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Journal of Chromatography A, 916 (2001) 51–59

JOURNAL OF CHROMATOGRAPHY A

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Ionene-coated sulfonated silica as a packing material in the packed-capillary mode of electrochromatography

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Abstract

An aliphatic ionene (2–10-ionene) has been selected as a modifier to prepare a novel polymer-coated packing material for capillary electrochromatography. The packing material was produced by dynamical modification of a commercially available sulfonated silica Exsil-100 SCX. Strong ion-exchange interactions in the capillary packed with ionene-modified sulfonated silica have been demonstrated by the example of the retention of *p*-aminobenzoic acid. The total calculated anion-exchange capacity of the sorbent in the capillary was about $4 \cdot 10^{-9}$ mol. A fast separation (about 15 min) of several aromatic acids was achieved with the packing material. The highest number of theoretical plates obtained was about 120 000. Limits of detection of the aromatic acids were 2–5 μg/ml. The advantages and lacks of the approach are discussed briefly.

Keywords: Electrochromatography; Stationary phases, electrochromatography; Ionene-modified stationary phases; Silica, ionene-modified; Ionenes; Benzoic acids

1. Introduction

Capillary electrochromatography (CEC) is a rather new and promising technique that has been applied to a variety of analytes ranging from acidic to basic compounds [1,2].

Up to now in CEC a few types of ion-exchange stationary phases have been used. It should be noted that research on CEC using strong anion-exchange (SAX) packings for the separation of inorganic anions or small organic molecules has rarely been reported. Strong anion-exchange CEC has been used for the separation of inorganic anions by Haddad and co-workers [3,4], for inorganic anions including iodide, iodate and perrhenate by Li et al. [5], for sulfate, sulfite and thiosulfate by Kitagawa et al. [6], or for aromatic acids by Ye et al. [7].

Recently, Ye et al. [8] reported the modification of a cation-exchange material with a cationic surfactant and the use of the resulting hydrophobic layer on this packing as a stationary phase in CEC. Separations carried out with this material were mainly based on partitioning between the mobile phase and the hydrophobic layer; on the other hand, one might also think of using such stationary phases in an ion-exchange mode, but this has not yet been investigated in detail.

Cationic surfactants are well-known as additives to the carrier electrolyte for the analysis of low-molecular mass anions in the (normal) mode of capillary zone electrophoresis (CZE) to reverse the electroosmotic flow (EOF); in addition, such surfactants may change the separation selectivity of CZE by an

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0021-9673/01/$ – see front matter
PII: S0021-9673(00)01172-9

ion-pairing mechanism. Typically used surfactants include hydrophobic tetraalkylammonium compounds or cationic polymers such as poly(1,1-dimethyl-3,5-dimethylenepiperidinium chloride) [9], poly(1,1-dimethyl-3,5-dimethylenepyrrolidinium chloride) [10], hexadimethrine chromate [11], polyethyleneimine [12], poly(diallyldimethylammonium chloride) [13,14], polyallylamine [15] or polymeric dyes [16].

Previous works [9–16] demonstrated that cationic polymers (polyelectrolytes) have certain advantages as capillary conditioning reagents. Beyond these applications they might be used in an open-tubular or packed-mode of CEC. In the latter case sulfonated packing materials (i.e. sulfonated silica for HPLC) dynamically coated by water-soluble cationic polymers might be very promising stationary phases. The formation of a so-called ''polyelectrolyte complex'' leads to ultimate high ability of absorption of the polymer on the sulfonated packing materials. In ion chromatography it was found that the aliphatic and aromatic ionenes are perfect modifiers of sufonated silica and show excellent results as anion-exchangers [17–19]. In this paper the first attempt to use polyelectrolyte-modified anion-exchangers in CEC is discussed.

2. Experimental

2.1. Instrumentation

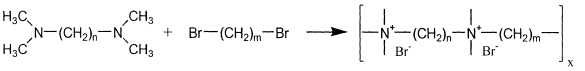
All CEC experiments were performed with a HP^{3D}CE instrument (Agilent, Waldbronn, Germany), equipped with a diode array detector and connected to a HP^{3D}CE Chemstation (Agilent) for data processing. An external pressure of 5 bar was applied to both ends of the column using helium gas. Samples were injected electrokinetically for 6 s at −5 kV. Temperature was kept at 23±0.2°C. All buffers were filtered through 0.45 μm filter and sonicated for 3 min in an ultrasonic bath before use. Fused silica capillaries (75 μm I.D.×360 μm O.D.) obtained from Polymicro Technologies (Phoenix, AZ, USA) were used throughout this work. The viscosity of the polymer solution was determined at 30°C with an Ubbelohde dilution viscometer.

2.2. Reagents

Deionised high-purity water (Milli-Q plus 185, Millipore, Bedford, MA, USA) was used to prepare all solutions. Reagent grade potassium salts for the buffer preparation, aromatic acids and tris(hydroxymethyl)aminomethane (Tris) were obtained from Merck (Darmstadt, Germany) and used to prepare the stock solutions (10–100 m*M*). *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine obtained from ICN Pharmaceuticals (Costa Mesa, CA, USA) and 1,10-dibromodecane purchased from Fluka (Buchs, Switzerland) were used as reagents for the synthesis of the ionene. Exsil 100-SCX sulfonated silica (5 μm) (Alltech/Exmere, Chester, UK) was used as the matrix for the preparation of the packing material for CEC. Strong anion-exchanger Nucleosil 100-5 SB (spherical 5 μm, anion-exchange capacity is about 1 mequiv./g) was obtained from Macherey–Nagel (Düren, Germany). Solvents (acetone, dimethylformamide) were spectral grade and used without further purification. Acetone and thiourea were used as EOF markers.

2.3. Synthesis of ionenes

The multiple Menshutkin reaction (N-alkylation) [20] was used for synthesising ionene:



The polymerisation was carried out in a dimethylformamide solution using equimolar monomers at 40°C. The total concentration of monomers was 1.0 mol/l and the reaction time was 48 h. The polymer was precipitated by pouring the reaction mixture into a large excess amount of acetone and dried under vacuum at 25°C for 3 days. The polymer obtained was a hygroscopic white powder with a yield of about 80%. The average molecular mass of the 2,10-ionene was 10 400 (determined by viscosity measurements).

2.4. Capillary pre-treatment and packing procedure

Uncoated capillaries were purged by a syringe with 0.1 *M* NaOH and water; afterwards the capillaries were flushed with air to remove water.

Pre-treated fused-silica capillaries were packed using a slurry-packing technique similar to that described by Hilder et al. [3]. Frits were generated by heating a narrow band (about 3 mm) of the capillary at 450°C for 12 s. During the heating, the capillary was flushed with water at 200 bar. After formation of the frits, the excess of the packing material was flushed out of the capillary and the packing quality was checked with a microscope. Finally, a detection window was fabricated 10 mm behind the end frit, the capillary was cut to a total length 33 cm, mounted in the cartridge of the CE instrument, and conditioned with running buffer containing polymer modifier by a HPLC pump for 30 min at about 150 bar. Next, the cartridge was installed into the HP^{3D}CE instrument. The procedure produces dynamically coated anion-exchangers and prevents the formation of small bubbles in the middle of the packed bed of the capillary. The structure of the polymer-coated material is presented in Fig. 1.

3. Results and discussion

3.1. Choice of polyelectrolyte and running buffer

Ionenes are polymers with quaternary nitrogen atoms in the backbone. The range of substances that fulfil this definition is wide. In the present study, we considered polyammonium salts which are the most commonly called as ionenes. The term ‘‘ionenes’’ was introduced by Noguchi and Rembaum [21]. There are several different approaches to name ionenes depending on the composition of functional groups between quaternary nitrogen atoms. The common formula of an ionene can be expressed as $-[R_2N^+-(CH_2)_n-NR_2^+-(CH_2)_m]-_X$. Almost all the ionenes are produced by a polycondensation reaction from two monomers; thus, their names are composed from two parts determined by the monomers. For instance, aliphatic ionenes are denoted with two numbers like *n–m* ionene, where *n* and *m* are the numbers of CH_2 groups between the nitrogen atoms.

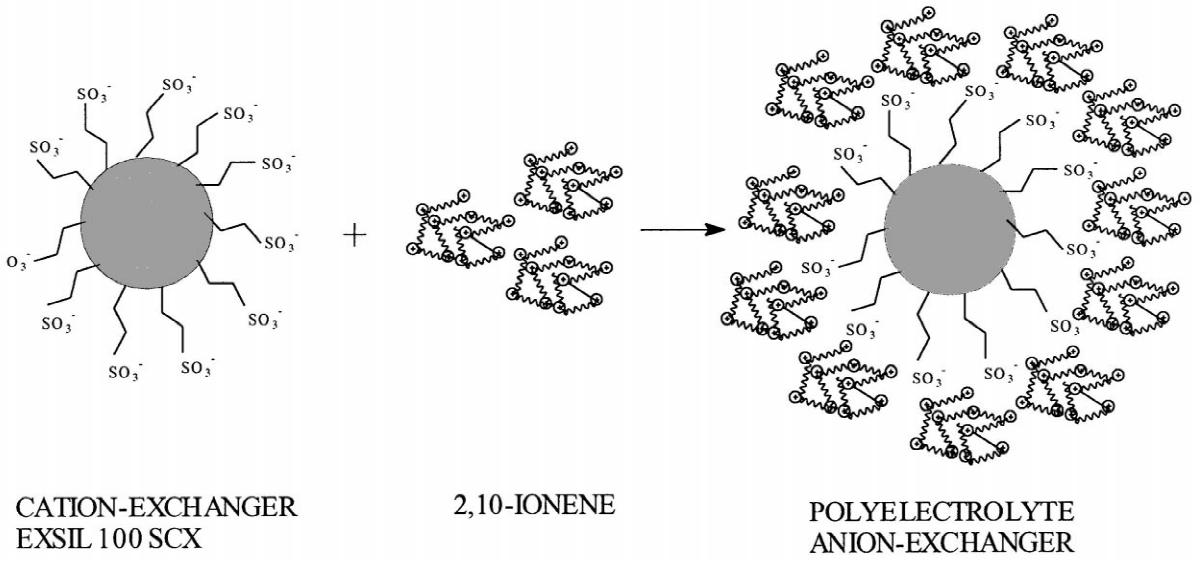


Fig. 1. The structure of polyelectrolyte modified anion-exchangers.

It is assumed that *n* is the aliphatic part of a diamine and *m* is from a dibromide (or diiodide). Nevertheless, many authors do not use this non-strict rule and indicate the smaller part first (like 3–6 ionene).

It is rather simple to synthesise ionenes with different flexibility of the chains by variation of the number of methylene groups between the nitrogen atoms in the backbone. The increase of the distance between the nitrogen atoms leads to a lower density of charges in the molecules, to a change of the conformation (from rod-like to globular polymers), and to an increase of hydrophobicity. Thus, from this point of view, ionenes are practically an ideal choice to test polymers with different (and smoothly changing) structure as modifiers in capillary electrophoresis and electrochromatography. 2–10-Ionene is one of the most hydrophobic aliphatic ionenes and has a globular structure [22]. The ionenes of such types have been tested previously [17] in ion chromatography and show good separation selectivity.

In the case of packed-capillary mode of CEC, a phosphate buffer containing 0.1% of 2–10-ionene with phosphate anions as counter-ions, 1 m*M* of KH_2PO_4, and Tris for pH adjustment to 6.53 was selected as a running buffer. At this pH the analytes employed in this work are present as their anions. Phosphate is transparent in the UV range and direct detection of aromatic acids could be performed.

3.2. Sorption of ionenes on capillary walls and sulfonated silica

Sorption of ionenes can take place on the packing material as well as on the inner surface of the capillary (the latter fact is well known for reversing the EOF in CEC). Unfortunately, so far it is not clear how much ionenes might be adsorbed on the inner surface in comparison with the packing material. Therefore, the following calculations and experiments have been carried out.

Taking a capillary of 50 cm total length $\times$ 100 μm I.D., the inner surface area *S* is $1.57 \cdot 10^{14}$ nm^2. It is reasonable to suppose that one average molecule of polymer takes the size of 1 nm^2 [22]. Assuming that the polymer molecules fully cover the walls of the capillary and form a monomolecular layer, $1.57 \cdot 10^{14}$ molecules of the polymer are needed to cover the inner surface of the capillary. To estimate the extent of sorption, an aromatic ionene named as 3–X-ionene was used. The ionene was synthesised and characterised previously [18]. The average molecular mass of the 3–X-ionene was 8000. The molecular mass of one branch of the polymer is 394 (bromide is the counter-ion). Therefore, one molecule of the polymer has $8000/394 \approx 20$ branches, and 40 bromide anions as counter-ions. Thus, theoretically the number of bromide ions *N* present at the inner surface would be:

$$N = 1.57 \cdot 10^{14} \cdot 40$$
$$\approx 6.3 \cdot 10^{15} \text{ bromide ions (about } 1 \cdot 10^{-8} \text{ mol).}$$

One might try to replace the bromide ion by another competing ion and to collect and quantify the released bromide ions. This would allow an estimation of the amount of polymer adsorbed on the inner surface. If the replacement is 100% and if the bromide is collected in a volume of 1 ml, than the bromide concentration C_{Br} would be:

$$C_{Br} = 1 \cdot 10^{-8} / 1 \cdot 10^{-3} = 1 \cdot 10^{-5} \text{ mol/l} = 800 \text{ ppb.}$$

Such a concentration level can be determined by modern analytical methods, e.g., by ion chromatography, so that a verification of the calculations seems to be possible.

For the purpose of the verification, the following experiments were made: an empty capillary of 50 cm total length $\times$ 100 μm I.D. was flushed with 0.1 *M* NaOH (2 min), water (3 min), a 10 m*M* solution of 3–X-ionene (5 min), and water (3 min). After this procedure, a 10 m*M* solution of sodium sulfate was pumped through the capillary, and 1 ml of liquid dropping from the outlet was collected (for about 20 min). The collected fraction was injected into an ion chromatograph to determine bromide. The chromatograms of the solutions are presented in Fig. 2. One can see that about 100% of the counter-ions bromide in the molecule of 3–X-ionene exist in a free form and should be replaced with sulfate ions in the experiment (Fig. 2b). Secondly, the observed concentration of bromide in the collected liquid is about 200 ppb, i.e. four times less than calculated (Fig. 2c). Thus, the total anion-exchange capacity of the hollow capillary modified by the solution of ionene is about $2.5 \cdot 10^{-9}$ mol. One should keep in mind that upon adsorption of the polymer on the charged inner

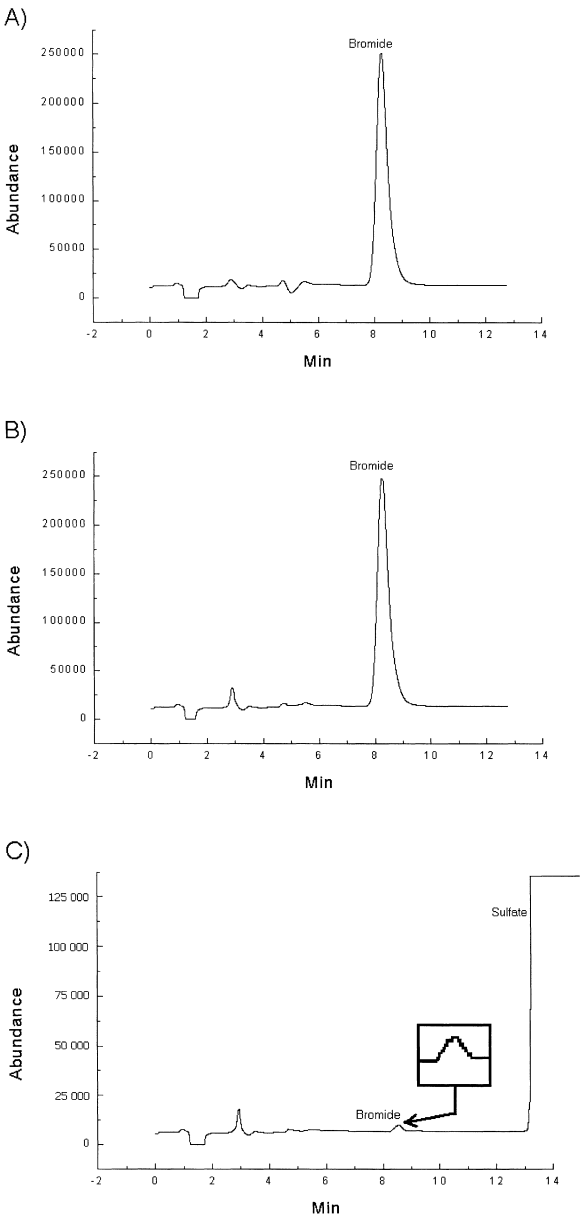


Fig. 2. Estimation of the amount of ionene adsorbed on the inner surface of a capillary using suppressed ion chromatography for the determination the counter-ion bromide. A = A standard solution of bromide (0.2 m*M*), B = 0.1 m*M* solution of 3–X-ionene (0.2 m*M* of bromide as counter-ion), C = solution obtained from replacing bromide of adsorbed ionenes by sulfate. Separation column: Waters IC-Pak Anion HR. Eluent 2 m*M* $NaHCO_3$–1.5 m*M* Na_2CO_3. Flow-rate 0.9 ml/min. Sample loop 20 μl.

surface, the molecule partially loses its conformation acquired in solution; thereby, it may collapse and form a more densely packed layer of adsorption. This could even result in an increasing amount of adsorbed polymer (compared with the calculated value) [23]. In our case an opposite phenomenon occurs. This might be accounted for by an irregular covering of the walls by the polymers; however, it should also be noted that some of the positive charges in the molecule of the polymer are not available, as they have reacted with the silanol groups of the surface of the walls forming the polyelectrolyte complex. Only ''uncompensated'' charged nitrogen atoms produce anion-exchange capacity and have bromide counter-ions. This would mean that in the case of a rod-like polymer like 3–X-ionene, a part of positive charges in the molecule are not available due to the formation of polyelectrolyte complexes on the capillary walls, and some charges are ''free'' and reverse the EOF. It is also possible to estimate a total anion-exchange capacity of the packing materials in the capillaries that have been used in our experiments in the packed-capillary mode of CEC. The volume of the packed bed of the capillary can be calculated as:

$$V = \pi R^2 H = 3.14 \cdot (37.5 \cdot 10^{-3})^2 \cdot 30$$
$$= 132\,468 \cdot 10^{-6} \approx 4 \cdot 10^{-2}\ \mathrm{mm}^3$$

where R is the radius of the capillary, H is the length of the packed part (30 mm). For the following calculations it is supposed that the bulk density of the silica ρ is 0.4 g/ml (according to product information from Merck). Hence, the mass m of the silica in the capillary is equal to:

$$m = V \cdot \rho = 5 \cdot 10^{-2}\ \mathrm{mg}$$

The anion-exchange capacity of polyelectrolyte anion-exchangers produced by treatment of the sulfonated silicas by ionenes is usually $8 \cdot 10^{-2}$ mmol/g (or $8 \cdot 10^{-8}$ mol/mg) [17,18]. Thus, the anion-exchange capacity (Q) of the packed bed in the capillary is:

$$Q = 8 \cdot 10^{-8} \cdot 5 \cdot 10^{-2} = 4 \cdot 10^{-9}\ \mathrm{mol}$$

These calculations may include some errors, but should result in a correct order of magnitude and can

be useful for understanding the separation conditions in the capillary.

3.3. The characterisation of the sorbents and retention of benzoic acids

In the case of dynamically coated polyelectrolyte packing materials the polymer molecules are immobilised at the surface of the matrix due to strong electrostatic interactions. Because of the formation of very stable polyelectrolyte complexes it is possible to use these sorbents in ion chromatography without additives of the polymer in the mobile phase [17]. In CEC there is one additional factor affecting the stability of the complex, namely the applied voltage. Indeed, negatively charged particles of the matrix and positively charged molecules of the polymer should migrate into opposite directions. This leads to a destruction of the polyelectrolyte complex and the sorbents could lose their anion-exchange properties. Therefore it is necessary to add a certain amount of the polymer modifier to the running buffer. The

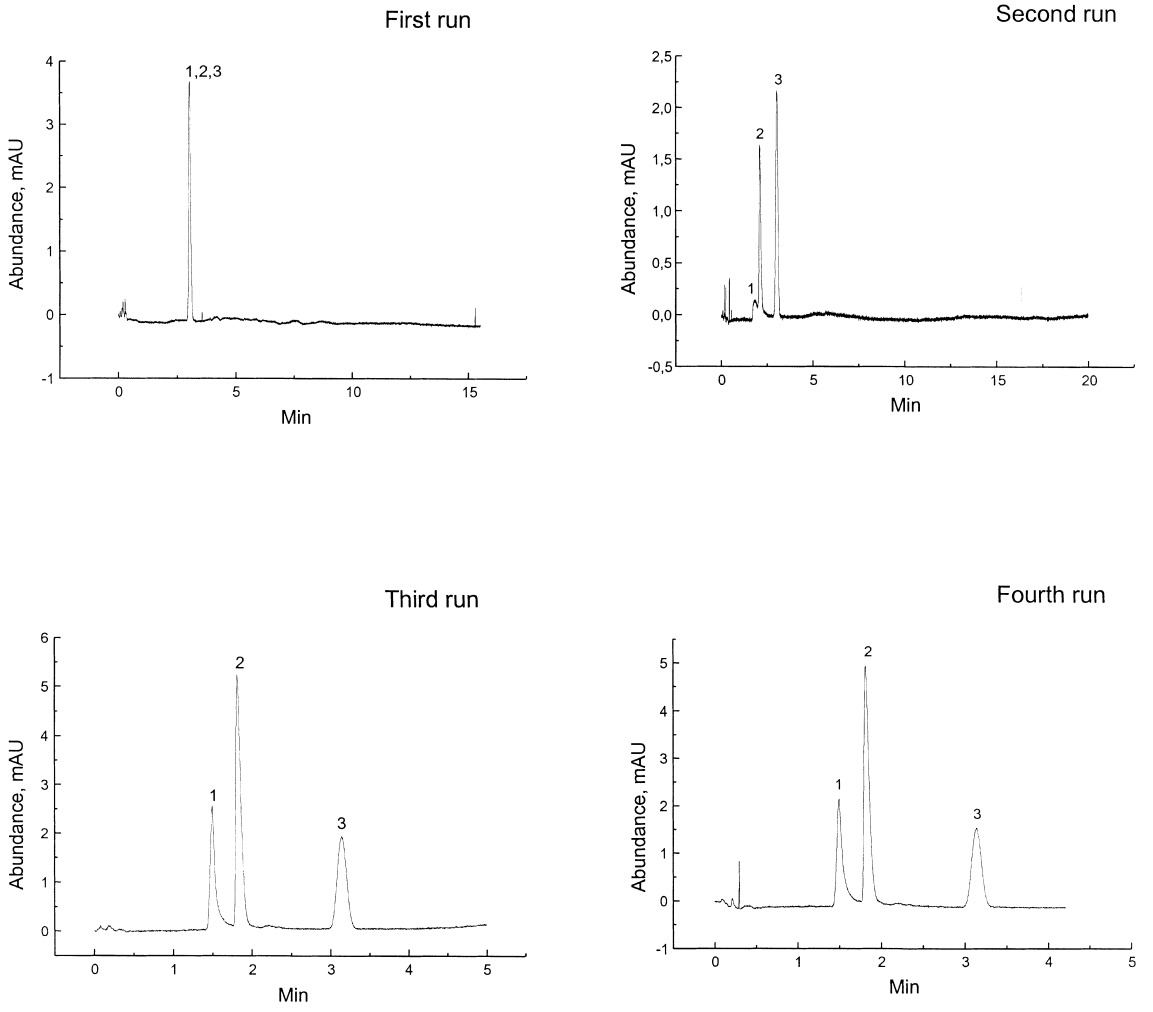


Fig. 3. Behaviour of ion-exchange CEC for the separation of benzoic acids. Packing material: Exsil-100 SCX (5 μm), modified by 2–10-ionene. Mobile phase: 0.1% solution of 2–10-ionene (PO_4 form)–1 m*M* KH_2PO_4, pH adjusted to 6.5 with Tris. Capillary 33 cm (3 cm packed bed)×75 μm. Applied voltage −15 kV. Injection: −5 kV for 6 s. Sample concentration at 0.5 m*M*, UV detection (254 nm). Peaks: (1) EOF, (2) benzoic acid, (3) *p*-aminobenzoic acid.

experiments demonstrated that the optimal concentration of the ionene in the running buffers is 0.1%. If the concentration of the ionene in the buffer is less than 0.1%, a continuous decrease of the migration times of the analytes was observed. Nevertheless even with 0.1% of modifier, the reproducible results could be only observed after 3–4 injections (Fig. 3). Apparently, applying voltage to the polyelectrolyte-modified packing materials leads to a significant re-equilibration of the molecules of the polymer at the surface of the sulfonated silica. Afterwards the number of reproducible runs was limited by eventual bubble formation in the packing material (which seems to be a general problem in CEC).

A linear increase of anion-exchange capacity with an increase of the length of the packed part in the capillary was observed. As could be predicted, the increase of the migration times depends on the constant of ion-exchange for each individual anions. For example, in the case of *p*-aminobenzoic acid the ion-exchange interactions with the functional groups of the anion-exchanger are much stronger than those for nitrate. It results in a significant increase of the retention of the acid vs. length of layer of the sorbent in the capillary. But under the conditions of the experiments the amount of the ionene adsorbed was small and the total ion-exchange capacities of the produced polyelectrolyte sorbents are lower than those for common commercially available anion-exchangers. The electropherograms of *p*-aminobenzoic acid on Nucleosil 100-5 SB and dynamically coated polyelectrolyte sorbents are presented in Fig. 4. Under the same conditions the retention times of the acid were 43 and 3.5 min, respectively. Thus, using such anion-exchangers with small ion-exchange capacity could be promising to reduce the total time of analysis.

It should be noted that the calculated anion-exchange capacity of the open capillary modified by ionenes and the capacity of the material in the packed capillary are about equal. However, there is a principle difference. In the case of a hollow capillary, anion-exchange interactions could involve the molecules at the surface of the capillary walls. But in the case of a packed capillary, the anion-exchange mechanism affects all the molecules of the injected samples. This means that open-tubular CEC will work only in capillaries with very small inner

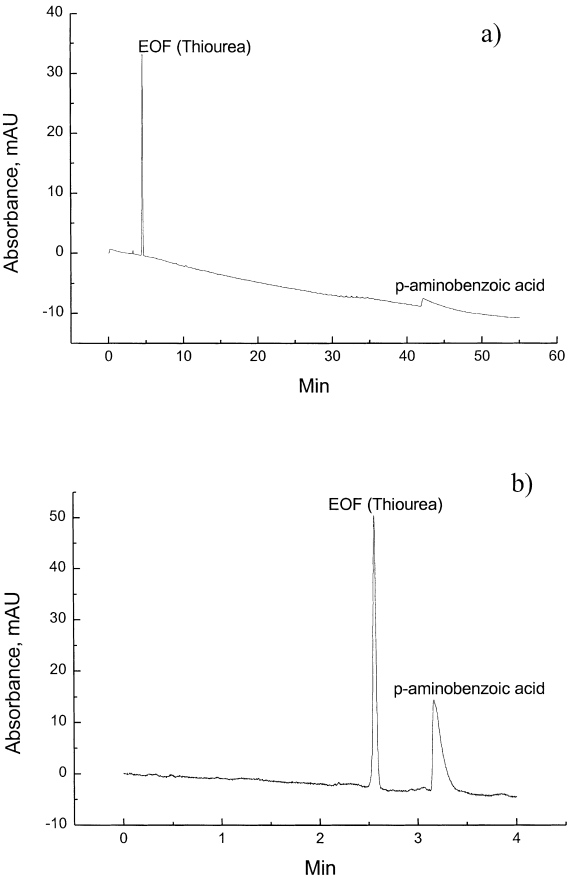


Fig. 4. Retention of *p*-aminobenzoic acid on different packing materials. (a) Nucleosil 100-5 SB (5 μm). Mobile phase: 1 m*M* KH_2PO_4, pH adjusted to 6.5 with Tris. (b) Exsil-100 SCX (5 μm), modified by 2–10-ionene. Mobile phase: 0.1% solution of 2–10-ionene (PO_4 form)–1 m*M* KH_2PO_4, pH adjusted to 6.5 with Tris. Other experimental conditions as for Fig. 3.

diameter, whereas in the present case the packed part plays the major role.

Fig. 5 illustrates this fact. Under the conditions of the experiments the aromatic acids are present as anions. In the case of a hollow capillary, the acids move through the capillary at a high rate. Anion-exchange interactions are minor and, therefore, the acids migrate more rapidly than a neutral marker (acetone). On the other hand, when the packed capillary was used, the ion-exchange interactions are much stronger. It results in a significant shift of the migration times of the acids (into a region after the EOF). The highest number of theoretical plates

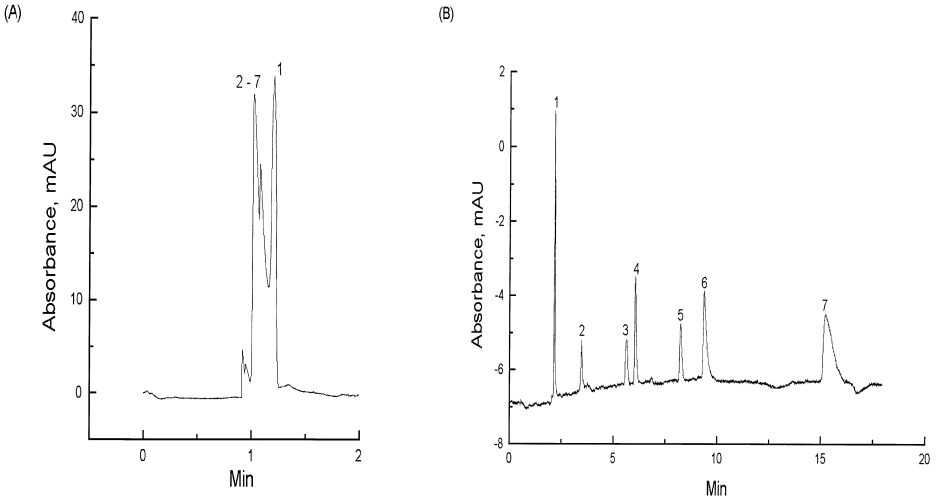


Fig. 5. Electropherograms of several aromatic acids in CZE (A) and CEC (B) modes. Capillary 33 cm (16 cm packed bed)×75 μm. Mobile phase: 0.1% solution of 2–10-ionene (PO_4 form)–1 m*M* KH_2PO_4–10% acetonitrile, pH adjusted to 6.4 with Tris. Applied voltage −20 kV. Injection: −5 kV for 5 s. Sample concentration at 0.5 m*M*, UV detection (254 nm). Peaks: 1=EOF (acetone), 2=*p*-hydroxybenzoic acid, 3=salicylic acid, 4=benzoic acid, 5=*o*-bromobenzoic acid, 6=*p*-aminosalicylic acid, 7=*p*-aminobenzoic acid.

obtained was about 120 000. Limits of detection of the aromatic acids were 2–5 μg/ml.

4. Conclusions

It has been shown that dynamic modification of strong cation-exchangers by a solution of a water-soluble polymer containing anion-exchange functional groups is a promising way to create new stationary phases for CEC. Apparently, the major advantage of the scheme is the possibility to control the retention of the solutes by changing the nature of polymer modifier. Unfortunately, the polymer modifier has to be present in the mobile phase, which excludes the use of aromatic ionenes. However, it should be noted that due to the formation of very stable complexes between the silica matrix and the polymer molecules, the concentration of the modifier in the running buffer can be considerably lowered in comparison with the use of tetraalkylammonium salts [8].

Acknowledgements

This research was funded by the Austrian Science Foundation (FWF) under grant No. M552-CHE.

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