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Capillary electrophoresis of methyl-substituted phenols in acetonitrile

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Abstract

The separation of mono- and dimethylphenols by capillary electrophoresis in pure acetonitrile was investigated. In acetonitrile, uncharged phenols interact with background electrolyte anions forming negatively charged complexes, which can be separated from each other by capillary electrophoresis. The background electrolyte anions tested were acetate, bromide and chloride. The calculated formation constants for phenol–anion complexes were highest with acetate and smallest with bromide. Complex formation was found to be sensitive to traces of water in the background electrolyte. The separation of methylphenols was also carried out in acetonitrile at high pH using background electrolytes prepared from diprotic acids and tetrabutylammonium hydroxide. At high pH the phenols were partly dissociated, providing an additional mechanism for the separation. All methylphenols were separated with the use of malonate background electrolyte. However, this approach was prone to interference from methanol resulting from the tetrabutylammonium hydroxide solution.

Keywords: Background electrolyte composition; Phenols; Acetonitrile; Methylphenols; Alkylphenols

1. Introduction

Acetonitrile (ACN) is an interesting solvent for capillary electrophoresis (CE). ACN has a number of physicochemical properties that may be of benefit in CE separations. Owing to the relatively low viscosity of ACN, the mobilities of ions (except H^+ and OH^-) are higher in ACN than in other solvents used in CE [1,2]. While high mobilities allow very rapid separations, they also mean that the mobilities of the background electrolyte (BGE) ions are high as well, and the electric current may then be high. Like other organic solvents, ACN affects the ionisation of acids [2–4]. Owing to the low solvation of their ionic forms in ACN, acids are much weaker in ACN than in water. Furthermore, the relative acid strengths in ACN are often different from those in water, and this feature can be utilised when the separation of weakly acidic analytes is difficult to achieve in aqueous media. Clearly, the ionisation behaviour of BGE constituents is also different in ACN, and this needs to be taken into consideration when selecting a suitable BGE. Interactions that are very weak or non-existent in water and other amphiprotic solvents may be strong in ACN. Homo- and heteroconjugation are perhaps the most common examples of interactions that are unfavourable in amphiprotic solvents but very common in ACN [2–5]. ACN can thus be used in many interesting CE investigations, as has already been demonstrated in various studies (see, e.g., Refs. [6–24]).

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Okada applied heteroconjugated anion formation in ACN as a separation mechanism for uncharged acids (phenols, benzoic acids and phenyl alcohols) [8,10,25,26]. The mechanism is based on the weak solvation of small anions in ACN, which is an extremely poor hydrogen bond donor. Thus, stabilisation of small anions tends to occur by reaction with other hydrogen bond donors. Where the donor compound is other than the conjugate acid of the anion, the reaction is called heteroconjugation. In Okada's work, uncharged analytes (phenols, etc.) acted as hydrogen bond donors to BGE anions, namely bromide, chloride, methanesulfonate, nitrate, perchlorate and tetrafluoroborate. The resulting negatively charged analyte–anion complexes could be separated by CE due to the differences in heteroconjugate formation. Heteroconjugated anion formation has also been utilised by Miller et al. [17], in their case for the separation of phenols as analytes with carboxylates as BGE anions. They extended the investigation to cover running conditions where the phenols were partly dissociated, thus combining heteroconjugation and dissociation as separation mechanisms. Recently, Vaher et al. [24] applied acetates, bis(trifyl)amide and hexafluorophosphate for the separation of benzoic acids and phenols by a heteroconjugation method.

Methylphenols are weak acids with fairly similar acidic strengths in water. As noted above, ACN offers useful possibilities for the CE investigation of weakly acidic analytes. The aim of the present work was to investigate the separation of mono- and dimethyl-substituted phenols in ACN. Unsubstituted phenol was included to allow comparison of the results with literature data. Heteroconjugated anion formation with different anions was investigated under conditions where the phenolic analytes were uncharged. In addition, the separation of partly dissociated phenols was investigated. A detailed discussion of the two separation approaches is presented.

2. Experimental

2.1. Chemicals

All chemicals were used as received unless otherwise noted. Acetonitrile (ACN, HPLC grade, far-UV) was from Lab-Scan (Dublin, Ireland). Methanol (HPLC grade) was from Mallinckrodt Baker (Deventer, Netherlands). Acetic acid (glacial), malonic acid and oxalic acid were purchased from Aldrich (Milwaukee, WI, USA). The following chemicals were from Fluka (Buchs, Switzerland): 2,3-dimethylphenol, 2,4-dimethylphenol, 2,5-dimethylphenol, 2,6-dimethylphenol, 3,4-dimethylphenol, 3,5-dimethylphenol, 2-methylphenol, 3-methylphenol, tetrabutylammonium acetate (TBAAc, electrochemical grade), tetrabutylammonium bromide (TBABr, electrochemical grade), tetrabutylammonium chloride (TBACl, electrochemical grade), tetrabutylammonium hydroxide (TBAOH; 1.0 mol/l in MeOH) and tetrabutylammonium perchlorate (TBAP, electrochemical grade). Tetrabutylammonium bimalonate (TBAHMal) was prepared by neutralisation of malonic acid with TBAOH in MeOH using potentiometric titration. The solution was evaporated to dryness under vacuum and the product was recrystallised from ethyl acetate. Phenol was from Merck (Darmstadt, Germany), 4-methylphenol from Sigma (St. Louis, MO, USA), pyrene from EGA-Chemie (Steinheim, Germany) and Hydranal-Coulomat AD Karl Fischer reagent from Riedel-de Haën (Seelze, Germany). Before use, malonic acid, oxalic acid, TBAAc, TBABr, TBACl and TBAHMal were dried in a vacuum desiccator containing phosphorus pentoxide.

2.2. Apparatus

An HP ^{3D}CE instrument (Hewlett-Packard, Waldbronn, Germany) was used in CE experiments. Uncoated fused-silica capillaries [58.5 cm (effective length 50.0 or 8.5 cm)×50 μm I.D.×375 μm O.D.] were purchased from Composite Metal Services (The Chase, Hallow, Worcestershire, UK). Samples were introduced by pressure injection (50 mbar for 1 s). The capillary cassette was thermostatted at 25.0 °C with forced air cooling and the tray temperature was maintained at 25.0±0.5 °C with an external water cooling device. UV detection was carried out at 200 nm. Running voltages were 10–20 kV, with resulting currents of 2–25.5 μA (unless stated otherwise). To keep the migration times of analytes reasonably short, CE runs with the highest chloride and acetate

concentrations were performed with use of short-end injection (effective capillary length 8.5 cm).

2.3. Procedures

Electrophoretic mobilities of analytes were calculated using the migration times of the analyte peak and the EOF marker (pyrene) and the instrumental parameters. The electrophoretic mobilities of analytes are averages of three replicate measurements (except where the effect of traces of water was investigated) with a relative span in most cases less than 1%. In a few cases the relative span was higher (maximum 2.8%). Capillary temperature was estimated by the method of Knox and McCormack [27] (a thermal conductivity of 0.1877 W m^{-1} K^{-1} for ACN [28] and 10 m/s as the flow velocity of the cooling air were used in calculations). For mobility measurements, the estimated temperatures at the centre of the capillary were in the range 25.1–26.4 °C. These temperatures were relatively close to the programmed value of the capillary cassette (25.0 °C), which meant that temperature did not play a significant role in the experiments.

Stock solutions of phenols were prepared in pure ACN at a concentration of 100 mmol/l of each analyte. Dilution to the final concentration (0.1 mmol/l each) was done with ACN. Pyrene (0.2 mmol/l) was used as a marker for EOF.

BGEs prepared from TBABr, TBACl and TBAP were made by weighing an appropriate amount of salt and dissolving it in ACN. Acetate and bimalonate BGEs were prepared by mixing equimolar amounts of acid and the respective tetrabutylammonium salt. Because of their hygroscopic nature, TBAAc and TBACl were weighed in closed vessels. Malonate and oxalate BGEs were prepared by half neutralisation of the acid with TBAOH (1.0 mol/l in MeOH).

ACN, like many other organic solvents, is slightly hygroscopic and thus absorbs water from the laboratory air. Although special care was taken to minimise the time in which vessels were open, contact of the BGEs with air could not be totally avoided. Accordingly, the water content of the BGEs was somewhat increased during preparation and handling of the solutions. The amount was dependent on the humidity of the laboratory air, which varied slightly from day to day. The water content of the solutions was measured by the Karl Fischer method using a 756 KF Coulometer from Metrohm (Herisau, Switzerland). Pure ACN used for the preparation of the BGEs contained 0.007–0.012% (w/w) water, and the freshly prepared BGEs contained 0.012–0.055% (w/w) water. The BGE in the running vials was removed with a syringe immediately after the electrophoretic experiments, and the water content was measured. In all cases the water content of the BGEs after use was less than 0.1% (w/w). The water content of selected BGEs is noted in the text below. Fresh BGE and sample solutions were introduced for every CE run unless stated otherwise. BGEs were prepared daily.

Non-linear curve fittings were performed with Microcal Origin 5.0 (Microcal Software, Northampton, MA, USA).

3. Results and discussion

In water, mono- and dimethyl-substituted phenols are weaker acids than unsubstituted phenol (see Table 1) owing to the acid-weakening effect of the methyl group [34]. Unsubstituted phenol and 3,5-dimethylphenol are very weak acids in ACN (their pK_a values are around 27, see Table 1). Ionisation constants for the other mono- and dimethyl-substituted phenols in ACN are not available in the literature. However, in view of the relatively linear dependency of the pK_a values of other phenols in water and ACN (see, e.g., Refs. [31–33]), it is reasonable to assume that the pK_a values of the other methylphenols are about 27 or higher. Thus, it is safe to assume that these phenols are uncharged if the pH of the BGE is below 25. As already discussed in the Introduction, ACN has a poor ability to donate hydrogen bonds to anions. The BGE anion (X^-) thus tends to accept hydrogen bond from an uncharged acid molecule (HA), forming a heteroconjugated complex:

$$X^- + HA \rightarrow AHX^- \quad (1)$$

Negatively charged complexes (AHX^-) can be separated by CE [8,10]. In the present work, BGE anions used for the heteroconjugation were chloride, bromide, acetate, perchlorate and bimalonate. Since the alkali metal salts are only sparingly soluble in ACN,

Table 1
Ionisation constants (pK_a) of analytes in water and acetonitrile at 25 °C

Analyte	Abbreviation	pK_a (water) [29]	pK_a (ACN)
Phenol	Ph	9.99	26.6 [31], 26.65 [32], 27.2 [33]
2-Methylphenol	2MePh	10.28	–
3-Methylphenol	3MePh	10.09	–
4-Methylphenol	4MePh	10.26	–
2,3-Dimethylphenol	23MePh	10.50	–
2,4-Dimethylphenol	24MePh	10.58	–
2,5-Dimethylphenol	25MePh	10.41 [30]	–
2,6-Dimethylphenol	26MePh	10.59	–
3,4-Dimethylphenol	34MePh	10.32	–
3,5-Dimethylphenol	35MePh	10.15	27.0 [32]

–, value not reported in the literature.

the BGE anions were added as tetrabutylammonium (TBA) salts.

3.1. Separation based on heteroconjugated anion formation

Separation of phenols based on heteroconjugation with chloride ion is presented in Fig. 1. The BGE was 10 mmol/l TBACl in ACN. Hydrochloric acid, the conjugate acid of chloride, is a much stronger acid (pK_a 8.9 in ACN [2]) than the analyte phenols, which ensures that no proton transfer from phenol to chloride should take place under the present conditions (for a detailed discussion, see Ref. [10]). Accordingly, the separation of phenols took place solely due to the heteroconjugation with chloride ions. As can be seen from Fig. 1, nine of the 10 phenols were separated from each other; only 2,3-dimethylphenol and 2,5-dimethylphenol were co-migrating. The strongest heteroconjugate formation took place with unsubstituted phenol (migrating last) and the weakest with 2,6-dimethylphenol (migrating first). Probably the protected hydroxyl group was responsible for the weak heteroconjugate formation of the latter. Interestingly, the migration order correlates rather well with the acid strength of the phenols in water (Table 1), unsubstituted phenol being the strongest and 2,6-dimethylphenol the weakest. A change in the BGE anion concentration did not improve the separation to any considerable extent.

The migration order of phenols with bromide as the BGE anion was much the same as with the chloride system. Separation at a lower bromide concentration was not as good as with the chloride system, but all the analytes were separated with 30 mmol/l TBABr (Fig. 2), although many of them were not baseline separated. Concentrations higher than 30 mmol/l were not used because Joule heating would have been increased. Although Joule heating could have been avoided by using lower electric field strengths, this would have led to unacceptably long migration times.

Acetate, which has been used for heteroconjugated anion formation by Miller et al. [17], was the next BGE anion investigated. Acetate BGE was an equimolar mixture of acetic acid and TBAAc. Theoretically, the pH of the solution was equal to the pK_a value of acetic acid, which is 22.3 in ACN [33]. The pH of the acetate BGE was therefore much lower than the pK_a values of phenols (pK_a values around 27, see above). This means that the phenols were uncharged, and their separation in acetate BGE was based on heteroconjugate formation with acetate ions. The electropherogram recorded with the acetate BGE is shown in Fig. 3. The separation pattern of the analytes was rather similar to that of 10 mmol/l TBACl (see Fig. 1) except that the resolution of 2,3-dimethylphenol-3,4-dimethylphenol and 2-methylphenol-4-methylphenol was worse. Slight triangulation of the two or three last analyte peaks was observed with the acetate system due to the electromigration dispersion. The repeatability of the EOF was very good, probably due to the buffering ability of the acetate BGE.

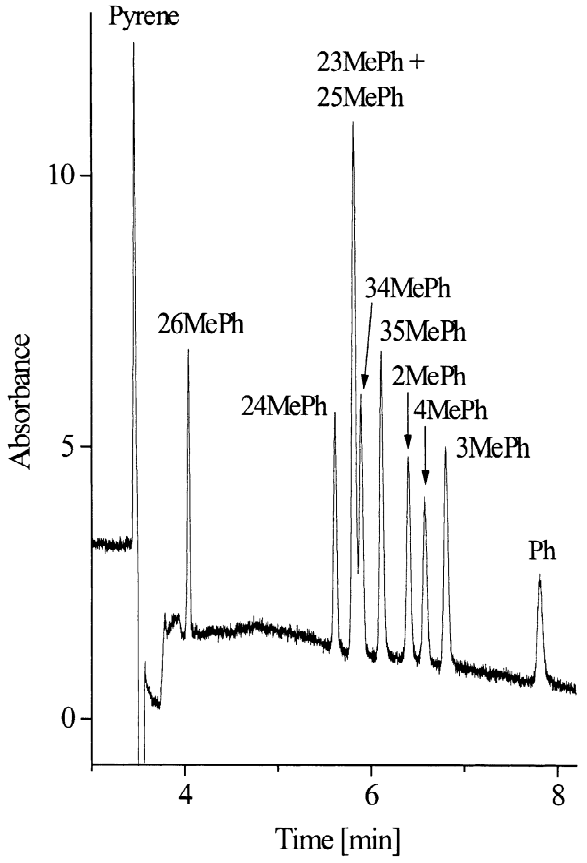

Fig. 1. Electropherogram of phenol and methylphenols in ACN. BGE: 10 mmol/l TBACl in ACN. CE: UV detection 200 nm, voltage +20 kV, current 18.7 μA, injection 50 mbar·s, uncoated capillary 58.5 cm (effective length 50 cm), capillary cassette temperature 25.0 °C. Pyrene was used as a marker for EOF. For identification of acronyms, see Table 1.

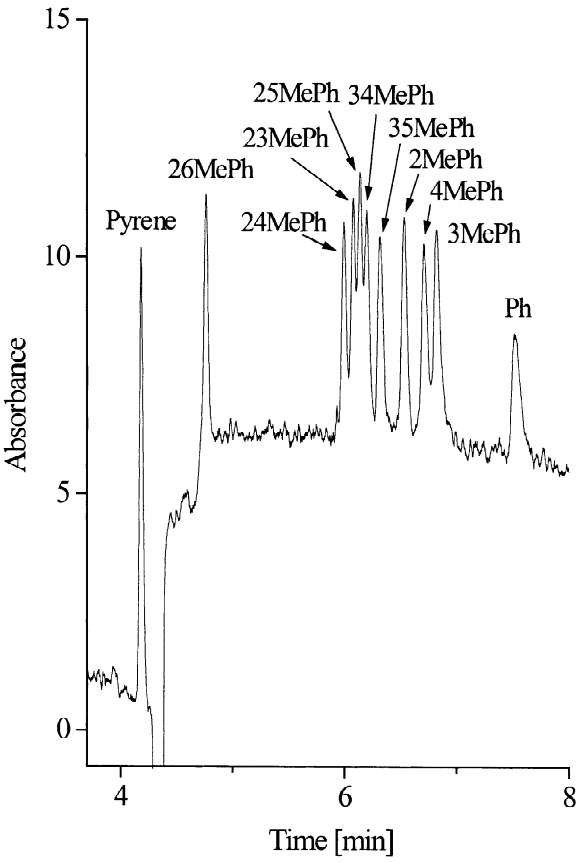

Fig. 2. Separation of phenol and methylphenols in ACN. BGE: 30 mmol/l TBABr in ACN. Voltage +20 kV, current 25.5 μA, estimated temperature at the centre of the capillary 27.0 °C. Other parameters as in Fig. 1. For identification of acronyms, see Table 1.

As reported by Okada [8], heteroconjugate anion formation in ACN also takes place between phenols and perchlorate ion. Although the analytes were clearly separated from the EOF marker peak with the perchlorate BGE, almost all were co-migrating (data not shown). In the hope of achieving better separation we increased the concentration of TBAP to 50 m*M* (using 30 kV), but this caused an unacceptably high current of 66.4 μA. The corresponding estimated temperature at the centre of the capillary was 32.7 °C (see Experimental for details), which was clearly higher than the programmed temperature of the capillary cassette (25.0 °C). Despite the high concentration of TBAP, no noticeable improvement in separation was achieved. An electropherogram similar to that with the perchlorate BGE was obtained with bimalonate BGE (equimolar mixture of malonic acid and TBAHMal; $pK_{a,1}$ of malonic acid: 15.3 [35]). Accordingly, perchlorate and bimalonate BGEs were not used further.

3.2. Effect of traces of water

The presence of water and methanol (and other compounds capable of donating hydrogen bonds) in acetonitrile BGE hinders the heteroconjugated anion formation between uncharged analytes and the BGE anions [10,17]. This is because water and methanol compete with the uncharged analyte as hydrogen

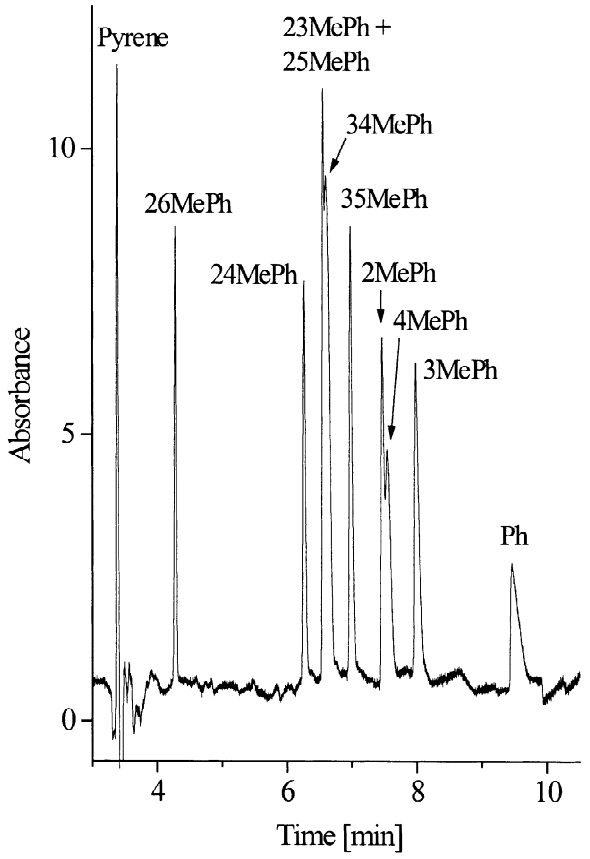

Fig. 3. Electropherogram of the analytes with acetate as BGE anion. BGE: 15 mmol/l acetic acid and 15 mmol/l TBAAc in ACN. Voltage +20 kV, current 12.7 μA. Other parameters as in Fig. 1. For identification of acronyms, see Table 1.

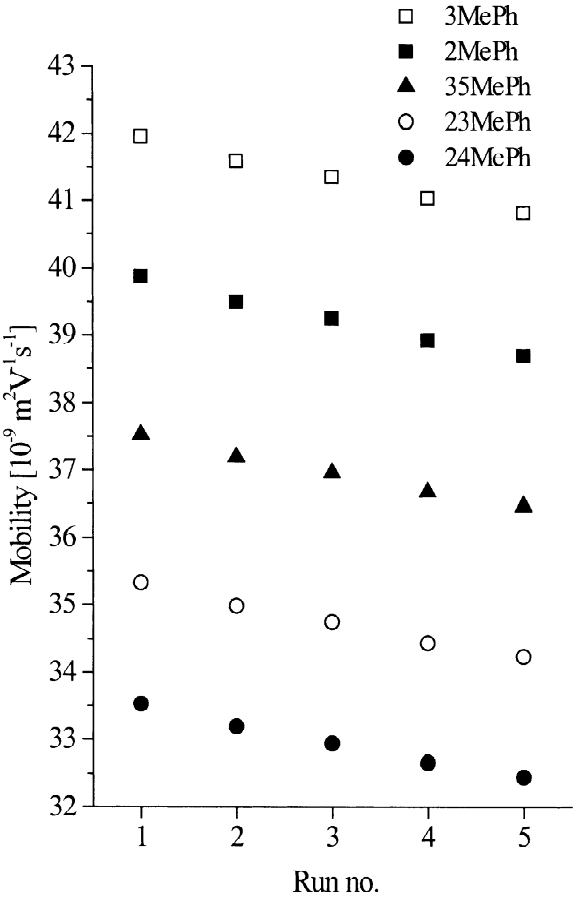

Fig. 4. Electrophoretic mobilities of the analyte–acetate complexes in five consecutive runs without replacement of the BGE between runs. BGE: 15 mmol/l acetic acid and 15 mmol/l TBAAc in ACN. See text for details.

bond donors to the BGE anion. Although running BGE vials were closed with a cap, the water content of the BGE increased slightly during the run. This was confirmed by measuring the water content of the BGE before and after the run (see Experimental). It was of interest to investigate how much the mobility of the analyte–anion complexes was affected by a very small increase in water content of the BGE. Fig. 4 presents the mobilities in five consecutive runs performed with the same BGE. The initial water content of the acetate BGE was 0.016% (w/w) and the water content after five runs was 0.063% for the inlet vial and 0.079% for the outlet vial. As can be seen from Fig. 4, the mobilities decreased from run to run: the mobilities in the final run were 2.7–3.2% lower than the corresponding mobilities in the first run. For 2,6-dimethylphenol the respective decrease was 4.5% (data not shown). Since the mobility of the EOF remained approximately constant within each run, we can conclude that the change in mobilities was due to a decrease in heteroconjugation. The whole series presented in Fig. 4 was repeated with fresh BGE, and the results of the two series were very similar. Accordingly, it is clear that even traces of water have a noticeable effect on the mobilities of heteroconjugated complexes. This was one reason why fresh BGEs were routinely used throughout this work.

3.3. Formation constants of heteroconjugation

In order to approximate the degree of heteroconjugation, we determined the formation constants for phenol–anion complexes assuming 1:1 complexation. The formation constant (K_f) of the heteroconjugated complex described in Eq. (1) is given by

$$K_f = \frac{[AHX^-]}{[X^-][HA]} \quad (2)$$

where the square brackets indicate the concentrations of the species. The molar fraction of the AHX^- complex is

$$\alpha = \frac{[AHX^-]}{[HA] + [X^-]} \quad (3)$$

Substituting $[AHX^-]$ from Eq. (2) into Eq. (3) allows the electrophoretic mobility (μ_{ep}) of the complex to be described as

$$\mu_{ep} = \alpha\mu_{AHX^-} = \frac{K_f[X^-]}{1 + K_f[X^-]} \cdot \mu_{AHX^-} \quad (4)$$

where μ_{AHX^-} is the limiting mobility of the complex. Note that Eq. (4) is valid only when [HA] is much lower than $[X^-]$.

Electrophoretic mobilities of the analyte–anion complexes were measured at anion concentrations ($[X^-]$) of 2.5–30 mmol/l. The anion concentration is 25–300 times greater than the analyte concentration. Thus, Eq. (4) can be used for approximation of the heteroconjugate formation constants. Eq. (4) was fitted to the mobilities measured at different chloride, bromide and acetate concentrations (six data points, except four for bromide). Fitted curves for 2-methylphenol with acetate, bromide and chloride as BGE anions are presented in Fig. 5, and the formation constants and limiting mobilities obtained from the curve fitting for all analytes are given in Table 2. As Table 2 shows, heteroconjugation of the analytes was strongest with acetate and weakest with bromide. In all systems the heteroconjugate formation constants were smallest for 2,6-dimethylphenol and highest for phenol. The mobility curves for 2-methylphenol (Fig. 5) show that, with acetate as anion, the limiting mobility was almost obtained in the concentration range investigated. On the other hand, only a slight curvature was observed for the corresponding plot with bromide. Clearly, the relatively narrow concentration range used for bromide was the reason for the rather large error in the limiting mobilities of bromide complexes (see Table 2). As noted above, the bromide concentration was not increased further because it would have led to long migration times of the complexes. However, even the present data allow the conclusion that complex formation was much weaker with bromide than with acetate and chloride.

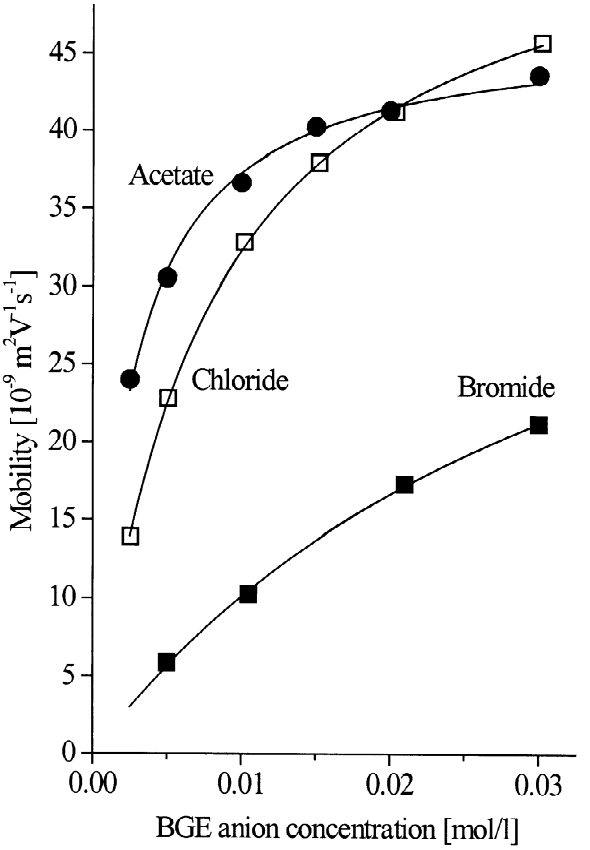

Fig. 5. Mobilities of 2-methylphenol–anion complexes versus BGE anion concentration. BGE anions are acetate, bromide, and chloride. Curve fittings are according to Eq. (4).

As discussed above, even traces of water in the BGE affect the mobilities of the analyte–anion complexes. The maximum water content of the BGE after the CE run was 0.056% for the chloride, 0.045% for the bromide and 0.067% for the acetate

Table 2
Heteroconjugate formation constants (K_f) and limiting mobilities (μ_{AHX^-}) of analyte–anion complexes derived from curve fitting according to Eq. (4)

Analyte	BGE anion					
	Chloride		Bromide		Acetate	
	K_f	μ_{AHX^-}	K_f	μ_{AHX^-}	K_f	μ_{AHX^-}
Ph	168 (2)	62.2 (0.3)	31 (5)	55.3 (5.4)	611 (25)	51.7 (0.4)
2MePh	130 (3)	57.2 (0.5)	27 (3)	47.4 (2.8)	399 (24)	46.7 (0.6)
3MePh	149 (4)	57.7 (0.5)	29 (4)	49.8 (4.5)	492 (23)	47.6 (0.4)
4MePh	139 (1)	57.2 (0.2)	29 (3)	47.7 (2.9)	448 (23)	46.0 (0.5)
23MePh	118 (2)	52.8 (0.4)	29 (2)	39.7 (1.3)	321 (24)	42.9 (0.8)
24MePh	109 (1)	51.7 (0.3)	25 (2)	41.7 (2.3)	287 (23)	41.6 (0.8)
25MePh	116 (1)	52.9 (0.2)	24 (2)	45.0 (2.4)	345 (23)	42.4 (0.7)
26MePh	31 (1)	43.1 (0.7)	6 (3)	45.1 (15.6)	74 (13)	30.2 (2.4)
34MePh	122 (3)	52.9 (0.5)	26 (4)	44.2 (4.1)	348 (28)	42.9 (0.8)
35MePh	128 (3)	54.2 (0.5)	27 (3)	44.8 (3.5)	400 (22)	43.8 (0.5)

Standard deviations are given in parentheses. Formation constants are in l/mol and limiting mobilities are in 10^{-9} m^2 V^{-1} s^{-1}.

system (Table 2). The effect of such small differences in water content of the BGEs on the mobilities is within experimental error (the relative span of mobilities was typically below 1%). Comparison of the results with the literature data shows that the formation constants derived for chloride and phenol/monomethylphenols were higher than those reported by Okada [10]. The formation constant of 151 l/mol for the phenol–chloride heteroconjugate found by Kolthoff and Chantooni [36] is close to the value 168±2 derived in the present work.

3.4. Separation of phenols at high pH

As discussed above, the ionisation constants of phenol and its methyl-substituted derivatives are assumed to be about 27 in ACN. In order to achieve even partial dissociation of such weak acids, the pH of the BGE should be above 25. Buffers prepared from oxalic acid ($pK_{a,2}$ 27.7 [35]) and malonic acid ($pK_{a,2}$ 30.5 [35]) were selected as BGEs for investigation. Theoretically, a solution prepared from an equimolar mixture of mono- and ditetraalkylammonium salts of a diprotic acid should have a pH equal to $pK_{a,2}$. Unfortunately, tetraalkylammonium salts of oxalic acid and malonic acid are not commercially available. Furthermore, the salts, especially ditetraalkylammonium salts, are not easily synthesised in pure form (they also contain relatively large amounts of water and monotetraalkylammonium salt; see, e.g., Ref. [35]). Accordingly, oxalate and malonate BGEs were prepared, as in Miller et al. [17], from the respective acid and tetrabutylammonium hydroxide. A clear limitation of this approach is that TBAOH is available only as a concentrated solution, typically prepared in MeOH or water (there is no ACN solution available). Thus, a second solvent is introduced to the BGE when solutions are prepared with TBAOH.

Electropherograms of the analytes with the oxalate and the malonate BGEs are presented in Figs. 6 and 7, respectively. Both BGEs contained 0.75% (v/v) MeOH resulting from the hydroxide solution. The electropherogram of the analytes with oxalate BGE (Fig. 6) is very similar to the electropherograms obtained with the chloride (Fig. 1) and acetate BGEs (Fig. 3). This suggests that the heteroconjugation mechanism dominated the migration of the analytes rather than dissociation of the analytes. Probably, the 0.75% MeOH present in the BGE reduced the pH from the theoretical value of 27.7 to a considerably lower value. This explanation is supported by the reported decrease in the pH of dicarboxylic acid buffers in ACN by several pK units with the addition of 0.75% MeOH [35,37].

Almost all the analytes were baseline separated with malonate BGE (Fig. 7); only 2,3-dimethylphenol and 3,5-dimethylphenol were partially overlapping. Although the pH of the BGE was probably lower than the theoretical value of 30.5 (see

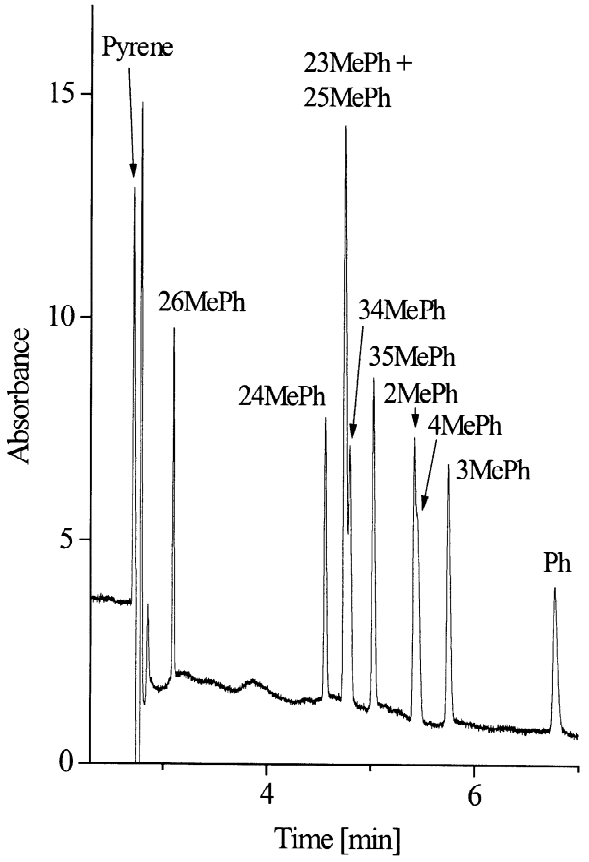

Fig. 6. Electropherogram of the analytes with oxalate BGE (5 mmol/l oxalic acid and 7.5 mmol/l TBAOH in ACN). The BGE also contained 0.75% (v/v) MeOH (see text for details). Voltage +20 kV, current 6.65 μA. Other parameters as in Fig. 1. For identification of acronyms, see Table 1.

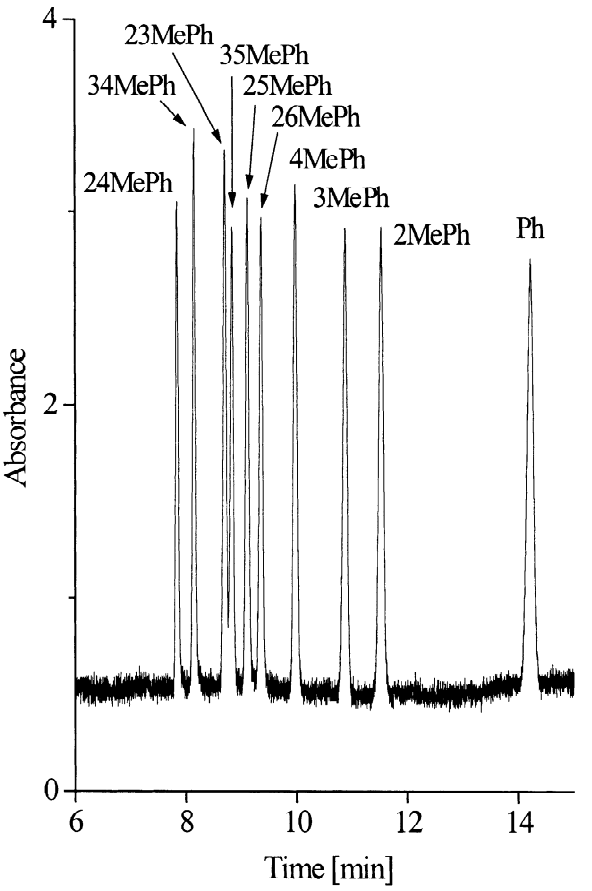

Fig. 7. Separation of the analytes with malonate BGE (5 mmol/l malonic acid and 7.5 mmol/l TBAOH in ACN). The BGE also contained 0.75% (v/v) MeOH (see text for details). Voltage +20 kV, current 6.65 μA. Other parameters as in Fig. 1. For identification of acronyms, see Table 1.

discussion above), it was high enough to cause partial dissociation of some of the phenols. This was evident from the difference in the migration order of the phenols in malonate BGE and in the other BGEs.

Evidently, the 0.75% (v/v) MeOH present in the system not only affected the dissociation of the BGE acid and the analytes, but also reduced heteroconjugate formation of the analytes and BGE anions. This was demonstrated using acetate buffer in ACN (Fig. 3) with 0.75% (v/v) MeOH added. The mobilities measured with this BGE were 26–40% lower than the respective values without MeOH (data not shown). Interestingly, the mobilities with the system containing 0.75% MeOH were highly repeatable even without replacing the BGE after each run.

4. Conclusions

(i) Uncharged phenol, methylphenols and dimethylphenols were separated as heteroconjugated anions in ACN. Heteroconjugated anion formation was most efficient with acetate, bromide and chloride as BGE anions.
(ii) Even traces of water in the BGE affected the heteroconjugation of phenols and BGE anions.
(iii) Heteroconjugate formation constants were

largest with acetate and smallest with bromide as BGE anion.

(iv) Separation of phenols was also successfully carried out in high-pH BGEs where analytes were partly dissociated. All 10 phenols were then separated with malonate BGE.

(v) The presence of a small amount of MeOH, resulting from the TBAOH used in the preparation of high-pH BGEs, affected the separation system noticeably.

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