditions	Buffer	Migration time (min)						
		pH	γ-acid	I-acid	amino-F-acid	dioxy-I-acid	amino-I-acid	di-I-acid
Without overpressure	Borate[a]	7.0	5.8	5.4	5.9	5.3	12.6	6.7
		7.5	5.8	5.8	6.0	5.8	13.7	7.1
		8.0	5.7	5.7	5.7	5.7	11.9	6.7
		8.5	6.3	5.9	6.0	6.3	14.1	7.4
		9.0	9.2	8.8	8.2	9.6	29.5	11.1
	Phosphate[b]	9.5	7.1	6.7	6.6	7.3	19.5	8.5
Overpressure 2000 Pa applied	Borate-phosphate[c]	7.0	5.4	5.3	5.6	5.3	N/A	N/A
		9.0	6.1	6.0	5.9	6.1	N/A	7.2
	Buffer	pH	H-acid	Chromotropic acid	W-acid	Koch acid		
Without overpressure	Borate[a]	7.0	5.5	5.8	6.6	10.7		
		7.5	6.5	6.7	8.0	14.0		
		8.0	7.7	7.3	9.2	17.3		
		8.5	14.9	10.3	16.9	43.2		
		9.0	17.8	10.8	19.1	34.1		
Overpressure 2500 Pa applied	Borate[a]	7.0	4.6	4.8	5.4	7.8		
		7.5	5.3	5.4	6.2	9.3		
		8.0	5.9	5.7	6.8	10.0		
		8.5	8.8	7.0	9.4	13.6		
		9.0	10.0	7.4	10.4	13.5		
Overpressure 2000 Pa applied	Borate-phosphate[c]	8.0	9.03	9.03	11.17	22.91		
		8.5	9.09	7.86	10.29	17.32		

Voltage, +25 kV; temperature, 35°C.
[a] 0.025 mol/l borate buffer+0.01 mol/l β-CD; capillary, 75 cm (60 cm to detector)×50 μm I.D.
[b] 0.025 mol/l phosphate buffer+0.01 mol/l β-CD; capillary, 75 cm (60 cm to detector)×50 μm I.D.
[c] 0.025 mol/l borate–phosphate buffer (1:1)+0.01 mol/l β-CD; capillary, 75 cm (60 cm to detector)×75 μm I.D.

Table 1 summarizes the migration times of the zones of the naphthalenesulphonic acids in all the working electrolytes tested.

3.2. Effects of pH on the separation

The separation of unsubstituted naphthalenesulphonic acids does not depend significantly on pH because they are fully ionised over a broad pH range. On the contrary, the amino-substituted naphthalenesulphonic acids are weak and their ionisation can be controlled by adjusting the pH of the mobile phase or of the working electrolyte. Consequently, their migration times depend significantly on pH (Table 2), as illustrated by Fig. 7 for I-, γ-, amino-F-, di-I-, amino-I- and dioxy-I-acids (occurring in technical I-acid) in the working electrolyte containing 0.025 mol/l borate with an addition 0.01 mol/l β-cyclodextrin. These acids could not be successfully separated in phosphate, borate and mixed phosphate–borate buffers at pH between 7 and 9 without the addition of β-cyclodextrin. The separation is improved using more basic electrolytes (borate buffers containing β-cyclodextrin), where the ionisation of the acids is enhanced, but the migration times increase. I-acid, γ-acid, amino-F-acid and dioxy-I-acid are not completely resolved and the migration order is changed in the working electrolytes as the pH is varied from 7 to 8.5. At pH =9, the separation is improved dramatically (Fig. 8B) with respect to the separation at pH=8.5 (Fig. 8A) and all the six acids occurring in technological samples of I-acid can be successfully separated. The time of analysis is approximately 30 min due to the presence of disulphonic amino-I-acid.

Similar behaviour was observed for amino- and hydroxynaphthalenesulphonic acids occurring in technological samples of H-acid (H-, chromotropic, W- and Koch acids. As shown in Fig. 9, the increase of the migration times of amino substituted H-, W- and Koch acids with increasing pH is much more significant than that of hydroxy-substituted chromotropic acid without an amino group (Table 2). Good separation could be achieved again only in working electrolytes containing β-cyclodextrin and the resolution of the individual acids was better in a borate or in a mixed phosphate–borate buffer than in a phosphate buffer (see migration times in Table 2).

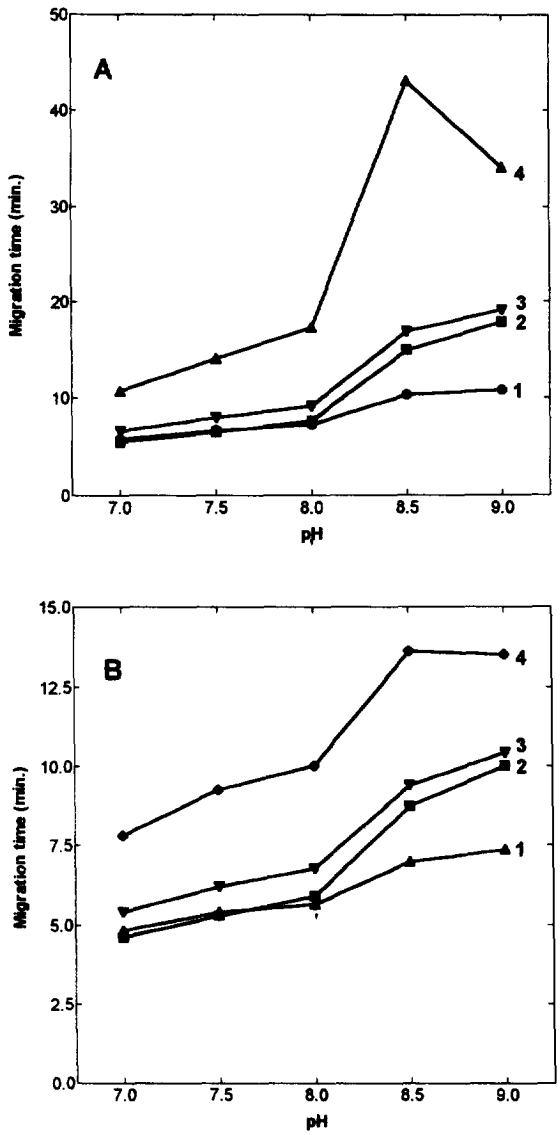


Fig. 9. Dependence of migration times of mixture of naphthalenesulphonic acids occurring in technological samples of H-acid on the pH of working electrolyte without overpressure (A) and with overpressure 2500 Pa applied across the capillary (B). Chromotropic acid (1), H-acid (2), W-acid (3) and Koch-acid (4). Capillary: 75 cm (60 cm to detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l +0.01 mol/l β-cyclodextrin; voltage +25 kV; capillary temperature 35°C.

Changing pH from 7 to 8.5 resulted in improved separation and reversed elution order of chromotropic and H acids (Fig. 10A and B). High migration time of the trisulphonic Koch acid was reduced

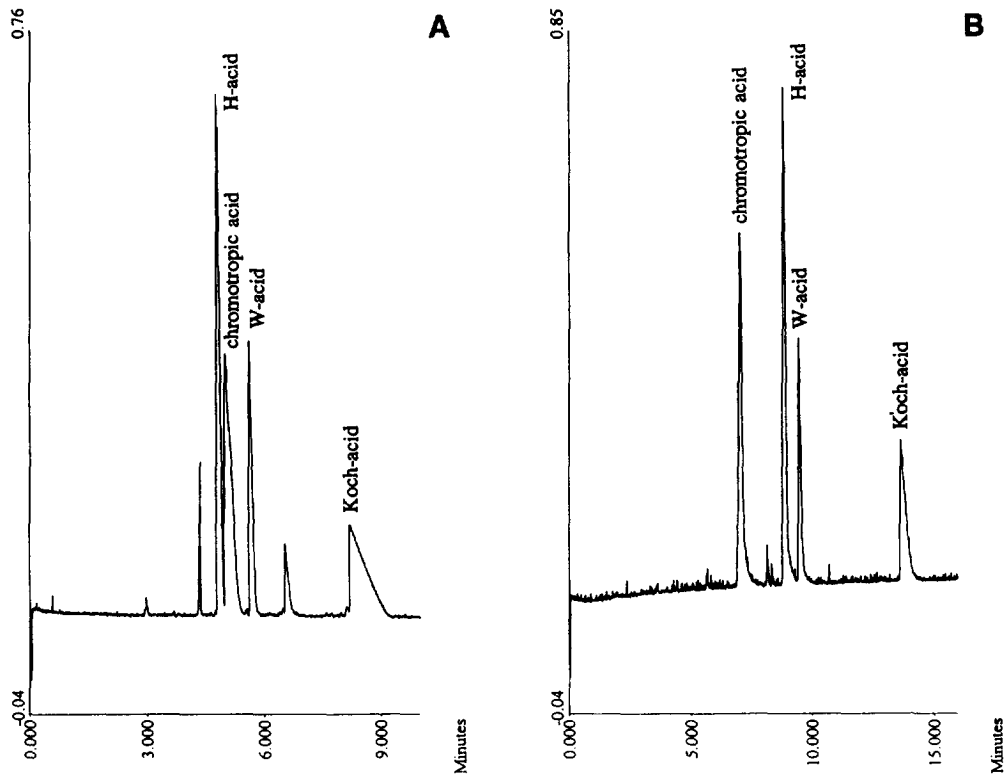


Fig. 10. Separation of a mixture of naphthalenesulphonic acids occurring in a technological sample of H-acid in working electrolyte with pH=7.0 (A) and pH=8.5(B). Borate buffer, 0.025 mol/l (pH=7.0, A, and pH=8.5 B) +0.01 mol/l β-cyclodextrin; overpressure 2000 Pa (A) and 2500 Pa (B) is applied across the capillary; for other conditions, see Fig. 2. Compounds as in Fig. 1.

under elevated pressure 2500 Pa applied across the capillary (Fig. 9 and Table 2), and good separation of all acids investigated was achieved at pH=8.5 (Fig. 10).

4. Conclusions

The separation of the isomeric unsubstituted naphthalene sulphonic acids by CZE is dramatically improved in the presence of cyclodextrins. Working electrolytes with borate buffers provide better separations than with phosphate buffers. The size of the cavity in cyclodextrins affects the separation very significantly. In a borate buffer containing β-cyclodextrin, complete separation of disulphonic acids can be accomplished, but trisulphonic acids can be better resolved in the working electrolyte with addition of γ-cyclodextrin and best separation of the isomeric naphthalenemono- to -tetrasulphonic acids is achieved in borate buffers containing a mixture of β- and γ-cyclodextrins.

The pH of the working electrolyte does not affect significantly the separation of strong unsubstituted naphthalenesulphonic acids, but affects the ionisation and has a dramatic effect on the separation selectivity and on the migration times of aminonaphthalenesulphonic acids. In borate buffers with addition of 0.01 mol/l β-cyclodextrin, all amino-, hydroxy- and aminohydroxynaphthalenesulphonic acids occurring in technological samples of I- and H-acids can be separated.

To decrease the time of the analysis, elevated pressure can be applied across the capillary without a significant loss of resolution. Under these conditions, calibration curves of the individual sample components were linear, and quantitative analysis of industrial samples containing some of the individual

sulphonic acids in concentrations of 0.1% or even less, related to the major sample component, was possible.

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