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Investigations on the behaviour of acidic, basic and neutral compounds in capillary electrochromatography on a mixed-mode stationary phase

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Abstract

This work describes the separation of acidic, basic and neutral organic compounds as well as inorganic anions in a single run by capillary electrochromatography employing a stationary phase which exhibits both strong anion-exchange and reversed-phase chromatographic characteristics. The positive surface charge of this stationary phase provided a substantial anodic electroosmotic flow. The analytes were separated by a mixed-mode mechanism which comprised chromatographic interactions (hydrophobic interactions, ion-exchange) as well as electrophoretic migration. The influence of ion-exchange and hydrophobic interactions on the retention/migration of the analytes could be manipulated by varying the concentration of a competing ion and/or the amount of organic modifier present in the background electrolyte. Additionally the effects of pH changes on both the chromatographic interactions as well as the electrophoretic migration of the analytes were investigated.

Keywords: Electrochromatography; Mixed-mode retention; Anion exchange; Stationary phases, CEC

1. Introduction

In addition to well-established capillary electroseparation techniques like capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC) has received increasing attention in recent years and suitable instrumentation has become commercially available. CEC can be seen as a hybrid technique which combines the characteristics of CZE and high-performance liquid chromatography (HPLC). It offers some of the advantages of both methods, such as the high separation efficiency of CZE resulting from the flat flow profile generated by the electroosmotic flow (EOF) and the corresponding reduction of eddy diffusion. From the chromatographic standpoint, CEC offers the availability of a variety of stationary phases, so that the most suitable chromatographic interaction required to solve a specific separation problem can be used. Focusing on the literature published so far, most of the CEC applications describe the separation of neutral analytes on reversed-phase (RP) stationary phase materials. Under these circumstances the analytes have no electrophoretic mobility and the electrophoretic portion of the separation mechanism is restricted to

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the use of the EOF to drive the eluent towards the detection end of the capillary. The separation selectivity is therefore similar to that encountered in RP-HPLC [1–8]. Interest in the behaviour of charged analytes in CEC is growing and a number of publications have reported the determination of such solutes, generally using RP stationary phases [7,9,10]. A mixed-mode retention mechanism results in which both electrophoretic mobility and hydrophobic interactions with the stationary phase contribute to the overall retention of the analytes, which to be suitable for this approach should have some hydrophobic characteristics. Recently Lurie et al. showed the separation of acidic, basic and neutral organic compounds on a C_8 RP stationary phase in a single run using a CEC step gradient [11].

In addition to the RP chromatographic supports mentioned above ion-exchange stationary phases have been introduced into CEC for the analysis of charged organic solutes [12–15] or inorganic ions [16–18]. Recently, so called mixed-mode stationary phases functionalised with alkyl chains (providing hydrophobicity) and cation-exchange [19–24] or anion-exchange sites [25] have also been presented. These chromatographic supports show separation selectivities that are different from those obtained on conventional RP columns. Djordevic et al. achieved a similar result by packing a capillary with a blend of a RP material and bare silica [26]. Mixed-mode stationary phases containing sulfonic acid groups have also been employed as a means to provide a strong and stable EOF even when low-pH electrolytes are used [24,27,28].

In this study the separation of acidic, basic and neutral organic analytes, together with inorganic anions, using a silica-based mixed-mode stationary phase functionalised with C_6-alkyl chains and quarternary ammonium groups is presented. This approach takes advantage of the electrophoretic, hydrophobic and anion-exchange properties of the analytes studied. Due to the positive charge of the anion-exchange sites, this type of packing provides a substantial anodic EOF. The electrophoretic, RP and ion-exchange chromatographic characteristics of the analytes were manipulated using procedures such as varying the pH of the background electrolyte (BGE), changing the concentration of the competing ion in the BGE, or varying the amount of organic solvent present in the BGE. Using these approaches enabled the separation of all test analytes to be achieved in a single run.

2. Experimental

2.1. Instrumentation

Experiments were performed using a HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany), equipped with a diode array detector and connected to a HP 3D-CE Chemstation (Hewlett-Packard) for data processing. A pressure of 10 bar was applied to both ends of the column using helium gas and the column was thermostatted at 25°C during all separations. Samples were injected eletrokinetically at −5 kV for 3 s.

2.2. Materials and reagents

Fused-silica capillaries (75 μm I.D.×360 μm O.D.) obtained from Polymicro Technologies (Phoenix, AZ, USA) were used throughout this work. Water was purified using a Milli-Q water system (Millipore, Bedford, MA, USA). The columns were packed with 3 μm silica-based mixed-mode strong anion-exchange/reversed-phase material (C_6/SAX, 190 m^2 g^{-1}, 8 nm pore size, 2.6% carbon, functionalised with *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride and finally end-capped with C_6 hexyltrimethoxysilane; Xtec Consultants). All chemicals used were of analytical-reagent grade.

BGEs were prepared either from tris(hydroxymethylamino)methane (Tris) or bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane (Bis-Tris) and titrated to the appropriate pH using hydrochloric acid before dilution with acetonitrile. For experiments involving different concentrations of the competing ion the chloride content of the BGEs was adjusted by addition of NaCl. All BGE solutions were filtered through a 0.45-μm membrane filter of Type HA (Millipore) and degassed before use.

2.3. Column preparation

Untreated fused-silica capillaries were packed

using a slurry packing technique similar to that published previously [18]. The packed column (35 cm total length, 25 cm packed bed) was conditioned before use by passing BGE through the column for at least 1 h using a HPLC pump. The column was then mounted in the HP cartridge and conditioned further with BGE using 10 bar inlet pressure and −10 kV overnight. After BGE changes this procedure was repeated.

3. Results and discussion

3.1. Effects of BGE pH

As an initial step, the CEC behaviour of acidic, basic and neutral aromatic substances, as well as two inorganic anions, was investigated using BGEs within a pH range of 6.0 to 8.5 prepared either from 10 m*M* Bis-Tris (pH 6.0 and 7.0) or 10 m*M* Tris (pH 8.0 and 8.5). The set of test substances was selected as follows: two organic acids [benzoic acid (B) and salicylic acid (Sal)], two aromatic bases [pyridine (Py) and aniline (An)], three phenols [phenol (Ph), 4-methoxyphenol (4-Me) and 4-chlorophenol (4-Cl)], two toluenes [toluene (Tol) and 4-nitrotoluene (4-NT)] and two inorganic anions [nitrate (NO_3^-) and iodide (I^-)]. Fig. 1 shows the dependence of the mobilities for the selected analytes on the pH of the carrier electrolyte (10 m*M* Bis-Tris or Tris, respectively with HCl used for pH adjustment and 60% of acetonitrile). Effective mobilities (observed mobilities minus EOF) of the solutes were calculated according to the equation commonly used in CZE and thiourea was employed as an unretained marker. Thiourea was chosen for EOF determination because acetone proved to be unsuitable when lower acetonitrile concentrations were used. Methanol provided very similar results compared with thiourea but was rejected because of the inferior peak shapes obtained. Fig. 1 shows the effective mobilities obtained for the selected analytes as well as the magnitude of the EOF as a function of the pH of the BGE. All data points relate to at least three consecutive measurements and showed excellent reproducibility (standard deviations below $4 \cdot 10^{-4}$ mm^2 V^{-1} s^{-1}). As can be seen, a slight decrease in the anodic EOF was encountered when the pH of the BGE was raised. This may be attributed to the increased number of negatively charged silanol groups at higher pH values, thereby lowering the effective positive charge on the capillary wall. The effective mobilities of the neutral solutes were very small and were almost unaffected by the pH changes, demonstrating that the hydrophobic interactions were independent of the pH of the BGE. In contrast, negatively charged analytes like organic acids and inorganic anions showed distinctly higher negative mobilities when the pH of the BGE was increased. The peaks of B and Sal were shifted from the back of the electrochromatogram to a position in front of the neutral solutes by changing from pH 6.0 to 7.5. Since these analytes were fully deprotonated over the pH range studied, the observed behaviour may be attributed to decreases in the extent of ion-exchange interactions as a result of the presence of increased levels of hydroxide (acting as an anion-exchange competing ion) in the BGE at the higher pH values.

3.2. Effects of changes of the concentration of competing ion in the BGE

To study the influence of the concentration of competing ion in the BGE on the behaviour of the test analytes, a 5 m*M* Bis-Tris, pH 6.5 BGE containing 55% acetonitrile was used. To this BGE (which already contained 2.5 m*M* of chloride originating from the HCl used for pH adjustment) NaCl solution was added. This resulted in overall chloride concentrations between 2.5 m*M* and 22.5 m*M*. Fig. 2 depicts the influence of chloride concentration on the behaviour of the test analytes. As expected, the magnitude of the negative EOF decreased with increasing ionic strength of the BGE, and for the same reason a slight decrease in effective positive mobility was encountered for the toluenes, phenols and the bases when the ionic strength was raised. Ionised species like B and Sal were affected much more strongly by the increase in chloride concentration. The peaks obtained for these analytes moved from the end of the electrochromatogram (no extra chloride added to the BGE) to a position between the inorganic anions and the other analytes (for chloride concentrations above 7.5 m*M*). This can be explained by decreased ion-exchange interactions between the positively charged functional groups of the

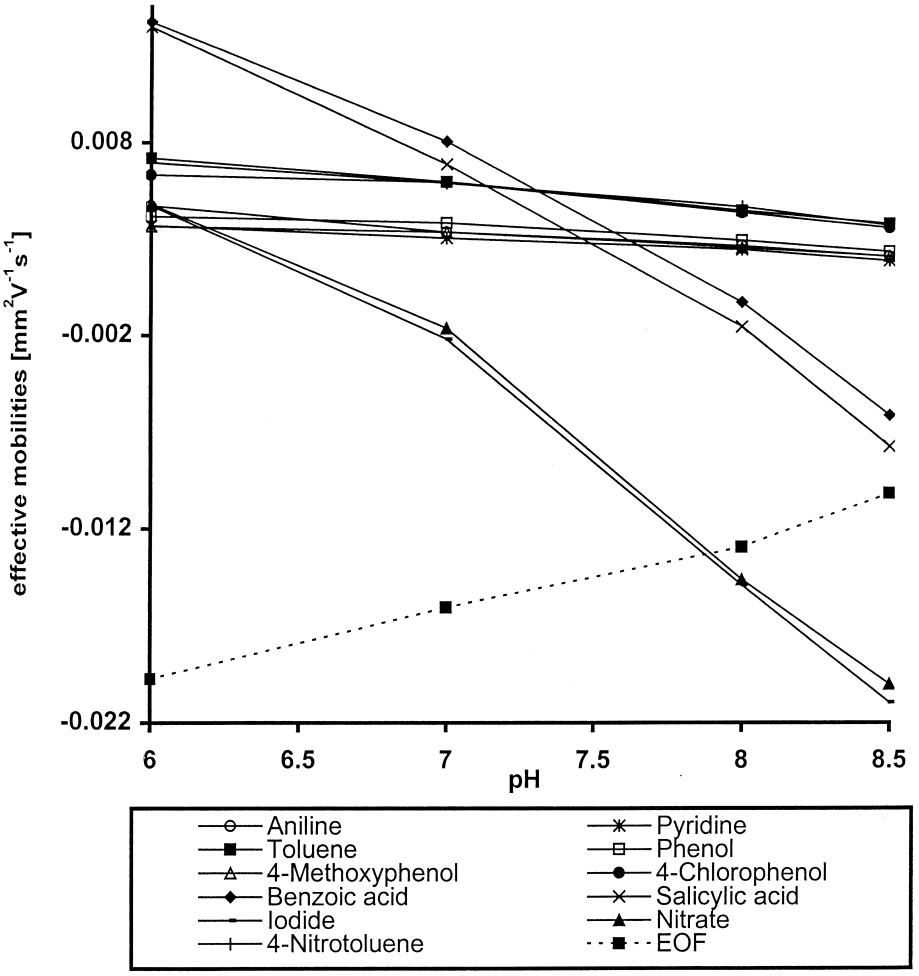

Fig. 1. Dependence of the effective mobilities of the test analytes on the pH of the BGE. Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with C_6/SAX. Mobile phase: 10 m*M* Bis-Tris (pH 6.0 and 7.0) and 10 m*M* Tris (pH 8.0 and 8.5)–60% acetonitrile, pH adjusted with HCl. Voltage: −20 kV.

stationary phase and these negatively charged analytes, resulting from the increased concentration of the competing ion (chloride) in the BGE. The same effect was obtained for nitrate and iodide using BGEs with chloride concentrations between 2.5 m*M* and 12.5 m*M*. At higher competing ion concentrations the decrease of mobility due to increased ionic strength of the BGE overruled this effect and longer retention times were observed for these analytes [29].

3.3. Effect of the concentration of acetonitrile in the BGE

The amount of acetonitrile added to a 10 m*M* Bis-Tris, pH 6.5 BGE was varied over the range 40–80% in order to manipulate the extent of hydrophobic interactions occurring between the analytes and the stationary phase. As can be seen from Fig. 3 the magnitude of the EOF and the effective mobility of iodide were virtually constant with increased

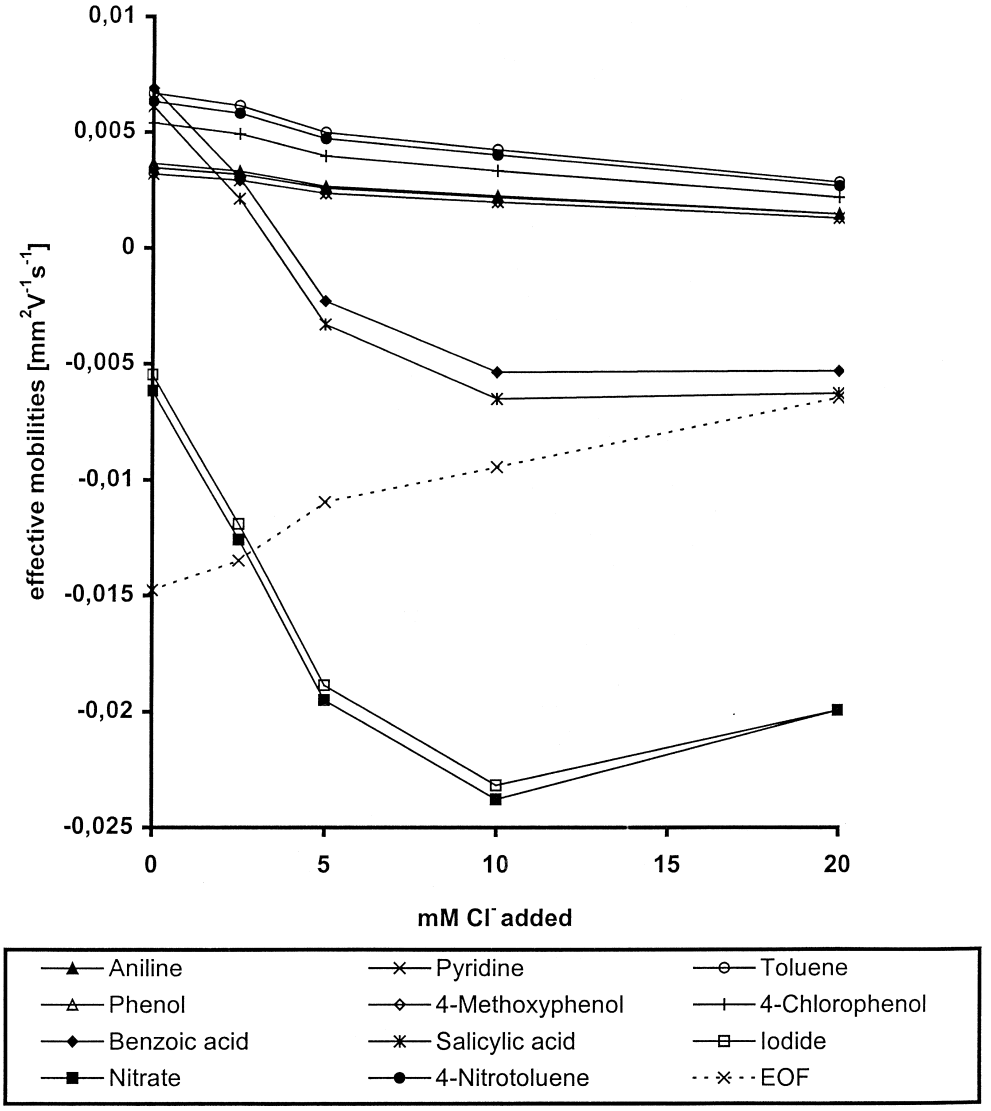

Fig. 2. Dependence of the effective mobilities of the test analytes on the concentration of the competing ion (chloride) in the BGE. Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with C_6/SAX. Mobile phase: 5 m*M* Bis-Tris (pH 6.5 with HCl)–60% acetonitrile; chloride concentration adjusted by addition of NaCl solution. Voltage: −20 kV.

acetonitrile concentrations. On the other hand the effective negative mobility of nitrate was reduced substantially and a reversed elution order between nitrate and iodide was obtained at acetonitrile concentrations above 55%. This behaviour is similar to that found in ion chromatography when eluents containing organic solvents such as acetonitrile are employed and can be attributed to changes in the solvation of these analytes [30,31]. For the organic acids it can be seen that B (pK_a 4.19) showed a steady decrease in effective negative mobility due to a reduction in its degree of ionisation caused by the

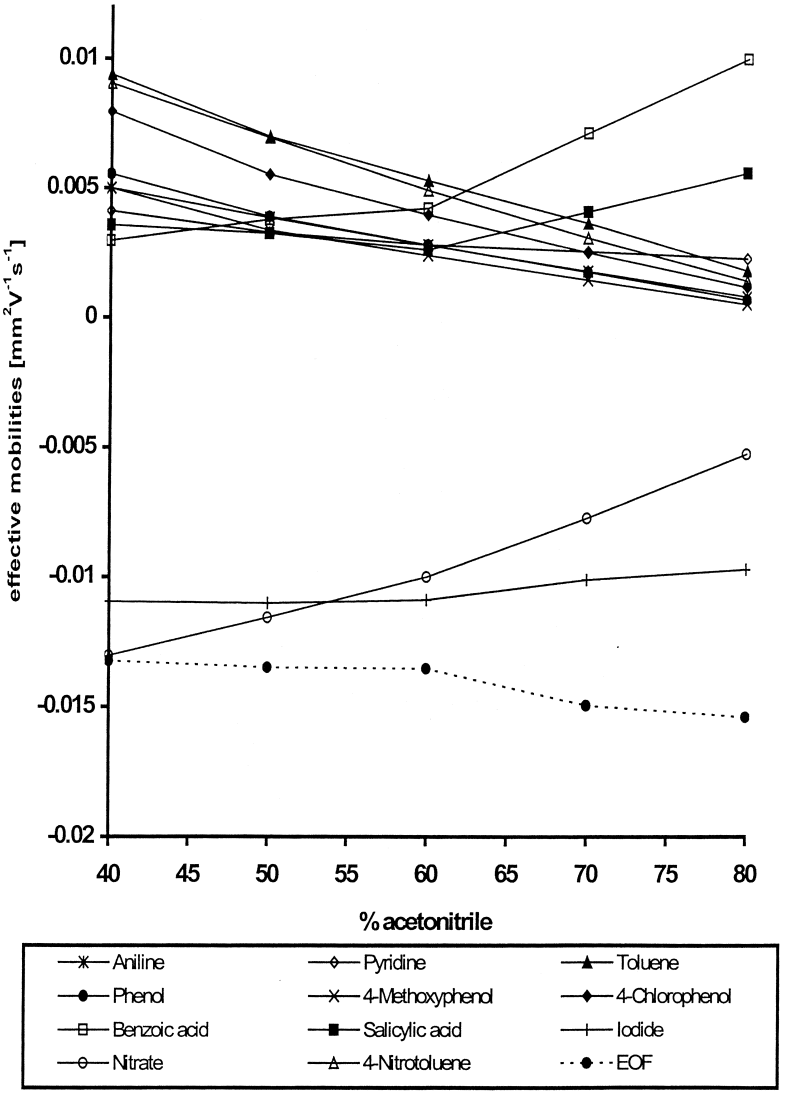

Fig. 3. Dependence of effective mobilities of the test analytes on the concentration of acetonitrile added to the BGE. Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with C_6/SAX. Mobile phase: 10 m*M* Bis-Tris (pH 6.5 with HCl)–40–80% acetonitrile. Voltage: −20 kV.

increased amount of organic solvent present in the BGE. In the case of the more acidic Sal (pK_a 2.97) this behaviour occurred only at acetonitrile levels above 60%. Furthermore, the peaks for these two analytes in the electrochromatogram moved significantly when acetonitrile levels were changed. Finally, the presence of acetonitrile caused a reduction in the hydrophobic interactions, so that uncharged analytes or those carrying a small charge showed decreased effective positive mobilities (and hence reduced retention times), as might be expected from results obtained on RP stationary phases.

3.4. Separation of the test mixture

A separation of the test mixture of inorganic

anions, organic acids, organic bases, and neutral compounds using the C_6/SAX mixed-mode stationary phase in a single run of less than 22 min duration is shown in Fig. 4. The BGE contained 2.5 m*M* of NaCl [added to the 5 m*M* Bis-Tris (pH 6.5) and 45% acetonitrile] in order to shift the peaks for the two organic acids (B and Sal) to shorter retention times and to improve their resolution from the basic and neutral analytes. Symmetrical peaks were obtained even for the two bases without any addition of an amine modifier to the BGE, as is commonly used to avoid peak tailing [11]. This fact may be explained by the screening effect of the positively charged functional groups attached to the stationary phase, which effectively reduce the level of interaction between the basic solutes and the silica base material [25]. Some peak tailing was observed for B and Sal which can be attributed to the fact that their elution was governed by several different retention mechanisms, namely electrophoresis, ion-exchange and hydrophobic interactions with the alkyl chains on the stationary phase. Peak efficiencies obtained for most of the analytes were high (e.g., 197 000 plates m^{-1} for Phe) but were substantially less for strongly interacting substances (e.g., 7500 plates m^{-1} for Sal).

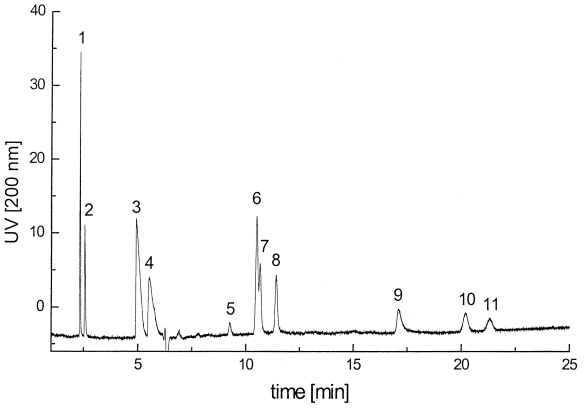

Fig. 4. Separation of the test mixture of inorganic anions, organic acids, organic bases and neutral compounds. Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with C_6/SAX. Mobile phase: 5 m*M* Bis-Tris (pH 6.5 with HCl)–45% acetonitrile; 2.5 m*M* chloride added. Voltage: −20 kV. Peaks: 1=NO_3^-; 2=I^-; 3=B; 4=Sal; 5=Py; 6=An; 7=4-Me; 8=Phe; 9=4-Cl; 10=4-NT; 11=Tol.

4. Conclusions

This work has demonstrated the feasibility of using a C_6/SAX stationary phase for the simultaneous separation of inorganic anions, organic acids, organic bases and neutral analytes in a single CEC run. Several mechanisms including electrophoretic migration, ion-exchange and hydrophobic interactions are involved in the separation of the test solutes. Separation selectivities could be manipulated by changing the electrolyte composition in order to influence the contribution of each of the above retention mechanisms to the separation. This was achieved by changing the pH, the concentration of the competing ion, and the amount of acetonitrile present in the electrolyte.

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