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Effects of substituted cyclodextrins on the separation of aromatic sulphonic acids by capillary zone electrophoresis

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Abstract

Effects of the addition of various additives in the working electrolyte on the selectivity of capillary electrophoretic separation of naphthalenesulphonic acids used as intermediates in the production of synthetic dyes were investigated. Cyclodextrins form inclusion complexes with various compounds and are not only excellent chiral selectors, but have been also successfully applied for separations of positional isomers. In this work, methyl-β-cyclodextrin, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin, (2-hydroxypropyl)-β-cyclodextrin and (2-hydroxypropyl)-γ-cyclodextrin were studied as isomeric selector additives and compared with unsubstituted β-cyclodextrin. In addition to the size of the cyclodextrin cavity, the number and type of the substituents in the cyclodextrin molecules strongly affect the separation of isomeric naphthalenesulphonic acids, but the effect of the substituted cyclodextrins on the separation selectivity is different for various types of sulphonic acids. Best separations of non-substituted naphthalenesulphonic acids were achieved in a borate buffer with methyl-β-cyclodextrin, whereas the running buffer with non-substituted β-cyclodextrin provides superior separation of amino and hydroxynaphthalenesulphonic acids. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Buffer composition; Cyclodextrins; Sulfonic acids; Naphthalenesulfonic acids; Organosulfur compounds

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 broad pH range, except for aminosulphonic 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 the separation of organic acids [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 usually elute close to the column dead volume with little separation and often even with 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 in reversed-phase

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HPLC with mobile phases containing strong electrolytes (salts) in concentrations 0.1–1 mol/l, where ionic interactions of the acids with non-reacted 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 10 isomeric naphthalene mono- to tetrasulphonic acids could be completely separated [12,13]. The acids elute 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 be separated, too. 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 of 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].

Because of complete ionization and similar mobilities, the separation of isomeric naphthalenesulphonic acids by CZE with common working electrolytes is often difficult. In pure borate buffers only non-substituted naphthalenesulphonic acids with different numbers of the SO_3H groups are separated. To overcome this problem, cyclodextrins (CDs) can be added to working electrolytes. Cyclodextrin additives to working electrolytes are widely used as chiral selectors for separation of optical isomers. In addition to being excellent chiral selectors, cyclodextrins should form inclusion complexes of different strengths also with various positional isomers [30] of naphthalenesulphonic acids and may enhance significantly the selectivity of CZE separation of isomeric naphthalenesulphonic acids. 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 [26,31], we found significant effect of the addition of β-or γ-cyclodextrin to the working electrolyte on the separation selectivity of various non-substituted and amino- or hydroxy-substituted naphthalenesulphonic acids. We applied the electrophoretic method with running buffer containing β-cyclodextrin to the analysis of aromatic sulphonic acids in technical samples of intermediates occurring in the production of dyes. For separation of some substituted naphthalenesulphonic acids the separation was better than in HPLC and we found linear response within the range 0.5–100 mg/l, with average error of 0.5%. However, the separation selectivity for some isomeric naphthalene di- and trisulphonic acids was still not at the optimum in running buffers with unsubstituted β-cyclodextrin. This was the reason why we studied in present work the effects of different types of substituted cyclodextrins on the separation selectivity of complex mixtures of non-substituted naphthalene mono- to tetra-sulphonic acids and of hydroxy- and amino-naphthalenesulphonic acids in technological samples occurring in the production of synthetic dyes.

2. Experimental

2.1. Chemicals

The standards and technical samples of non-substituted and substituted naphthalene mono- to tetrasulphonic acids used as acid dyes intermediates were obtained from Synthesia, Pardubice — Semtín, Czech Republic. Their structures are given in Fig. 1.

The individual standards and synthetic mixtures

were dissolved at appropriate concentrations (0.2–0.5 mg/ml) in the working electrolytes used. To each sample, thiourea was added as the marker of the electroosmotic flow.

Sodium tetraborate, boric acid and sodium hydroxide (all analytical grade) were obtained from Lachema (Brno, Czech Republic). The cyclodextrins used as the running buffer additives (all of analytical grade) were obtained from Fluka (Buchs, Switzerland): β-cyclodextrin (B-CD) methyl-β-cyclodextrin (M-B-CD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-B-CD), heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-B-CD), (2-hydroxypropyl)-β-cyclodextrin (HP-B-CD), and (2-hydroxypropyl)-γ-cyclodextrin (HP-G-CD).

Water, deionized, was double distilled in glass with addition of potassium permanganate. Working electrolytes for CZE were prepared by dissolving the buffer components in water. The pH was adjusted by mixing the buffers components in appropriate ratios using an OP 208 pH meter (Radelkis, Budapest, Hungary). Borate buffers, 0.025 mol/l, pH 9, with addition of 0.01 mol/l cyclodextrin were used as working electrolytes in all experiments. All buffers were filtered using a Millipore 0.45-μm filter and degassed by ultrasonication before use.

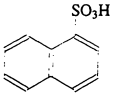
2.2. Apparatus and methods

A Crystal 310 capillary zone electrophoresis system (ATI Unicam, Cambridge, UK) equipped with a variable-wavelength detector was used. A fused-silica capillary, 75 cm (60 cm effective length to the detector×50 μm I.D.); J&W, Folsom, USA) was 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) every day before the start of the work and before the change of the running buffer. The temperature of the capillary was set at 35°C and the samples were introduced into the capillary using pressurized injection (0.1 min at 2500 Pa, which corresponds to the sample volume of 3 nl). 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 overpressure (2500 Pa) applied across the capillary in addition to the electric field, to speed up the separation. Single capillary was used for all experiments with various cyclodextrin additives during the period of 3 months, without significant loss of separation. The washing procedure in between the sets of experiments with various cyclodextrins was sufficient to establish new equilibrium conditions, as can be concluded from the reproducibility of the experimental migration times (see standard deviations in Table 1).

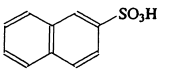
3. Results and discussion

Because the electroosmotic migration velocity is higher than the electrophoretic migration velocity of the acids tested, a positive potential was applied across the capillary, so that the acid ions migrated from the source to the detector at a velocity lower than that of the electroosmotic flow (EOF). 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. Based on our earlier results [26,31], we used working electrolytes containing 0.025 mol/l borate buffer of pH 9 with addition of 0.01 mol/l of various cyclodextrins. In pure phosphate or borate buffers, non-substituted naphthalenesulphonic acids are separated only into classes with different numbers of the $-SO_3H$ groups (1, 2 or 3). The migration times increase in the order mono- <di- <tri- <tetrasulphonic acids, with little or no separation of positional isomers (see migration times in Table 1). This agrees with the results reported earlier by Kok et al. [27], who were able to separate 1- and 2-naphthalenesulphonic 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 with little separation of monosulphonic acids.

Cyclodextrins are neutral polymers of glucose with a shape of a truncated cone. The size and the shape of the cavity in their molecules depend on the number of glucose units; the common cyclodextrins used are α-, β- and γ-cyclodextrins consisting of 6, 7



1-naphtalene sulphonic acid (1-NSA)



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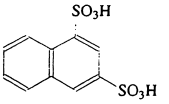
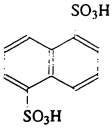


1,3-naphtalene disulphonic acid (1,3-NDSA)



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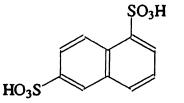

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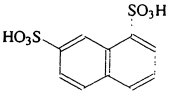
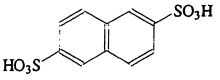


1,7-naphtalene disulphonic acid (1,7-NDSA)



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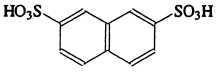
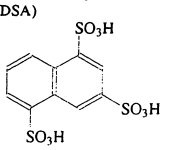
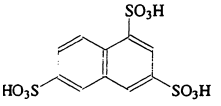


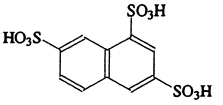
2,7-naphtalene disulphonic acid (2,7-NDSA)



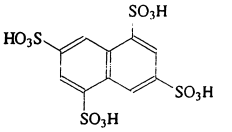
1,3,5-naphtalene trisulphonic acid (1,3,5-NTSA)



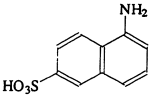
1,3,6-naphtalene trisulphonic acid (1,3,6-NTSA)



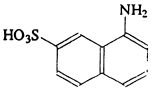
1,3,7-naphtalene trisulphonic acid (1,3,7-NTSA)



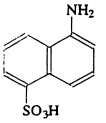
1,3,5,7-naphtalene tetrasulphonic acid (1,3,5,7-NTeSA)



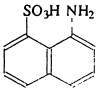
1-amminonaphtalene-6-sulphonic acid (Cleve-1,6-acid)



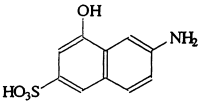
1-amminonaphtalene-7-sulphonic acid (Cleve-1,7-acid)



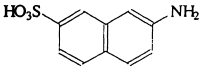
5-amminonaphtalene-1-sulphonic acid (Laurent acid)



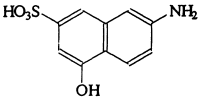
8-amminonaphtalene-1-sulphonic acid (peri acid)



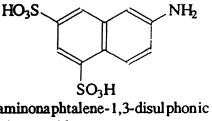
7-amino-1-hydroxynaphtalene-3-sulphonic acid (γ- acid)



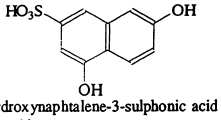
2-aminonaphtalene-7-sulphonic acid (amino-F-acid)



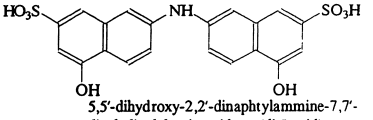
6-amino-1-hydroxynaphtalene-3-sulphonic acid (I-acid)



6-aminonaphtalene-1,3-disulphonic acid (amino-I-acid)



1,6-dihydroxynaphtalene-3-sulphonic acid (dioxy-I-acid)



5,5'-dihydroxy-2,2'-dinaphtylammine-7,7'-disulpdisulphonic acid (di-I-acid)

and 8 glucose units. The diameters of inner cavities are 5.5, 6.4 and 8.3 Å for α-, β- and γ-cyclodextrins, respectively. Polar –OH groups are placed outside the cavity whose 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. The polarity of the external surface of neutral cyclodextrins is affected by the substitution by various functional groups. The number and the size and polarity of the substituents influence the stability of the inclusion complexes and the selectivity of CZE separation.

The addition of cyclodextrins effect is expected to affect the migration of negatively charged solutes to the detector when positive polarity of high voltage is connected to the sample end of the capillary. 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, supposedly at a decreased migration velocity of bulkier complexed ions. The cyclodextrin complex formation can affect not only the total analysis time, but it can have even more important effect on the *relative* migration velocities of sample compounds controlling the selectivity of separation. Hence, the resulting separation should depend on the strength of the CD complexes and consequently on the substituent groups present in derivatized cyclodextrins, which may affect (increase or decrease) the separation selectivity and resolution of the mixtures of isomeric naphthalenesulphonic acids.

We measured the migration times of 22 naphthalenesulphonic acids in running buffers containing five different modified cyclodextrins. The results are given in Table 1, together with the earlier results for unmodified β-cyclodextrin and for the buffer without addition of cyclodextrin, for the comparison sake. To avoid excessively long migration times of polysulphonated acids (>30 min), it was necessary to apply elevated pressure 2500 Pa across the capillary during the electrophoresis. As shown in Table 1, the overpressure decreased considerably the migration times and causes some additional peak broadening (see, e.g., Fig. 4 of Ref. [1]), but, as demonstrated by the electropherograms in Figs. 2–6, this concerns mainly ''overresolved'' late peaks and has only minor effect on the resolution of isomers, as the overpressure did not affect significantly the selectivity of separation expressed as:

$$p = \frac{1 - t_{M(A)}/t_{M(B)}}{t_{M(A)}/t_{EOF} - 1}$$

Here, $t_{M(A)}$ and $t_{M(B)}$ are the migration times of the reference standard (A) and of the sample compound (B), respectively, and t_{EOF} is the time of the electroosmotic flow measured using thiourea as marker compound. It can be shown that the values of p are independent of the electroosmotic flow and are a measure of relative electrophoretic migration times of compounds A and B, so that we can attribute the changes in p induced by the CD additives principally to variations in the stability of the complexes formed with aromatic sulphonic acids. The electroosmotic flow only moderately changed when cyclodextrins with different size of the cavities and size and number of alkyl substituents in CD additives were used. Changes of EOF between repeated runs using

Fig. 1. The non-substituted acids were: (1) 1-naphthalenesulphonic acid (1-NSA); (2) 2-naphthalenesulphonic acid (2-NSA); (3) 1,3-naphthalenedisulphonic acid (1,3-NDSA); (4) 1,5-naphthalenedisulphonic acid (1,5-NDSA); (5) 1,6-naphthalenedisulphonic acid (1,6-NDSA); (6) 1,7-naphthalenedisulphonic acid (1,7-NDSA); (7) 2,6-naphthalenedisulphonic acid (2,6-NDSA); (8) 2,7-naphthalenedisulphonic acid (2,7-NDSA); (9) 1,3,5-naphthalenetrisulphonic acid (1,3,5-NTSA); (10) 1,3,6-naphthalenetrisulphonic acid (1,3,6-NTSA); (11) 1,3,7-naphthalenetrisulphonic acid (1,3,7-NTSA); (12) 1,3,5,7-naphthalenetetrasulphonic acid (1,3,5,7-NTeSA). The substituted naphthalenesulphonic acids were: (13) 1-aminonaphthalene-6-sulphonic acid (Cleve-1,6-acid); (14) 1-aminonaphthalene-7-sulphonic acid (Cleve-1,7-acid); (15) 5-aminonaphthalene-1-sulphonic acid (Laurent acid); (16) 8-aminonaphthalene-1-sulphonic acid (Peri acid); (17) 2-aminonaphthalene-7-sulphonic acid (amino-F-acid); (18) 7-amino-1-hydroxynaphthalene-3-sulphonic acid (γ-acid); (19) 6-amino-1-hydroxynaphthalene-3-sulphonic acid (I-acid); (20) 6-aminonaphthalene-1,3-disulphonic acid (amino-I-acid); (21) 1,6-dihydroxynaphthalene-3-sulphonic acid (dioxy-I-acid); and (22) 5,5′-dihydroxy-2,2′-dinaphthylammine-7,7′-disulphonic acid (di-I-acid).

Table 1
Migration times t_M and selectivities p of naphthalenesulphonic acids in the running buffers with various cyclodextrins

Acid	*N*		β-CD		Methyl-β-CD		Dimethyl-β-CD		Trimethyl-β-CD		Hydroxypropyl-β-CD		Hydroxypropyl-γ-CD	
	t_M (min)	*p*	t_M (min)	*p*	t_M (min)	*p*	t_M (min)	*p*	t_M (min)	*P*	t_M (min)	*P*	t_M (min)	*P*
Without overpressure:														
2-NSA	7.04±0.01		5.64±0.01		5.34±0.01		5.27±0.01		8.37±0.03		5.94±0.02		10.20±0.02	
1-NSA	7.04±0.01	0.00	7.32±0.01	0.74	6.80±0.01	0.83	6.79±0.01	0.81	9.83±0.02	0.22	7.77±0.01	0.81	10.70±0.02	0.05
1,3-NDSA	12.84±0.06	0.60	14.05±0.05	1.93	12.38±0.02	2.19	11.91±0.01	2.03	25.71±0.03	1.02	16.19±0.04	2.18	N/A	N/A
1,5-NDSA	12.84±0.06	0.60	15.14±0.04	2.03	12.67±0.02	2.23	12.77±0.02	2.13	27.26±0.02	1.05	17.03±0.04	2.24	N/A	N/A
1,6-NDSA	12.84±0.06	0.60	10.14±0.02	1.43	10.26±0.02	1.85	11.91±0.01	2.03	25.71±0.03	1.02	11.25±0.02	1.63	N/A	N/A
1,7-NDSA	12.84±0.06	0.60	15.87±0.04	2.08	13.97±0.03	2.38	13.29±0.02	2.19	28.76±0.03	1.07	19.11±0.16	2.38	N/A	N/A
2,6-NDSA	12.84±0.06	0.60	7.42±0.02	0.77	7.01±0.01	0.92	8.21±0.01	1.30	21.01±0.05	0.91	7.92±0.03	0.86	26.86±0.06	0.71
2,7-NDSA	12.84±0.06	0.60	7.12±0.01	0.67	7.30±0.01	1.04	8.54±0.01	1.39	24.87±0.06	1.01	7.92±0.03	0.86	28.24±0.06	0.74
1,3,5-NTSA	28.75±0.11	1.01	46.04±0.10	2.84	33.89±0.21	3.25	29.43±0.10	2.98	N/A	N/A	N/A	N/A	N/A	N/A
1,3,6-NTSA	28.75±0.11	1.01	17.02±0.04	2.16	18.14±0.02	2.72	21.83±0.06	2.76	N/A	N/A	20.67±0.18	2.46	N/A	N/A
1,3,7-NTSA	28.75±0.11	1.01	43.70±0.08	2.82	31.83±0.13	3.21	28.53±0.08	2.96	N/A	N/A	N/A	N/A	N/A	N/A
1,3,5,7-NTeSA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
t_{EOF} (min)	4.03±0.01		4.31±0.01		4.24±0.01		4.14±0.01		5.04±0.02		4.61±0.01		5.46±0.02	
Amino-F	6.80±0.05		5.73±0.01		5.39±0.01		5.25±0.01		6.72±0.02		5.53±0.02		8.37±0.03	
Cleve-1,6	6.70±0.01	−0.02	6.44±0.01	0.38	5.84±0.01	0.28	5.93±0.01	0.40	6.98±0.02	0.07	5.96±0.02	0.27	8.37±0.03	0.00
Cleve-1,7	6.80±0.05	0.00	7.27±0.01	0.74	6.50±0.01	0.62	6.40±0.01	0.63	6.92±0.02	0.05	6.72±0.01	0.67	8.82±0.03	0.07
Laurent	6.80±0.05	0.00	7.53±0.01	0.84	6.92±0.02	0.81	6.60±0.01	0.72	7.21±0.03	0.12	7.26±0.03	0.90	8.58±0.03	0.03
Peri	6.97±0.03	0.04	7.72±0.01	0.90	6.92±0.02	0.81	6.69±0.01	0.76	7.28±0.03	0.14	7.21±0.01	0.88	8.71±0.02	0.05
I	7.73±0.02	0.17	5.79±0.01	0.03	5.27±0.01	−0.08	4.96±0.01	−0.21	6.72±0.02	0.00	5.70±0.03	0.12	9.90±0.05	0.22
Gamma	7.45±0.03	0.12	5.95±0.01	0.13	5.27±0.01	−0.08	5.00±0.01	−0.18	6.44±0.01	−0.08	5.84±0.02	0.20	9.59±0.06	0.18
Dioxy-I	8.22±0.01	0.24	6.06±0.01	0.19	5.33±0.01	−0.04	5.05±0.01	−0.14	6.50±0.01	−0.06	5.77±0.01	0.16	10.54±0.05	0.29
Di-I	9.80±0.04	0.43	7.02±0.01	0.65	5.96±0.01	0.34	5.43±0.01	0.11	5.90±0.02	−0.25	6.25±0.03	0.44	7.80±0.02	−0.10
Amino-I	12.06±0.11	0.61	12.83±0.02	1.94	11.52±0.03	1.94	10.34±0.02	1.74	12.66±0.18	0.84	12.38±0.05	2.10	20.51±0.08	0.84
t_{EOF} (min)	3.96±0.01		4.45±0.01		4.23±0.01		4.09±0.01		4.30±0.02		4.37±0.01		4.90±0.02	
With overpressure (2500 Pa):														
2-NSA	5.57±0.04		4.66±0.01		4.46±0.01		4.44±0.01		6.52±0.02		4.77±0.02		7.04±0.01	
1-NSA	5.57±0.04	0.00	5.73±0.01	0.78	5.42±0.01	0.82	5.47±0.01	0.84	7.38±0.01	0.24	5.87±0.02	0.89	7.26±0.01	0.05
1,3-NDSA	8.65±0.07	0.61	9.15±0.03	2.03	8.45±0.02	2.19	8.36±0.01	2.09	13.84±0.03	1.10	9.75±0.03	2.44	15.05±0.02	0.78
1,5-NDSA	8.65±0.07	0.61	9.60±0.01	2.13	8.50±0.02	2.21	8.76±0.02	2.20	14.28±0.03	1.13	10.33±0.03	2.57	13.19±0.00	0.68
1,6-NDSA	8.65±0.07	0.61	7.47±0.01	1.56	7.42±0.01	1.85	8.35±0.01	2.09	13.84±0.03	1.10	7.72±0.02	1.82	13.19±0.00	0.68
1,7-NDSA	8.65±0.07	0.61	9.89±0.01	2.19	9.17±0.02	2.39	9.00±0.03	2.26	17.71±0.04	1.31	10.77±0.04	2.66	16.09±0.04	0.83
2,6-NDSA	8.65±0.07	0.61	5.79±0.01	0.81	5.56±0.01	0.92	6.35±0.02	1.34	12.32±0.01	0.97	5.97±0.02	0.96	12.34±0.01	0.63
2,7-NDSA	8.65±0.07	0.61	5.79±0.01	0.70	5.74±0.01	1.04	6.54±0.52	1.44	13.59±0.02	1.08	5.93±0.02	0.93	12.93±0.01	0.67
1,3,5-NTSA	13.71±0.17	1.02	16.31±0.05	2.95	14.89±0.06	3.25	14.18±0.12	3.07	38.85±0.26	1.72	19.73±0.11	3.62	N/A	N/A
1,3,6-NTSA	13.71±0.17	1.02	10.31±0.02	2.27	10.79±0.03	2.73	12.18±0.08	2.84	32.99±0.25	1.66	11.23±0.04	2.74	30.15±0.14	1.13
1,3,7-NTSA	13.71±0.17	1.02	16.00±0.06	2.93	14.48±0.06	3.21	13.94±0.10	3.04	37.48±0.25	1.71	18.90±0.10	3.57	N/A	N/A
1,3,5,7-NTeSA	20.91±0.30	1.26	26.20±0.16	3.40	23.27±0.16	3.75	22.41±0.10	3.58	N/A	N/A	N/A	N/A	N/A	N/A
t_{EOF} (min)	3.51±0.02		3.75±0.01		3.67±0.01		3.63±0.01		4.04±0.02		3.95±0.01		4.19±0.02	

Abbreviations of compound names as in Section 2.1. N, no cyclodextrin added to running buffer.

$$p = \frac{1 - t_{MA}/t_{MB}}{t_{MA}/t_{EOF} - 1}$$

B, sample compound; A, reference compound: 2-NSA for non-substituted naphthalenesulphonic acids; Amino-F for substituted acids.

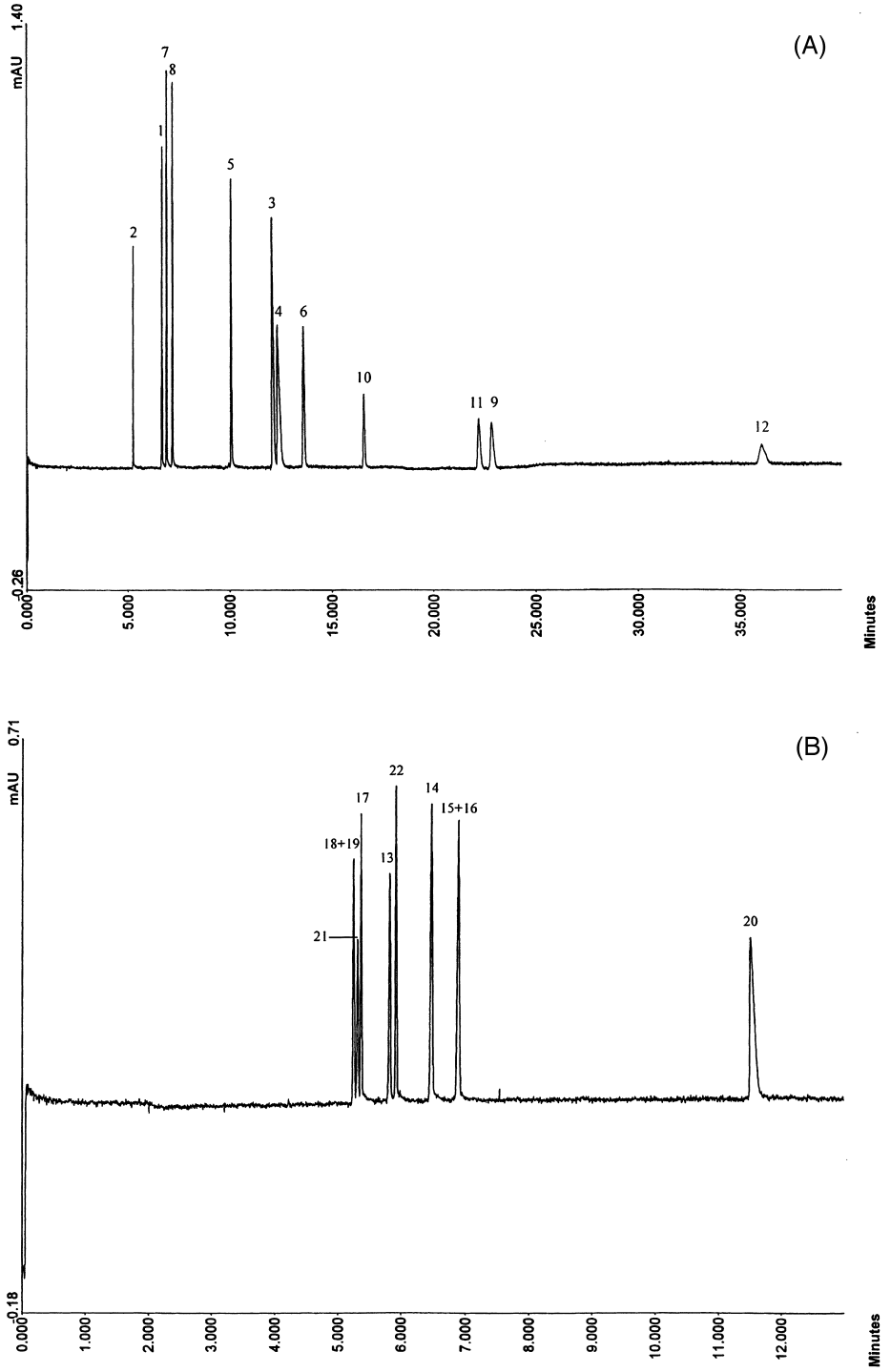


Fig. 2. (A) Separation of non-substituted naphthalenesulphonic acids in the working electrolyte with methyl-β-cyclodextrin. Capillary, 75 cm (60 cm to the detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l (pH 9.0)+0.01 mol/l methyl-β-CD. Voltage, +25 kV; detection, UV, 230 nm; capillary temperature, 35°C; injection at 2500 Pa, 0.1 min; overpressure, 2500 Pa from $t=15$ min. (B) Separation of substituted naphthalenesulphonic acids in the working electrolyte with methyl-β-cyclodextrin,without overpressure. For numbers of compounds, see Section 2.1.

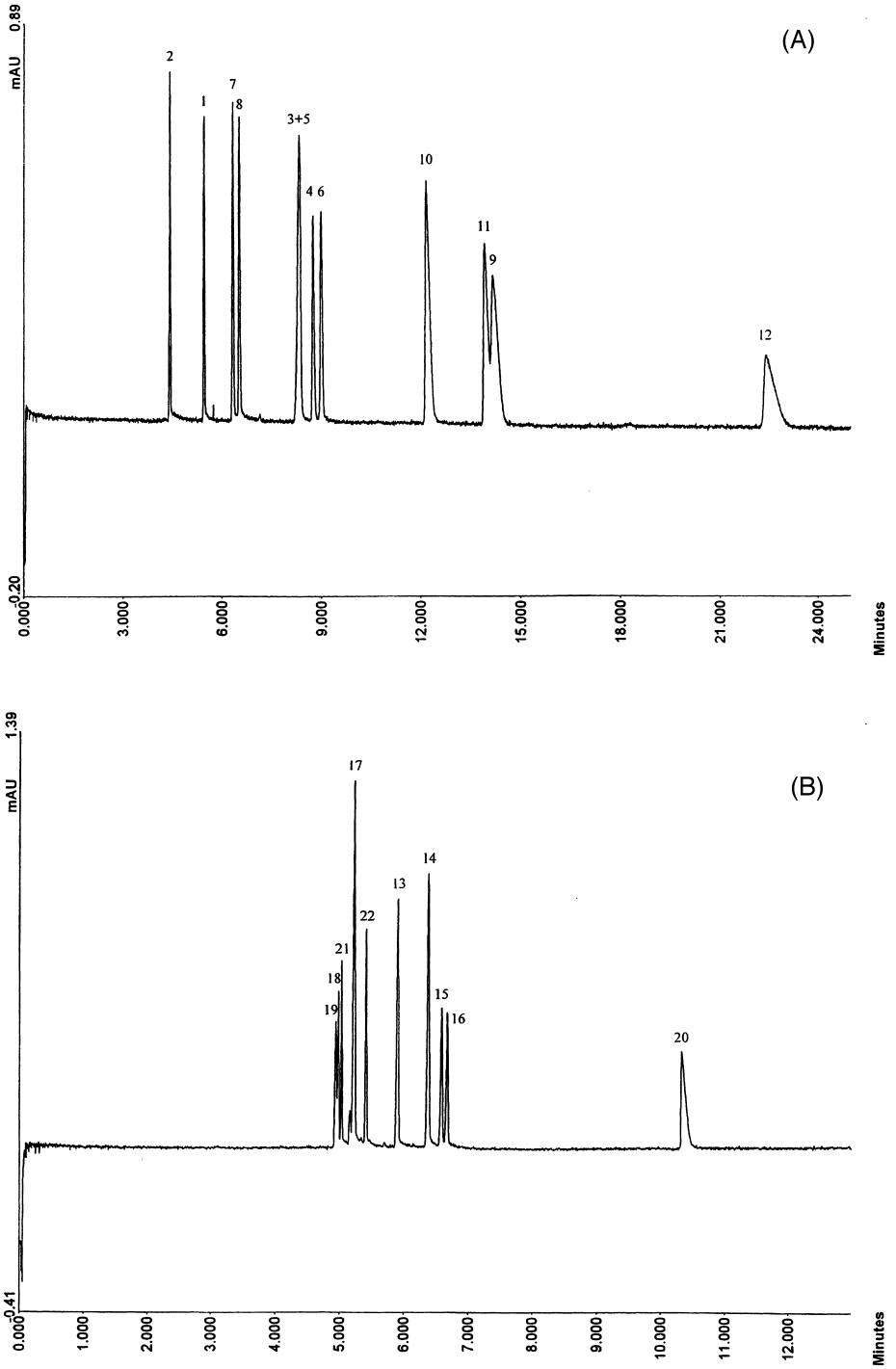


Fig. 3. (A) Separation of non-substituted naphthalenesulphonic acids in the working electrolyte with heptakis(2,6-di-*O*-methyl)-β-cyclodextrin. Capillary, 75 cm (60 cm to the detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l (pH 9.0)+0.01 mol/l dimethyl-β-CD. Voltage, +25 kV; detection, UV, 230 nm; capillary temperature, 35°C; injection at 2500 Pa, 0.1 min; overpressure, 2500 Pa. (B) Separation of substituted naphthalenesulphonic acids in the working electrolyte with heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, without overpressure. For numbers of compounds, see Section 2.1.

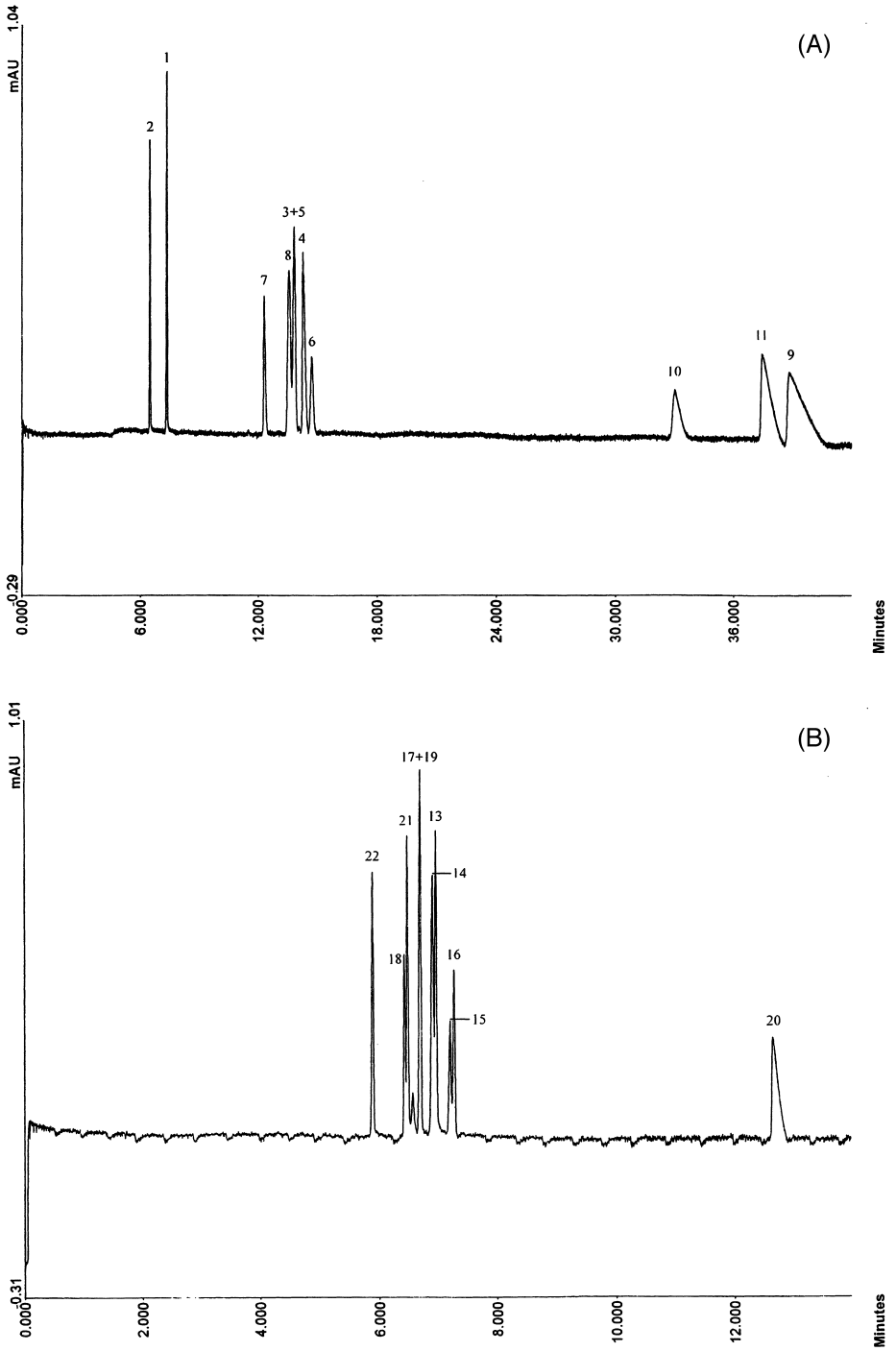


Fig. 4. (A) Separation of non-substituted naphthalenesulphonic acids in the working electrolyte with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin. Capillary, 75 cm (60 cm to the detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l (pH 9.0)+0.01 mol/l trimethyl-M-β-CD. Voltage, +25 kV; detection, UV, 230 nm; capillary temperature, 35°C; injection at 2500 Pa, 0.1 min; overpressure, 2500 Pa. (B) Separation of substituted naphthalenesulphonic acids in the working electrolyte with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin, without overpressure. For numbers of compounds, see Section 2.1.

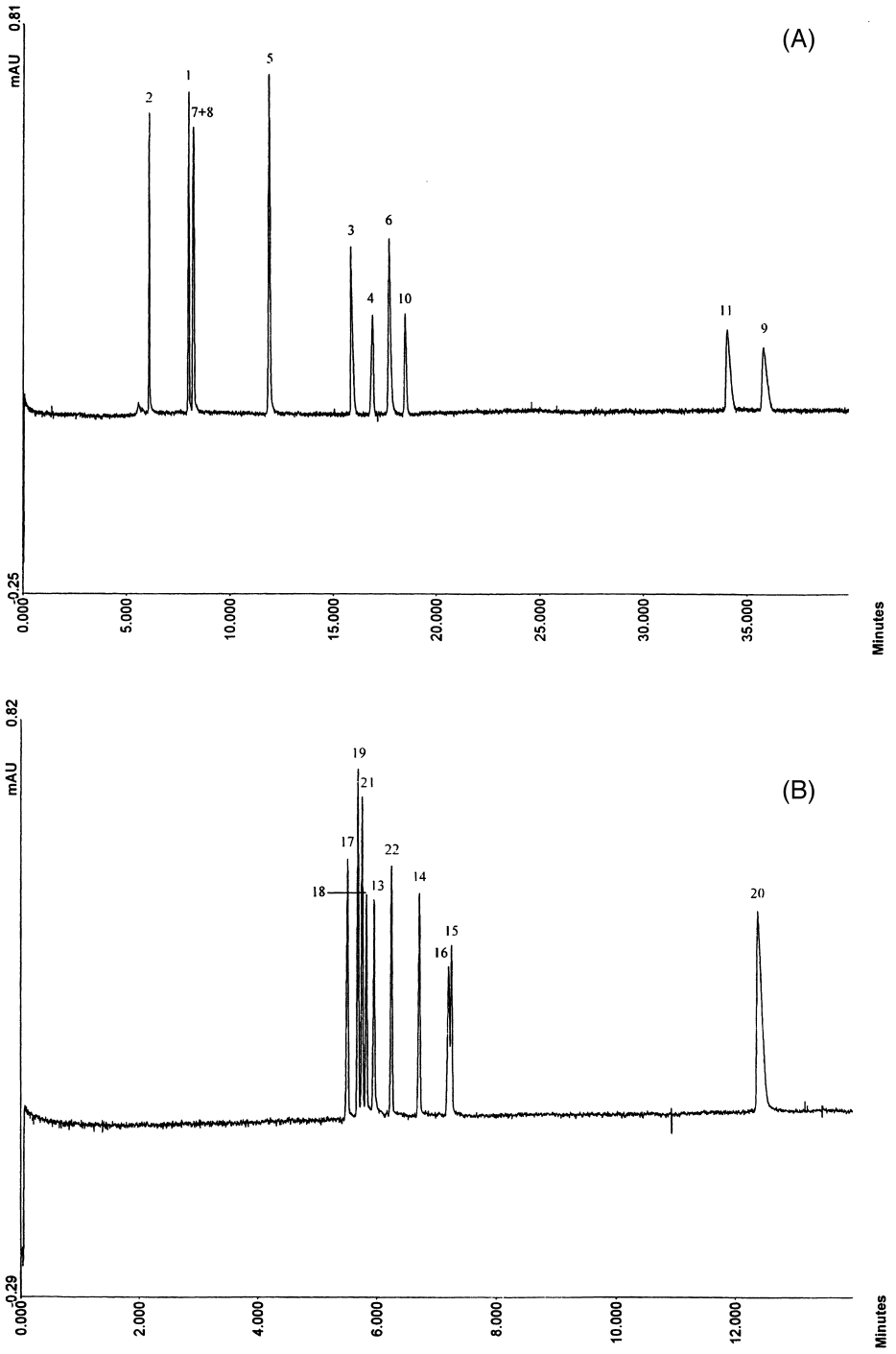


Fig. 5. (A) Separation of non-substituted naphthalenesulphonic acids in the working electrolyte with (2-hydroxypropyl)-β-cyclodextrin. Capillary, 75 cm (60 cm to the detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l (pH 9.0)+0.01 mol/l hydroxypropyl-β-CD. Voltage, +25 kV; detection, UV, 230 nm; capillary temperature, 35°C; injection at 2500 Pa, 0.1 min; overpressure, 2500 Pa from $t=13$ min. (B) Separation of substituted naphthalenesulphonic acids in the working electrolyte with (2-hydroxypropyl)-β-cyclodextrin, without overpressure. For numbers of compounds, see Section 2.1.

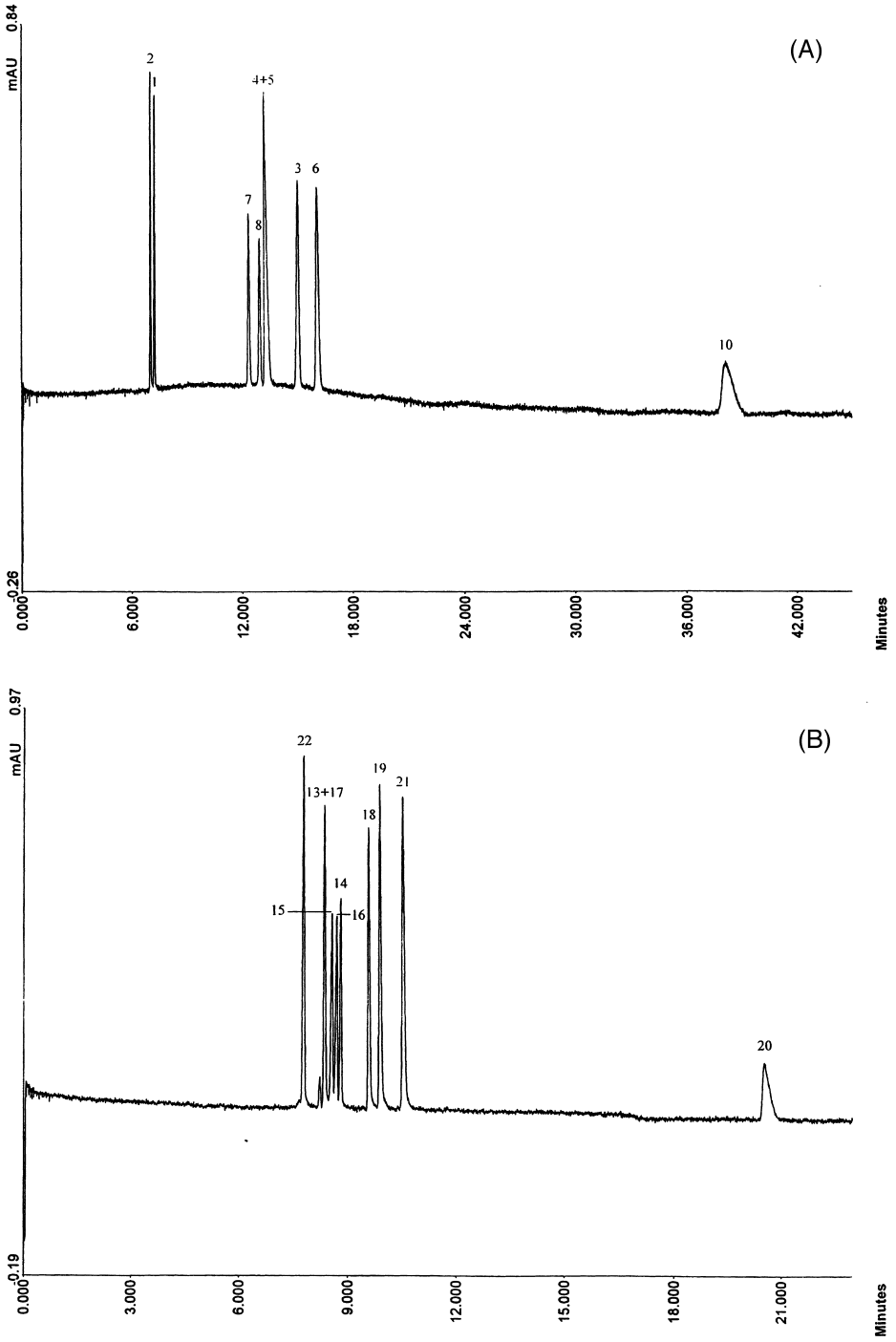


Fig. 6. (A) Separation of non-substituted naphthalenesulphonic acids in the working electrolyte with (2-hydroxypropyl)-γ-cyclodextrin. Capillary, 75 cm (60 cm to the detector)×50 μm I.D. uncoated fused-silica. Borate buffer, 0.025 mol/l (pH 9.0)+0.01 mol/l hydroxypropyl-CD. Voltage, +25 kV; detection, UV, 230 nm; capillary temperature, 35°C; injection at 2500 Pa, 0.1 min; overpressure, 2500 Pa. (B) Separation of substituted naphthalenesulphonic acids in the working electrolyte with (2-hydroxypropyl)-γ-cyclodextrin, without overpressure. For numbers of compounds, see Section 2.1.

the same running buffer were almost negligible (see standard deviations in Table 1).

However, the phenomena involved in the equilibria in the running buffers used are obviously more complex than originally anticipated, as — against the simple assumptions — the migration times of some sulphonic acids increased after addition of various CDs to the running buffer (Table 1). The complicating effects may be possibly attributed either to the adsorption of cyclodextrins on the inner walls of the capillary which may slowdown the migration of sulphonic acid complexes, or to the formation of multiply charged CD complexes with borate anions with increased electrophoretic mobilities towards the sample end of the capillary. Anyway, the addition of various CDs to the running buffer improved considerably the separation of some sample compounds, including isomeric naphthalenesulphonic acids.

In the running buffer containing β-cyclodextrin, mixtures of various naphthalenesulphonic acids were successfully separated. With elevated pressure applied across the capillary to speed up the analysis, all 12 naphthalene mono- to tetrasulphonic acids are well separated, except for isomeric 1,3,5- and 1,3,7-naphthalenetrisulphonic acids [31]. In this working electrolyte, mixtures of amino and hydroxynaphthalenesulphonic acids were successfully separated without overpressure.

The effects of the addition of various substituted cyclodextrins to the working electrolyte on the selectivity of separation are illustrated by the migration data in Table 1. In the borate buffer with methyl-β-cyclodextrin, the separation pattern is similar as in the buffer with non-substituted β-cyclodextrin, but the separation selectivity (Table 1) and the resolution are significantly better for the unsubstituted naphthalenesulphonic acids. The mixture of all 12 tested acids can be completely separated in acceptable time (Fig. 2A). On the other hand, the separation of amino and hydroxynaphthalenesulphonic acids impairs with respect to the separation in the running buffer with non-substituted β-cyclodextrin (Fig. 2B).

The separations of non-substituted and substituted naphthalenesulphonic acids in the working electrolytes with addition of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin are shown in Figs. 3 and 4. Significant increase of the migration times of non-substituted naphthalenesulphonic acids with increasing number of methyl substituents on the cyclodextrin ring in the latter running buffer is observed (Fig. 4), so that the analysis time in buffers with trimethyl-β-cyclodextrin is unacceptably long even if overpressure is applied. The quality of separation of non-substituted naphthalenesulphonic acids in the running buffer with addition of dimethyl-β-cyclodextrin is similar as in the running buffer with γ-cyclodextrin (see Ref. [31]). The separation of substituted naphthalenesulphonic acids impaired with respect to the running buffer with non-substituted β-cyclodextrin.

The electropherograms measured in the working electrolytes with addition of (2-hydroxypropyl)-β- and (2-hydroxypropyl)-γ-cyclodextrin are compared in Figs. 5 and 6. Here again, the migration times of non-substituted naphthalenesulphonic acids are too long and the resolution impairs in comparison to the running buffer with methyl-β-cyclodextrin. The separation selectivity of amino and hydroxynaphthalenesulphonic acids strongly depends on the size of the cyclodextrin cavity, however no essential improvement of the separation was observed with respect to the buffers with non-substituted β-cyclodextrin additive.

4. Conclusions

The separation of the isomeric unsubstituted naphthalenesulphonic acids by CZE is dramatically improved in presence of cyclodextrins. Capillary zone electrophoresis in running buffers with various cyclodextrin additives is suitable also for the separation of amino- and hydroxysubstituted derivatives of naphthalenesulphonic acids. To decrease the time of the analysis, elevated pressure can be applied across the capillary.

The size of the cyclodextrin and the type of the substituent in the cyclodextrin molecule strongly affect the separation of isomeric acids. The effect of the substituted cyclodextrins on the separation selectivity is different for various types of sulphonic acids.

Best separations of non-substituted isomeric naphthalene mono- to tetrasulphonic acids were achieved in a borate buffer with methyl-β-cyclodextrin,

whereas non-substituted β-cyclodextrin additive provides better separation of amino and hydroxynaphthalenesulphonic acids than all substituted cyclodextrins studied.

Acknowledgements

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References

[1] R.H. Stehl, Anal. Chem. 42 (1970) 1802.
[2] P. Jandera, J. Churáček, J. Chromatogr. 86 (1973) 423.
[3] E.S. Jacobs, R.J. Passarelli, J. Chromatogr. Sci. 13 (1975) 153.
[4] M. Singh, J. Assoc. Off. Anal. Chem. 60 (1977) 173.
[5] I.S. Kim, F.I. Sasinos, D.K. Rishi, R.D. Stephens, M.A. Brown, J. Chromatogr. 589 (1991) 177.
[6] D.P. Wittmer, N.O. Nuessle, W.G. Haney, Anal. Chem. 47 (1975) 1422.
[7] R. Gloor, F.L. Johnson, J. Chromatogr. Sci. 15 (1977) 413.
[8] C. Prandi, T. Venturini, J. Chromatogr. Sci. 19 (1981) 308.
[9] B.A. Bidlingmeyer, S.N. Deming, W.P. Price, B. Sachok, M. Petrusek, J. Chromatogr. 186 (1979) 419.
[10] P. Jandera, H. Engelhardt, Chromatographia 13 (1980) 18.
[11] P. Jandera, J. Churáček, J. Bartošová, Chromatographia 13 (1980) 485.
[12] P. Jandera, J. Churáček, J. Chromatogr. 197 (1980) 181.
[13] A. Ito, H. Hiyama, Kagaku to Kogyo (Osaka) 59 (1985) 54.
[14] X. Shen, R. Wang, Fenxi Huaxue 11 (1983) 836.
[15] S. Husain, R. Narishma, S.N. Alvi, R.N. Rao, J. Chromatogr. 596 (1992) 127.
[16] D.R. Wilder, G.W. Tindall, L.J. Cunningham, J.L. Little, J. Chromatogr. 635 (1993) 221.
[17] S.N. Croft, D. Hinks, J. Soc. Dyers Colour 108 (1992) 546.
[18] K.P. Evans, G.L. Beaumont, J. Chromatogr. 636 (1993) 153.
[19] S.N. Croft, D.M. Lewis, Dyes Pigment. 18 (1992) 309.
[20] P. Blatny, Ch.-H. Fischer, A. Rizi, E. Kendler, J. Chromatogr. A 717 (1995) 157.
[21] S. Razee, A. Tamura, T. Masujima, J. Chromatogr. A 715 (1995) 179.
[22] H. Lin, T. Zhu, Y. Zhang, S. Qi, A. Huang, Y. Sun, J. Chromatogr. A 724 (1995) 448.
[23] M. Masár, D. Kaniansky, V. Majdanová, J. Chromatogr. A 724 (1996) 327.
[24] D.A. Oxspring, E. O'Kane, R. Marchant, W.F. Smyth, Anal. Methods Instrum. 1 (1994) 196.
[25] W.C. Brumley, J. Chromatogr. 603 (1992) 267.
[26] P. Jandera, J. Fischer, V. Staněk, M. Kučerová, P. Zvoníček, J. Chromatogr. A 738 (1996) 201.
[27] S.J. Kok, E.H.M. Kloster, C. Gooier, N.H. Velthorst, U.A.Th. Brinkman, O. Zerbinati, J. High Resolut. Chromatogr. 19 (1996) 99.
[28] S.J. Williams, D.M. Goodall, K.P. Evans, J. Chromatogr. 629 (1993) 379.
[29] W.C. Brumley, C.M. Brownrigg, J. Chromatogr. 646 (1993) 377.
[30] S. Terabe, S. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr. 516 (1990) 23.
[31] J. Fischer, P. Jandera, V. Staněk, J. Chromatogr. A 772 (1997) 385.