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Journal of Chromatography A, 884 (2000) 277–285

JOURNAL OF CHROMATOGRAPHY A

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Behavior of basic compounds in ion-exchange capillary electrochromatography with low-pH carrier electrolytes

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Abstract

This work describes the separation of basic aromatic compounds by capillary electrochromatography employing acidic carrier electrolytes and bare silica as well as strong cation-exchange stationary phases. A mixed-mode separation mechanism was involved, comprising chromatographic interactions (adsorption, ion-exchange) as well as electrophoretic migration. The influence of ion-exchange on the retention/migration of the solutes could be manipulated according to procedures commonly employed in ion chromatography. These include variations of the eluting strength and/or the concentration of the competing ion present in the background electrolyte. Using this approach, separation times could be shortened and changes in selectivity could be achieved for a number of analytes.

Keywords: Electrochromatography; Background electrolyte composition; Mixed-mode chromatography; Aniline; Toluidines; Pyridine; Benzylamine; Aminopyridines

1. Introduction

Over recent years capillary electrochromatography (CEC) has received increasing attention as a method which complements well-established capillary electroseparation techniques such as capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MECC). The interest in this method has been intensified since suitable instrumentation for CEC has become available commercially. CEC offers a number of benefits over high-performance liquid chromatography (HPLC) or CZE. First, the plug-like profile of the electroosmotic flow (EOF) and even more important the reduction of the eddy diffusion helps to avoid the degree of band broadening of the analyte zones which is encountered in HPLC. Second, the independence of the flow velocity of the capillary diameter, the column length and also the particle size of the packing, allows the use of microbore capillaries and microparticulate chromatographic supports without the limitation of increased back pressure as occurs when liquid chromatographic techniques are employed. Finally, CEC offers additional separation selectivity by taking advantage of both the electrophoretic and the chromatographic properties of the solute. The literature published so far on CEC has concentrated mainly on the separation of neutral analytes using capillaries packed with C_{18} modified silica particles. In this case the electrophoretic portion of the separation mechanism is restricted to the use of the EOF instead of a mechanical pump to drive the mobile phase and the analytes towards the detection end of the capillary [1–8].

Recently, there have been several reports of the

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)01314-X

CEC separation of charged analytes by employing polar stationary phases such as ion exchangers [9–14] or bare silica [15,16]. In this case the analytes are separated according to their chromatographic properties (i.e. their interactions with the stationary phase) and also their electrophoretic properties (i.e. migration in the electric field). Wei et al. described CEC separations of basic compounds on bare silica using carrier electrolytes covering a pH range of 7.5–10.5 [15]. The mechanism involved was found to be multifunctional, including ion-exchange, reversed-phase and normal-phase interactions as well as electrophoretic migration of the analytes. Nevertheless, there have been no reports of systematic investigations dealing with the possibility of manipulating the extent of the ion-exchange contribution to the mixed electrophoretic/chromatographic mechanism involved in the CEC separation of basic aromatic compounds employing cation-exchange stationary phases.

The purpose of the present study was to investigate the behavior of these analytes on bare silica as well as on strong cation exchangers employing acidic carrier electrolytes. Under these conditions the analytes can be regarded as fully protonated and, therefore, strong interaction with the ionic sites of the exchanger should occur. These interactions can be manipulated using procedures commonly employed in ion chromatography (IC) [17], namely by changes in the composition of the background electrolyte (BGE), such as variation of the eluting strength or the concentration of the competing ion.

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 12 bar was applied to both ends of the column using helium gas and the column was thermostatted at 20°C during all separations. Samples were injected electrokinetically at −5 kV for 5 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 (Millipore, Bedford, MA, USA) system. The columns were packed with 3 μm silica (190 m^2/g; XTec Consultants, Clwyd, UK) or 3 μm silica-based strong cation-exchange material (SCX, capacity≈ 120 μequiv./g, 1% carbon; XTec Consultants) manufactured by reaction of triethoxysilylpropanesulfonic acid with the base silica described above. All chemicals used were of analytical reagent grade.

Phosphate buffers were prepared either from disodium hydrogenphosphate and titrated to the appropriate pH using phosphoric acid or from phosphoric acid and using lithium, sodium or potassium hydroxide for pH adjustment. All buffer solutions were filtered through a 0.45 μm membrane filter of Type HA (Millipore) and degassed before use. Acetone was used for the determination of the EOF.

2.3. Column preparation

Untreated fused-silica capillaries were packed using a slurry packing technique similar to that described by Hilder et al. [14]. The packed column (35 cm total length, 25 cm packed bed) was then mounted in the HP cartridge and conditioned with mobile phase before use. For CZE experiments capillaries with a total length of 35 cm (26.5 cm to the detection window) were used.

2.4. Procedures

Retention factor (k) values were calculated using the equation commonly used in liquid chromatography [18], using acetone as t_0 marker. The number of theoretical plates was determined by the peak width at half height method.

3. Results and discussion

3.1. Bare silica stationary phase

As an initial step, the CEC behavior of basic

aromatic substances using a column packed with bare silica was investigated. A set of nine basic aromatic test substances containing at least one amino group was selected as follows: Benzylamine (B), pyridine (Py), three isomeric aminopyridines [2-aminopyridine (2AP), 3-aminopyridine (3AP) and 4-aminopyridine (4AP)], aniline (An), and three isomeric toluidines [*o*-toluidine (*o*-Tol), *m*-toluidine (*m*-Tol) and *p*-toluidine (*p*-Tol)]. Considering that a bare silica stationary phase was used and the fact that the analytes existed predominantly as cations under the conditions used (pH 3–4.5), the retention behavior can be expected to be determined by both the electrophoretic mobility of the analytes and their interaction with the chromatographic stationary phase. In view of the low pH of the BGE electrolyte, the latter consists mainly of adsorption and to some extent also ion-exchange occurring at the deprotonated silanol groups [17,19]. Fig. 1 shows the dependence of the *k* values for the test analytes on the pH of a 5 m*M* NaH_2PO_4 running buffer containing 70% acetonitrile. The EOF, determined by the use of acetone as a neutral marker, was found to be in the range of 0.009 $mm^2\ V^{-1}\ s^{-1}$ (pH 3.0) and 0.012 $mm^2\ V^{-1}\ s^{-1}$ (pH 4.5) and standard deviations encountered for the *k* values were found to be in the range of 1–6%. It can be seen that *k* values increased when the pH of the BGE was raised and the fact that negative *k* values were obtained for all analytes expresses the important influence of the electrophoretic portion of the separation mechanism involved. In the case of the less basic analytes, changes in their charge/ionic radius ratios and, hence, their electrophoretic mobilities will occur additionally to increased ion-exchange interaction between the positively charged analytes and the increasing number of deprotonated silanol groups of the stationary phase when raising the pH of the carrier electrolyte. For the more basic solutes ($pK_a > 6$) e.g. aminopyridines no significant change in protonation will occur within the selected pH range. Therefore changes in retention obtained for these substances may be attributed mainly to the chromatographic portion of the mixed mode separation mechanism. Comparison of the CEC separation of the test analytes using a highly acidic carrier electrolyte (pH 3.0) with CZE experiments performed under the same conditions revealed similar migration/retention for most of the analytes orders except for the pairs *p*-Tol/Pyr and B/2AP as can be seen from Table 1. Focusing on these data it can be seen that employing these acidic conditions and a bare silica stationary phase the influence of chromatographic interactions is not strong enough to cause selectivity changes for most of the analytes.

3.2. Cation-exchange stationary phase

In contrast to the bare silica chromatographic support described above a strong cation-exchanger will provide ion-exchange interactions even when low-pH BGEs are employed. This can be seen clearly from Table 1, which compares the *k* values obtained for the analytes on bare silica and cation-exchange stationary phases, using a 5 m*M* NaH_2PO_4 BGE (pH 3.0) containing 70% acetonitrile. The EOF (0.014 $mm^2\ V^{-1}\ s^{-1}$) obtained was significantly higher than the one on the bare silica employing identical conditions. In this case the additional interactions between the positively charged solutes and the sulfonate groups of the ion-exchange material lead to higher *k* values, and a completely different retention order is achieved when the ion exchanger is used. Assuming that a mixed mode separation mechanism is involved, this demonstrates the presence of increased chromatographic interactions, in particular ion-exchange, leading to a reduced influence of electrophoretic migration on the separation.

3.3. Variation of the cation-exchange contribution

As already known from IC, ion-exchange interactions between the solute and the stationary phase can be influenced by variation of the eluent (or in the case of CEC the carrier electrolyte) composition. Different strategies include pH variations (influencing the charge of the solutes and in some cases if a weak ion-exchanger is used also the exchange capacity of the stationary phase) and variations of the eluting strength and/or the concentration of the competing ion. A series of experiments has been performed to study the retention or migration behavior respectively of the selected analytes using the 120 μeq/g strong cation-exchanger described above. Carrier electrolytes with a constant pH (3.0) but different eluting strength were used to manipulate the amount of ion-exchange interactions present in the mixed separation mechanism. This was achieved by

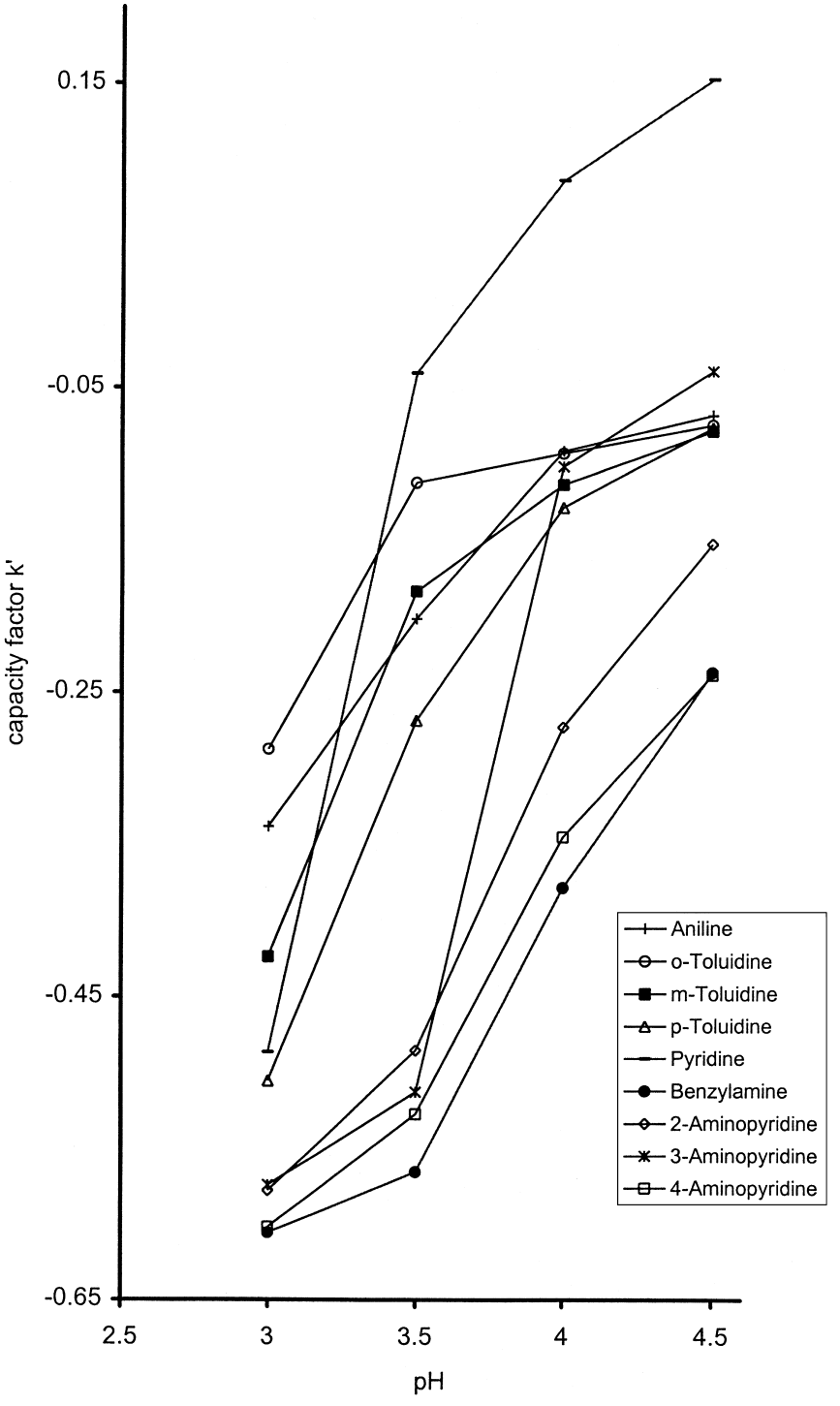

Fig. 1. Dependence of the *k* values obtained for basic aromatic compounds on the background electrolyte pH. Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with 3 μm silica. Mobile phase: 5 m*M* NaH_2PO_4–acetonitrile (30:70), pH adjusted with H_3PO_4. Voltage: +15 kV.

Table 1
Comparison of k values obtained on different stationary phases

Analyte	Retention factors		
	CZE	Silica	SCX
Aniline	−0.067	−0.34	2.06
o-Toluidine	−0.056	−0.29	1.99
m-Toluidine	−0.090	−0.42	2.16
p-Toluidine	−0.18	−0.50	2.33
Pyridine	−0.29	−0.49	3.27
Benzylamine	−0.57	−0.61	2.39
2-Aminopyridine	−0.61	−0.60	3.24
3-Aminopyridine	−0.56	−0.57	2.82
4-Aminopyridine	−0.57	−0.58	2.60

two different approaches. First phosphate buffers including competing ions with different eluting strength (namely Li^+, Na^+ and K^+) at a concentration of 5 m*M* have been prepared. In this way the chromatographic portion of the separation mechanism was varied whereas electrophoretic migration was kept constant and the EOF within a very narrow range (0.015–0.016 $mm^2\ V^{-1}\ s^{-1}$). Fig. 2 depicts the influence of the competing ion on the k values obtained for the investigated basic substances. Standard deviations obtained for the k values were typically in the range of 1–8%. As can be seen from this plot decreasing k values were obtained with increasing elution strength of the competing ion. Additionally selectivity changes were encountered An/*o*-Tol and 3AP/2AP/Py by changing the competing ion and with it the amount of ion-exchange interaction involved in the separation mechanism.

Another possibility to achieve variations in the ratio ion-exchange/electrophoretic migration present in the separation mechanism is to choose a competing ion and vary its concentration. Fig. 3 shows the retention factors obtained for the investigated analytes using pH 3.0 NaH_2PO_4 carrier electrolytes containing 70% of acetonitrile with Na^+ concentrations ranging from 5 m*M* to 25 m*M*. Reproducibility of the k values was found to be quite good with standard deviations between 1 and 3%. As can be deduced from this plot increased concentrations of the competing ion reduce the amount of ion-exchange interactions and with it the retention. Increased ionic strength also led to a decrease in EOF from 0.014 $mm^2\ V^{-1}\ s^{-1}$ (5 m*M* Na^+) to 0.012 $mm^2\ V^{-1}\ s^{-1}$ (25 m*M* Na^+) as might be expected.

Two chromatograms obtained for the basic aromatic compounds investigated in this study using a 10 m*M* (a) and a 20 m*M* (b) NaH_2PO_4 carrier electrolyte (pH 3.0) including 70% acetonitrile are depicted in Fig. 4. The 10 m*M* running electrolyte allowed the separation of all solutes of interest in approximately 30 min. Shorter analysis times could be achieved with the 20 m*M* electrolyte but in this case the pair *o*-Tol/An coeluted. Additionally resolution for the pair py/2AP could be enhanced using the 20 m*M* buffer leading to decreased chromatographic interactions and therefore increased influence of the electrophoretic portion of the separation mechanism. The number of theoretical plates per meter calculated for the chromatogram shown in Fig. 4(a) was found to be in the range of 95 000 (*o*-Tol) and 500 000 (*p*-Tol) for most of the peaks. Despite the tailing encountered for the more retained peaks due to increased chromatographic interactions more than 650 000 plates per meter were obtained for 4AP using the peak width at half height method. The less resolved pair Py/2AP showed lower efficiencies but this is mainly due to their very strong interaction with the stationary phase leading to increased peak tailing.

4. Conclusions

This work has demonstrated that various mechanisms including electrophoretic migration, adsorption and ion-exchange are involved in the separation of basic aromatic compounds by CEC on silica as well as on strong cation-exchange stationary phases. Whereas the silica packing provided similar retention orders as encountered in CZE experiments a completely different retention pattern was obtained with the SCX stationary phase. The ion-exchange interactions mainly occurring between the latter chromatographic support and the analytes could be manipulated by changing the composition of the carrier electrolyte, in particular type (Li^+, Na^+ and K^+) as well as the concentration (5–25 m*M* Na^+) of the competing ion. By this separation times could be influenced and also selectivity changes were achieved for most of the test analytes.

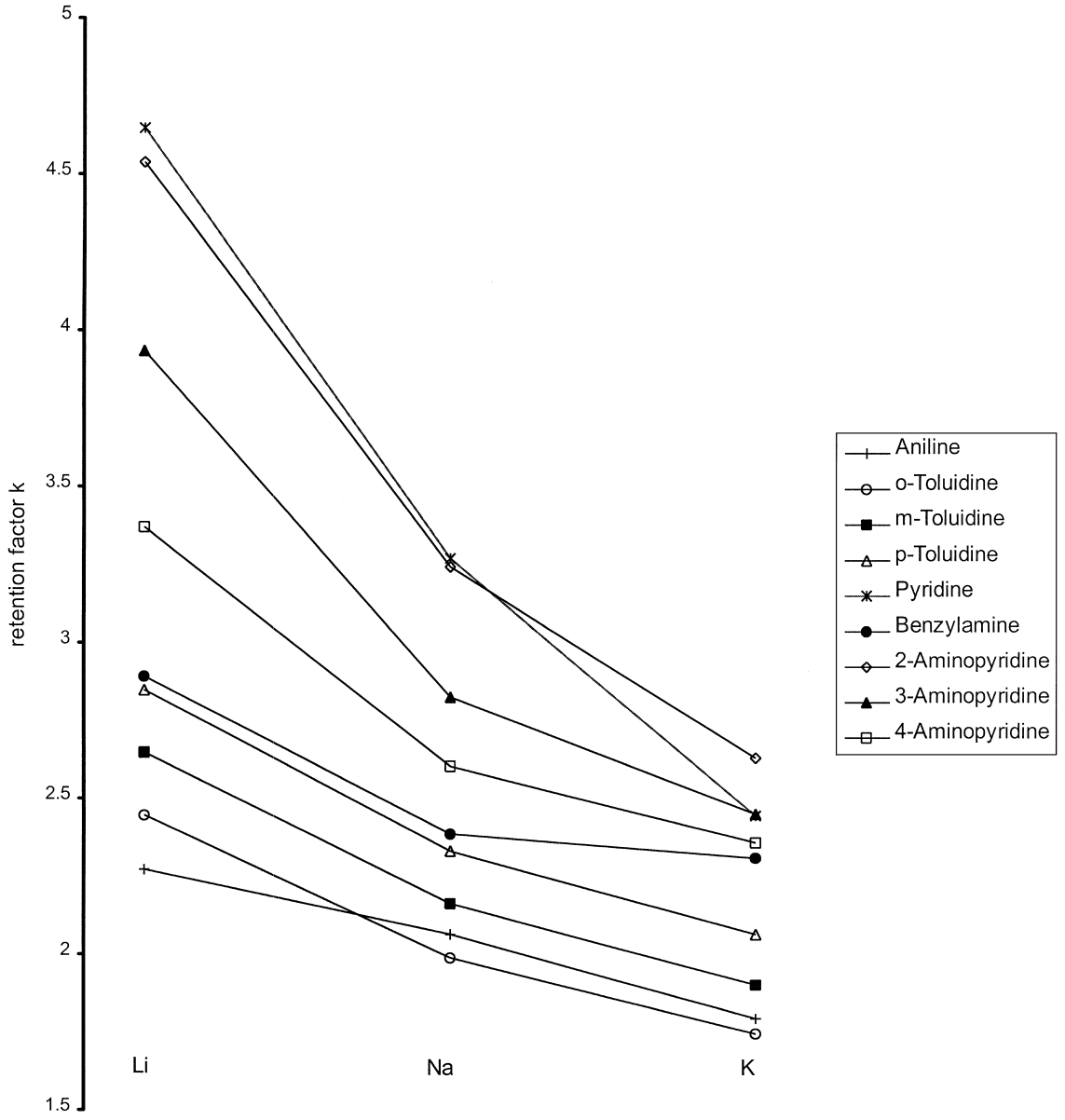

Fig. 2. Dependence of the *k* values obtained for basic aromatic compounds on the nature of the competing ion. Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with 3 μm SCX (capacity≈120 μequiv./g). Mobile phase: 5 m*M* LiOH/NaOH/KOH–acetonitrile (30:70) pH adjusted to 3.0 with H_3PO_4. Voltage: +15 kV.

Acknowledgements

This work was supported by the Austrian Science Fund (FWF) project number J 1622 CHE. Further the authors would like to thank Professor Peter Myers (Xtec Consultants Ltd.) for providing the

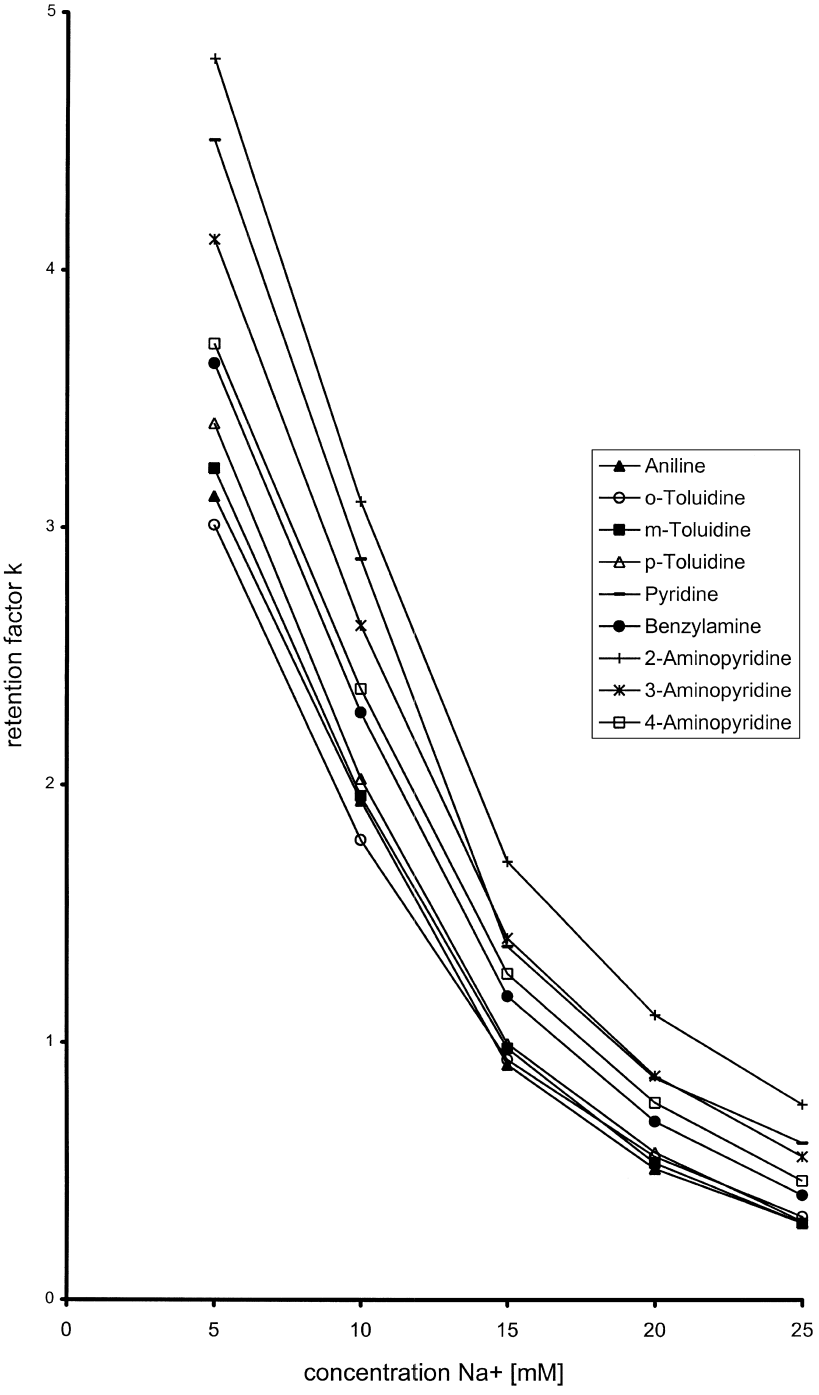

Fig. 3. Dependence of the *k* values obtained for basic aromatic compounds on the concentration of the competing ion. Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with 3 μm SCX (capacity≈120 μequiv./g). Mobile phase: 5 to 25 m*M* NaH_2PO_4–acetonitrile (30:70) pH adjusted to 3.0 with H_3PO_4. Voltage: +15 kV.

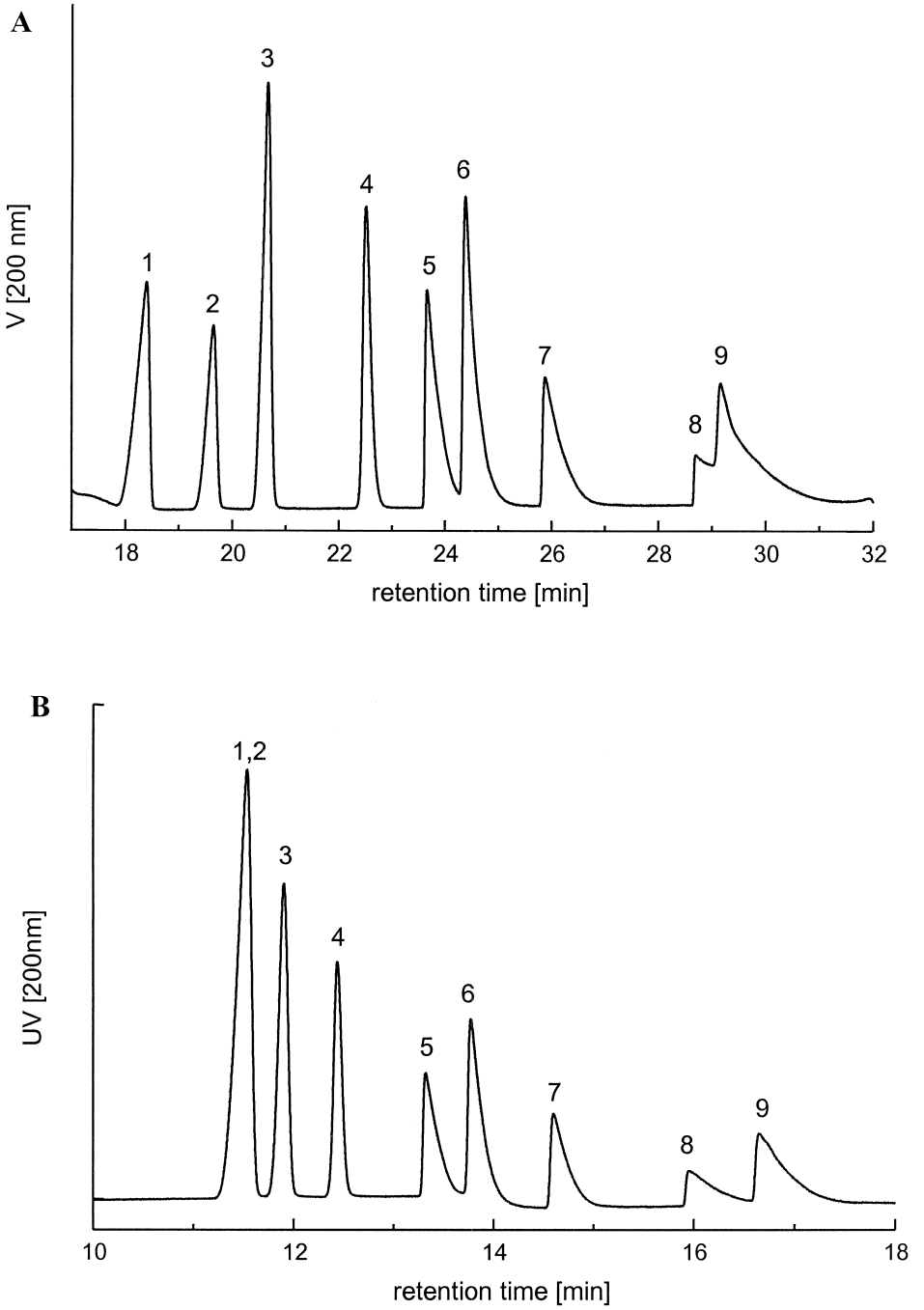

Fig. 4. Separation of a standard mixture Capillary: 35 cm (26.5 cm packed bed)×75 μm I.D., packed with 3 μm SCX (capacity≈120 μequiv./g). Mobile phase: (a) 10 m*M* NaH_2PO_4–acetonitrile (30:70), (b) 20 m*M* NaH_2PO_4–acetonitrile (30:70) pH adjusted to 3.0 with H_3PO_4. Voltage: +15 kV. Peaks: (1) *o*-Tol; (2) An; (3) *m*-Tol; (4) *p*-Tol; (5) B; (6) 4AP; (7) 3AP; (8) Py; (9) 2AP.

packing materials used in this study and Ms. Emily F. Hilder for help regarding the packing of the columns.

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