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Influence of buffer electrolyte pH on the migration behavior of phenolic compounds in co-electroosmotic capillary electrophoresis

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Abstract

The separation behavior of phenolic compounds with various substituents was investigated with co-electroosmotic capillary electrophoresis (migration of the analytes in the same direction as the electroosmotic flow). Co-electroosmotic flow conditions were set up when the electroosmotic flow direction was reversed by adding either a cationic surfactant (cetyltrimethylammonium bromide) or a polycation (1,5-dimethyl-1,5-diazaundecamethylene polymethobromide) to the buffer. An alkaline buffer electrolyte was chosen to ensure complete dissociation of the phenols (pH 10–12). It was shown that a separation of the chosen phenols was possible under co-electroosmotic conditions by optimizing the buffer pH.

1. Introduction

Capillary Electrophoresis (CE) has become a powerful tool for the separation of charged [1,2] and uncharged [3,4] compounds. Traditional CE methods, including micellar supported techniques like micellar electrokinetic (capillary) chromatography (MEKC, MECC) [5–7], make use of the fact, that anionic analytes migrate in the opposite direction of the electroosmotic flow (EOF). These methods may be called *counter*-electroosmotic methods. As a consequence, these techniques often cause rather long migration times of anions. Some early attempts have been made to change the ζ-potential of a silica surface by long-chained alkylammonium salts which, at concentrations slightly below the critical micellar concentration, form dimeric hemimicelles. These are attached with the charged head groups arranged in opposite directions orthogonal to the negatively charged surface [8]. The coating takes place even at moderate pH values due to low pK_A value of the silanol groups of 7.1 [9] which causes a change of the sign of the ζ-potential. This concept was applied to coat a fused-silica capillary with cetyltrimethylammonium bromide (CTAB) to obtain a positively charged inner surface and a reversed EOF [10]. Detailed investigations of the separation behavior of inorganic anions in hemimicellar coated capillaries have been carried out recently [11–13]. Another possibility to dynamically coat the capillary is the usage of coating agents [14] and polycations with more than one positive

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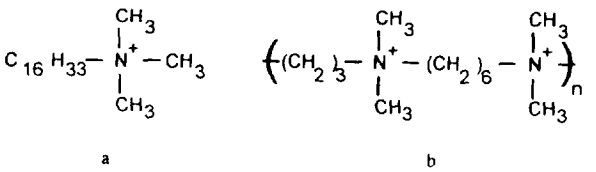

Fig. 1. Chemical structures of CTAB (a) and HDB (b).

functionality per molecule, e.g. 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (hexadimethrin bromide, HDB, Polybrene) [15]. This compound is also known to allow protein analysis by CE as it prevents cationic proteins from sticking to the wall. By using EOF modifiers, an EOF from the cathodic to the anodic side of the capillary is established. Using a power supply with reversed polarity ("negative power supply"), no hardware alterations of the CE system are required. As anionic species migrate into the same direction as the EOF this principle is called *co*-electroosmotic CE [16].

In this paper, CTAB and HDB are used as EOF modifiers. (Fig. 1).

We assumed that the application of co-electroosmotic methods on mixtures of organic acids (e.g. phenols) should result in fast separations of these compounds. In counter-electroosmotic CE run times can be shortened by applying higher voltages and using shorter capillaries. However, this reduction of run time results in a deterioration of resolution according to the Giddings–Jorgenson (see [1]) relation. Moreover, simple optimization of the buffer composition does not eliminate the problem of Joule's heat, as for fast separations with counter-electroosmotic conditions a high electroosmotic flow mobility has to be established, which requires the application of high voltages.

2. Experimental

2.1. Apparatus

A Quanta 4000 CE system connected with a system interface module and a personal computer was used. Data processing was performed with commercial chromatography software (Maxima 820). Uncoated, narrow-bore silica capillaries (AccuSep) with an inner diameter of 50 μm, a total length of 32 cm and an effective separation length of 24.5 cm each were used. All these devices and parts were obtained from Waters Chromatography, Division of Millipore, Milford, MA, USA.

2.2. Reagents

All reagents were of analytical grade. Phenol standard solutions were prepared by dissolving the various phenols (Sigma, Deisenhofen, Germany and Aldrich, Steinheim, Germany) in gradient-grade methanol (Fluka, Buchs, Switzerland). CTAB and HDB (Polybrene) were obtained from Sigma. Depending on the type of EOF modifier 0.7 m*M* CTAB or 0.001% (w/v) HDB were used. Buffer electrolyte mixtures with 15 m*M* of phosphate and 1.25 m*M* of tetraborate each and a pH of 10 to 12 were prepared from borax and disodium hydrogenphosphate (Merck, Darmstadt, Germany) by dissolving in ultrapure water from a Milli-Q system with a conductivity of 18 MΩ (Millipore, Bedford, MA, USA). The pH values were adjusted with 0.5 *M* NaOH. All buffer solutions were vacuum degassed with sonication prior to usage.

2.3. Procedure

Prior to usage the capillary was purged for approximately 15 min with a non-CTAB- or -HDB-containing buffer electrolyte with the same composition as the running buffer (purging buffer I). After this pretreatment the capillary was rinsed with the running buffer containing the EOF modifier (purging buffer II). Between the runs a purging sequence consisting of 1 min buffer I followed by 2 min buffer II was applied. The pre-conditioning of the capillary and the purging sequence between the runs were essential to obtain reproducible results. Injection was performed hydrostatically for 5 s. On-column UV detection was carried out at 254 nm.

3. Results and discussion

Phenols can be separated under neutral or moderately alkaline conditions with counter-electroosmotic methods [17–21]. As phenolic compounds can be considered to be weak acids, they dissociate at high pH values depending on their substituents. With co-electroosmotic flow conditions at high pH values a faster separation of these compounds should be possible.

In this investigation two different EOF modifiers were used (see Introduction). It appeared that alkylated phenols give rise to strong interactions with the aliphatic backbone of the EOF modifier. Likewise electrostatic interactions of the phenolates with the charged headgroups of the EOF modifier can be assumed. This causes peak zones to broaden and theoretical plate numbers to deteriorate. Thus, the separation of phenolic compounds at a high pH value above their pK_A values with CTAB as EOF modifier is limited to phenols with certain substituents.

The compounds for the standard mixture used in this paper were chosen with respect to their functional groups. Three phenolic acids, one phenolic aldehyde and eight alkylphenols were used (Table 1). The magnitude of the electrophoretic mobilities of phenolic compounds mainly depends on their pK_a values. Furthermore, the phenolic acids are doubly dissociated due to their acidic and phenolic functional groups and thus elute first.

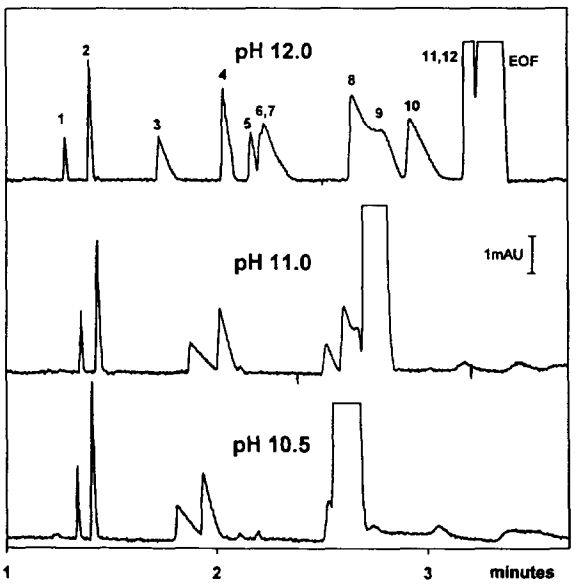

Fig. 2. Capillary electropherograms of a set of 12 phenols with 0.7 m*M* CTAB as EOF modifier at pH 10.5, 11.0 and 12.0. For conditions see Experimental section; for peak identification see Table 1. 10 kV.

Fig. 2 shows capillary electropherograms of a mixture of these 12 phenolic compounds acquired in the pH range of 10.5 to 12.0 with CTAB as EOF modifier. The phenolic acids are

Table 1
Phenolic compounds used in this study and corresponding pK_a values

Class	No.	Compound (IUPAC)	Trivial name	pK_a
Phenolic acids	1	4-Hydroxybenzoic acid		4.61, 9.31
	2	4-Hydroxy-3,5-dimethoxybenzoic acid	Syringic acid	4.20, 9.10
	3	4-Hydroxy-3,5-cinnamic acid	Sinapic acid	Not available
Aldehyde	4	4-Hydroxy-3,5-dimethoxybenzaldehyde	Syringaldehyde	Not available
Alkylphenols	5	3-Methylphenol	*m*-Cresol	10.09
	6	4-Methylphenol	*p*-Cresol	10.27
	7	2-Methylphenol	*o*-Cresol	10.32
	8	3,4-Dimethylphenol	3,4-Xylenol	10.36
	9	2,3-Dimethylphenol	2,3-Xylenol	10.54
	10	2,6-Dimethylphenol	2,6-Xylenol	10.63
	11	2,3,5-Trimethylphenol		10.69
	12	2,4,6-Trimethylphenol		10.99

doubly dissociated at this pH value and migrate in front of the EOF as the species with the highest electrophoretic mobilities. In the case of 4-hydroxy-3,5-cinnamic acid (sinapic acid), hydrophobic interactions of substituents with CTAB become obvious: in contrast to HDB as EOF modifier, which will be discussed later, this particular phenolic acid is clearly retarded compared to the other acids and partly overlaps with 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde). This is due to the aliphatic chain of the 4-hydroxy-3,5-cinnamic acid which strongly interacts with the cetyl core of the surfactant. The mobility of syringaldehyde is intermediate compared to the acids and the alkylphenols and is more or less independent on pH of the buffer electrolyte. Cresols, xylenols and trimethylphenols have the lowest mobility and cannot be separated under these conditions. Below pH 11 the dimethylphenols migrate only slightly in front of the EOF. Despite the fact that the pH value is above the respective pK_a value of the trimethylphenols, these compounds do not possess a net electrophoretic mobility large enough to separate at pH 11. Only at pH values above 12 the mono- and dimethylphenols show considerable electrophoretic mobilities. Nevertheless, no satisfactory separation of the isomers is possible. Moreover, high pH values above 12 are not desirable as they require high ionic strengths.

The observed low electrophoretic mobilities of the phenols, especially of the higher methylated species, are a result of mainly two effects: on the one hand the net electrophoretic velocities and on the other hand the formation of aggregates of free CTAB surfactant molecules and hemimicelles with the phenolic analytes in terms of hydrophobic interactions. This results in altered charge properties of the phenols and does significantly influence the migration behavior of the phenols. The poor peak shapes of the investigated methylphenols, which are observed with CTAB can be attributed to these interactions. In addition, the CTAB system is prone to changes in pH as the concentration of hemimicelles varies thus influencing the magnitude of the EOF.

A significant improvement in performance of the co-electroosmotic separation upon the CTAB system is achieved with HDB as EOF modifier. This type of EOF modifier also reverses the EOF by a dynamic coating. However, this coating is more stable and insensitive to minor changes in buffer pH than the CTAB system. Although some interactions of the analytes with the aliphatic sections of the EOF modifier as well as electrostatic interactions are assumable even with this modifier, the results are much more promising.

The performance of the separation mainly depends on the pH value of the buffer (Fig. 3). At pH 10.5 some peak zones coincide. As in the case of CTAB, the phenolic acids migrate with the highest mobility, followed by the aldehyde and the alkylphenols, which appear as unseparated and broad peaks with low mobilities. This is due to the fact that the pH value of the buffer is in the range of the pK_a values of the alkylphenols (see Table 1). In the HDB system sinapic acid does not interact with the EOF modifier. This implies that with HDB solvophobic interactions do not seem to play an

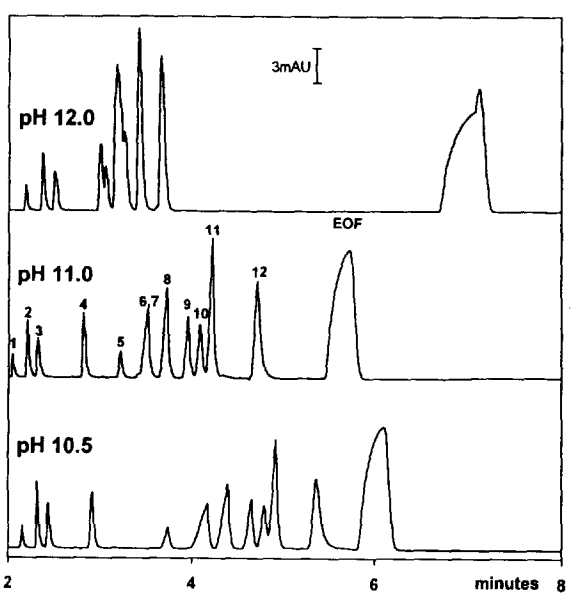

Fig. 3. Capillary electropherograms of a set of 12 phenols with 0.001% (w/v) HDB as EOF modifier at pH 10.5, 11.0 and 12.0. For conditions see Experimental section; for peak identification see Table 1. 10 kV.

important role. This becomes obvious when electropherograms, both acquired at pH 11, but with different EOF modifiers, are compared. Peak zones of the methylated phenols and separation efficiencies are improved dramatically as well as the electrophoretic mobilities of these analytes are also increased. This comparison proves the influence of the hydrophobic interactions in the surfactant based CTAB system on the migration behavior and the separation efficiency of the phenols. On the contrary, a satisfactory separation of the test mixture (except for *p*- and *o*-cresol) is possible at pH 11 and with HDB as EOF modifier. It is to mention that the migration order of the methyl phenols strictly coincides with the pK_a values of the specific isomeric class (mono-, di- and trimethylphenols), though a relation of migration order and pK_a value actually can only be drawn among the compounds of a single isomeric class as the mass-to-charge ratio is altered with additional substituents.

At pH 12 the separation deteriorates again as the electrophoretic mobilities of the methylated phenols become too high and some of these analytes coincide again. The dependence of the electrophoretic mobilities of the investigated phenols on the pH value of the buffer electrolyte take the expected course with both types of EOF modifier. In the CTAB system (Fig. 4) the mobilities of the cresols increase markedly above pH 11, corresponding to the pK_a, whereas the di- and trimethylphenols are retarded almost completely. Above pH 11.5 a measurable electrophoretic mobility occurs. The magnitude of the EOF steadily decreases over the investigated range. Anyway, even with this increased separation window the hydrophobic interactions of analytes and CTAB prevent a reasonable separation.

With HDB (Fig. 5), the effective electrophoretic mobilities of the phenolic acids slightly increase, whereas the mobility of the phenolic aldehyde remains constant until pH 11.5 is reached. Above this value an increased mobility is observed. The mobilities of the methylphenols steadily increase over the entire pH range 10.5–12. With the trimethylphenols this increase is more dominant above pH 11. The EOF remains relatively stable over the pH range 10.5–12.

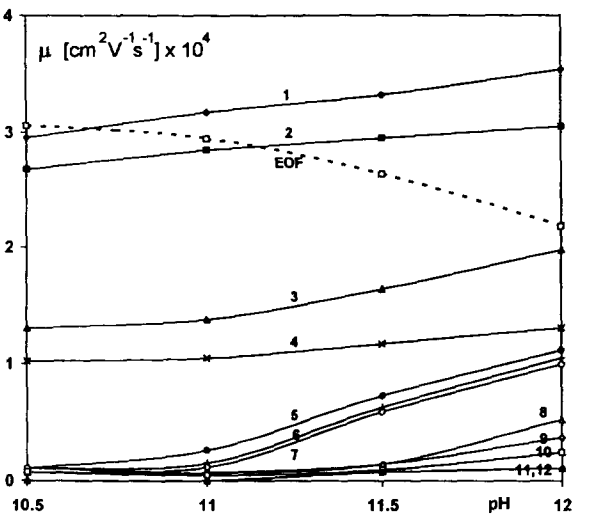

Fig. 4. Dependence of electroosmotic flow and effective electrophoretic mobilities (μ) of phenols on buffer pH. Conditions as in Fig. 2.

The dependence of the theoretical plate numbers of some selected phenols in the HDB system on the pH value is shown in Table 2. It proves that a pH value of approximately 11 is optimal for the co-electroosmotic separation of the selected phenols in terms of time of analysis, resolution and theoretical plate number.

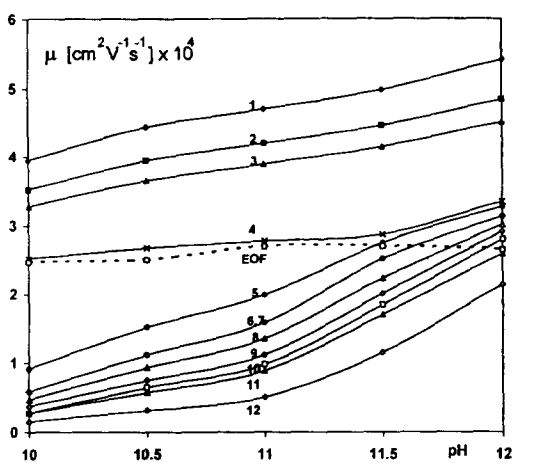

Fig. 5. Dependence of electroosmotic flow and effective electrophoretic mobilities of phenols on buffer pH. Conditions as in Fig. 3.

Table 2
pH dependence of theoretical plate numbers with HDB as EOF modifier

pH	Theoretical plate number				
	Syringic acid	Syringaldehyde	*m*-Cresol	2,3-Xylenol	2,4,6-Trimethylphenol
10.0	127 000	109 200	87 000	133 000	55 600
10.5	166 700	104 600	104 800	136 200	92 500
11.0	113 800	135 100	213 000	208 500	134 800
11.5	111 300	122 800	n.d.	161 200	104 000
12.0	109 900	101 000	104 900	n.d.	90 400

n.d. = Not determined.

4. Conclusions

As a general rule, a satisfactory co-electroosmotic separation of phenolic compounds is possible if the extent of interactions of anionic phenolates with the positively charged capillary wall and the cationic EOF modifiers molecules can be kept within certain limits. In this investigation electrostatic interactions of anionic phenolates with cationic species did not appear to be of a considerable disadvantageous effect. On the other hand, hydrophobic interactions of alkylated phenols with the aliphatic backbone of the EOF modifier had a significant influence on the separation. Especially in the case of CTAB as EOF modifier only selected species do not interact with the modifier. HDB does not have the disadvantageous effects of CTAB in terms of interactions with the analytes. This implies a different mechanism of the surface coating. It is conceivable that the polycation forms coils with the hydrophobic methylene groups directed to the inner side and the charged groups arranged on its surface. This may explain the low tendency to form hydrophobic aggregates of HDB with alkylated phenols. As run times are shorter and theoretical plate numbers are in the range or higher than comparable counter-electroosmotic methods, co-electroosmotic CE procedures should be used as suitable alternatives in special applications. Fig. 6 shows a fast co-electroosmotic separation of six selected phenols. Arising from this it may be stated that co-electroosmotic CE is a suitable method for the analysis of phenols. Although the interactions of some of the analytes with the EOF modifier limits the application to certain phenols, the addition of organic solvents to the buffer electrolyte enables the separation of analyte mixtures which cannot be analyzed with the system described above [22]. This increases the separation window as well as selectivity and performance of the method.

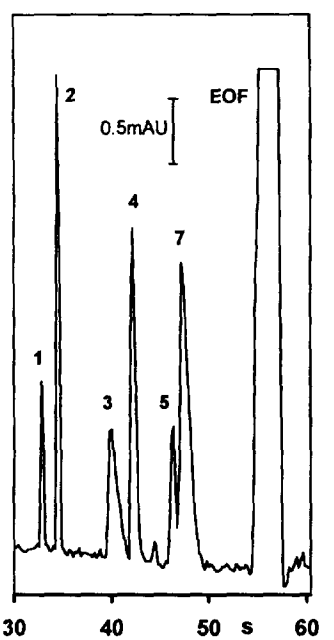

Fig. 6. Fast co-electroosmotic separation of six phenols with 0.7 m*M* CTAB as EOF modifier. Conditions as in Fig. 2. 25 kV, 90 μA.

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