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Capillary electrochromatography of tricyclic antidepressants on strong cation exchangers with different pore sizes

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Abstract

Four cation-exchange materials, possessing propanesulfonic acid ligands, for use in capillary electrochromatography were prepared from different commercially available 5-μm bare-silica particles ranging from 80 to 800 Å in pore size. The performance of the materials was investigated at different compositions of the mobile phase (pH, ionic strength, and acetonitrile content) using tricyclic antidepressants and related quaternary ammonium analogues as test analytes. The wide-pore materials promoted pore flow, but this had no positive influence on the performance. The small-pore (highest surface area) particles gave, as could be expected, the best selectivity.

Keywords: Electrochromatography; Stationary phases, CEC; Cation exchangers; Pore size; Tricyclic antidepressants

1. Introduction

The interest in capillary electrochromatography (CEC) has increased constantly during the last decade since Knox and Grant revived the technique in theory and practice [1–3]. By comparing the books of abstracts of HPCE 1996 and HPCE 2000 the share of CEC entries has increased almost five times. The use of electroosmotic flow (EOF) in chromatography has been reported as early as 1939 [4], but Pretorius et al. were the first to use EOF to propel the mobile phase through a narrow LC column [5]. They showed that the band broadening was substantially lower than that obtained in pressure-driven chromatography. In 1981 Jorgenson and Lukacs demonstrated the use of CEC on reversed-phase (RP) particles in a glass capillary [6], and the use of RP stationary phases in CEC has dominated since then.

In CEC the EOF originates not only from the capillary walls as in CZE, but mainly from the particles in the stationary phase [7]. Since the surface charge of silica is highly dependent on pH, analyzes at low pH can be troublesome as the silanol groups are dissociated to a low extent, hence, the EOF will be very low. Smith and Evans suggested a solution to this problem by using strong cation-exchange (SCX) particles instead [8]. Strong cation exchangers or mixed-mode stationary phases in CEC have a fairly high EOF even at low pH due to the low pK_a value of the sulfonate groups [7,9–14].

The solutes in the present study (tricyclic antidepressants) are notorious for giving severe peak tailing in reversed-phase chromatography due to

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mixed retention mechanisms: hydrophobic interaction with the alkyl chains, ion exchange at the charged silanol groups and hydrogen bonding to silanol groups [15]. The tailing can be suppressed by adding a small aliphatic amine to the mobile phase to mask the silanol groups [16–18]. Smith and Evans found not only that the EOF is high on SCX stationary phases but also that it was possible to separate basic analytes with low peak tailing and extremely high efficiencies [8]. The zone sharpening causing these high efficiencies has proven difficult to understand and to reproduce [19–21]. Studies on reversed-phase materials have shown that it is favorable to use particles with large pore size that can provide pore flow [23,24]. The efficiency increase is due to higher mass transfer rate and improved flow homogeneity.

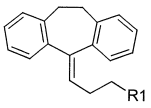
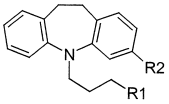
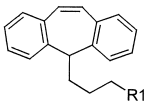
The aim of this study was to investigate the effect of the mobile phase composition on the electrochromatographic performance of tricyclic antidepressants and related quaternary ammonium compounds (Table 1) on four different strong cation exchangers prepared at our laboratory from commercially available bare silica particles differing in pore size. The effects of the mobile phase composition with regard to ionic strength, pH and content of acetonitrile were studied.

2. Experimental

2.1. Chemicals used during the capillary electroseparations

The buffer stock solutions with pH values between 2.8 and 7.5 were made from NaOH (Merck, Darmstadt, Germany), H_3PO_4 (Merck) and water (Milli-Q Water System, Millipore, Bedford, MA, USA). (The same buffer stock solutions were used through the whole series of experiments). The ionic strength was kept constant at 0.125 *M* in all the buffer stock solutions [22]. Mobile phases were made from buffer stock solution, water and acetonitrile (ACN) (Fisher Scientific, Leicestershire, UK). The pH in the buffer was measured before addition of acetonitrile. The basic mobile phase was made from buffer stock solution, pH 2.8–water–ACN (10:30:60, v/v); the ionic strength was 12.5 m*M* in the final mixture. In all other mobile phases only one parameter was changed at a time e.g. the percentage of ACN while the pH and ionic strength were constant. Stock solutions of the solutes, amitriptyline·HCl (AMI) (Merck Sharp and Dome, Rahway, NJ, USA), nortriptyline·HCl (NOR) (Pharmacia, Uppsala, Sweden), desipramine·HCl (DESI) (Ciba-Geigy, Basel, Switzerland), imipramine·HCl (IMI) (Hässle,

Table 1
Structures of the analytes

Structure	Name	R1	R2	Short
	Nortriptyline	$-NHCH_3$		NOR
	Amitriptyline	$-N(CH_3)_2$		AMI
	N-Methylamitriptyline	$-N(CH_3)_3^{\oplus}$		M.AMI
	Desipramine	$-NHCH_3$	–H	DESI
	Imipramine	$-N(CH_3)_2$	–H	IMI
	Clomipramine	$-N(CH_3)_2$	–Cl	CLOMI
	*N,N*-Dimethylprotriptyline	$-N(CH_3)_3^{\oplus}$		DM.PRO
	N,N-Dipropylprotriptyline	$-NCH_3(C_3H_7)_2^{\oplus}$		DP.PRO

Mölndal, Sweden), clomipramine·HCl (CLOMI) (Ciba-Geigy, Basel, Switzerland), *N,N*-dimethylprotriptyline bromide (DM.PRO), *N,N*-dipropylprotriptyline bromide (DP.PRO) and *N*-methylamitriptyline bromide (M.AMI), were made from 1 mg of the substance dissolved in 500 μl buffer and 1500 μl water. The quaternary ammonium derivatives of protriptyline and amitriptyline were synthesized previously in house as described by Borg and Schill [25]. As electroosmotic marker 1-phenyl-1,2-ethanediol (PED) (Aldrich, Milwaukee, WI, USA) was used, the stock solution was 10 m*M* in water–ACN (80:20). The sample solutions were typically made from 30 μl of each analyte solution, 60 μl EOF marker and 750 μl mobile phase or a mixture of water and ACN with the same percentage of ACN as the mobile phase. We did not observe any effect on peak appearance whether the water–ACN mixture or mobile phase was used to dilute the sample.

2.2. Instrumentation

The experiments were performed with fused-silica capillaries obtained from Polymicro Technologies (Phoenix, Arizona, USA). The dimensions of the capillaries were effective length 25 cm×100 μm I.D.×360 μm O.D. The length after the detector was 8.5 cm. The capillaries were mounted in a Hewlett-Packard 3D CE system (Agilent Technologies, Waldbronn, Germany). To suppress bubble formation both ends of the column were pressurized with nitrogen at 10 bar. Detection was carried out at 210 nm, sometimes also at 191 and 239 nm to ensure peak identity. A Jasco PU-980 HPLC (Jasco, Tokyo, Japan) pump was used to purge the capillary by using the constant pressure mode (100 kg/cm^2). The samples were electrokinetically injected at 1–6 kV for 2.5 s towards the cathode. The column was thermostated at 25°C. The voltage was 10 or 15 kV resulting in currents of 4–25 μA depending on the mobile phase.

2.3. Preparation of the stationary phases

The cation-exchange materials were made from bare silica from two different manufacturers, Nucleosil (Macherey-Nagel, Düren, Germany) and Zorbax (Agilent Technologies, Waldbronn, Germany). The characteristics of the bare-silica materials, as declared by the manufacturers, are presented in Table 2. To prepare the materials we used a three-step method modified from earlier published procedures [26–29]. The amounts of chemicals used for derivatisation were based on the denoted specific area of the bare silica. Calculating with approximately six silanol groups per square nanometer of silica equals $9.96 \cdot 10^{-6}$ mol of silanol groups per square meter [30]. All amounts in the following description are calculated for a material with a specific area of 100 m^2/g.

2.3.1. Activation of the silica

In a 100-ml round bottle equipped with a blade stirrer 1 g of silica particles was immersed in 50 ml of 0.1 *M* HNO_3 (Merck) and refluxed for 5 h. The mixture was allowed to cool and the silica gel was rinsed with water (five times) and then with methanol (three times) using a Millipore vacuum filter equipment (filter pore size 0.45 mm HVLP04700). The particles were dried overnight at 150°C.

2.3.2. Silanization of the activated gel

The dry particles were immersed in 30 ml of freshly dried toluene (Riedel de Haën, Seeze, Germany) and then 0.76 ml ($4 \cdot 10^{-3}$ mol) of (3-mercaptopropyl)-trimethoxysilane (3-MPS) (Sigma, St. Louis, MO, USA) was added. The mixture was kept overnight at 105°C under gentle stirring. The particles were filtered and carefully washed with methanol (12 times) and then allowed to dry for several hours on the filter.

Table 2
Characteristics of the 5-μm pure silica particles as denoted by the suppliers

Supplier	Pore size (Å)	Specific area (m^2/g)	Name after derivatization
Nucleosil	100	350	XN100
Nucleosil	500	35	XN500
Zorbax	80	180	XZ80
Zorbax	800	12	XZ800

2.3.3. Oxidation of thiolsilica

In the last step the cation-exchange function, propanesulfonic acid, was obtained by oxidation of the thiol group using in situ generated anhydrous trifluoroacetic acid peroxide. A mixture of 30 ml acteonitrile and 1.11 ml ($8{\cdot}10^{-3}$ mol) trifluoroacetic anhydride (TFAA) (Sigma–Aldrich, Steinheim, Germany) was thoroughly cooled on an ice bath before dissolving 1.55 g (equivalent to $16{\cdot}10^{-3}$ mol of H_2O_2) of urea hydrogen peroxide adduct (UHP) (Sigma). The thiolsilica was added and the reaction mixture was allowed to reach ambient temperature and was stirred overnight. Finally the silica material was washed with methanol, water and methanol again for five, three and five times, respectively. The particles were allowed to dry before packing in capillaries. The excess of peroxide in the filtrate was destroyed with saturated sodium hydrogen sulfite solution. The molar ratio between the reagents was 1:4:8:16 for silanol, 3-MPS, TFAA and UHP, respectively. We named the stationary phases XZ80, XN100, XN500 and XZ800, where X denotes that it is an ion exchanger, N or Z specifies whether the starting material was from Nucleosil or Zorbax and the final number equals the stated pore size in Å.

2.4. Column preparation

The columns were packed according to the method described in detail by Enlund and Westerlund [18]. The SCX stationary phases were, however more difficult to pack than reversed-phase materials. The particles had a much higher tendency to aggregate, therefore the slurry was ultrasonicated four times for 3 min with vortexing between every sonication before introduction into the packing chamber. These stationary phases also required higher temperature and longer (60 s) heating time to produce stable frits.

2.5. Evaluation of electrochromatograms

All data presented are mean values from three injections. When examining the effects of the mobile phase composition on peak symmetry, efficiency and resolution the injection was adjusted so that the peak size of an analyte was kept quite constant to avoid comparing a small peak with a larger, and maybe, overloaded peak. Different mobile phases produce different EOF and, hence, different amounts of the sample will be injected. The asymmetry factor (asf) was calculated at 10% of the peak height by dividing the widths at the rear and the front sides of the peak. The values for efficiency (N) and resolution (R_S) were calculated by HP CHEMSTATION using the following equations:

$$N = 5.54 \times \frac{t_R^2}{w_{0.5}^2} \tag{1}$$

$$R_S = \frac{2.35 \times (t_{R_B} - t_{R_A})}{2 \times (w_{0.5B} + w_{0.5A})} \tag{2}$$

where t_R is the retention time, $w_{0.5}$ is the width at half height. Crego et al. have characterized the elution parameters in electrochromatography and derived an expression for the retention time for ionized compounds [31]:

$$t_{R_{CEC}} = \frac{(1+k)}{\left[\dfrac{1}{t_{eo_{CEC}}} + \dfrac{V \times \mu_{ep_{CZE}}}{L_d \times L_{tot}}\right]} \tag{3}$$

where $t_{R_{CEC}}$ is the retention time in CEC, k is the retention factor, $t_{eo_{CEC}}$ is the retention time for the electroosmotic marker in CEC, $\mu_{ep_{CZE}}$ is the electrophoretic mobility of the analyte in CZE using the same electrophoretic conditions as in corresponding CEC experiments, L_d is the length of the capillary to the detector and L_{tot} is the total length of the capillary. Rearrangement of Eq. (3) gives the retention factor for charged solutes:

$$k = \left(\frac{t_{r_{CEC}}}{t_{eo_{CEC}}} + \frac{\mu_{ep_{CZE}}}{\mu_{obs_{CEC}}}\right) - 1 \tag{4}$$

where $\mu_{obs_{CEC}}$ is the observed mobility of an analyte in the CEC experiments. The effective mobility in CEC was defined as:

$$\mu_{eff_{CEC}} = \mu_{obs_{CEC}} - \mu_{eo_{CEC}} \tag{5}$$

3. Results and discussion

3.1. Effect of pH

Fig. 1 shows that the EOF is influenced to a much lower extent by pH, while keeping the ionic strength

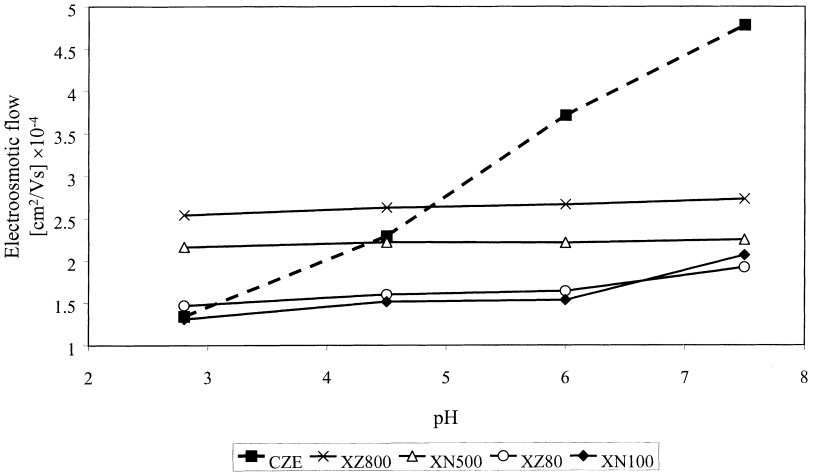


Fig. 1. Effect of pH on EOF in CZE compared to CEC on different SCX materials. The mobile phase consisted of buffer stock solution (pH 2.8–7.5)–water–ACN (10:30:60). The ionic strength in the final mixtures was approximately 12 m*M*. $L_d = 25$ cm, $L_{tot} = 34.5$ cm, Voltage 10 kV. Hydrodynamic injection in CZE (10 mbar×1 s) and electrokinetic injection in CEC (1–3 kV×2.5 s). EOF marker, 1-phenyl-1,2-ethanediol (PED).

constant on the SCX columns, compared to CZE in an untreated capillary. The EOF increases slightly with pH on the ion-exchange materials, probably due to the dissociation of underivatized silanol groups. This would increase the ion-exchange capacity, which was evident as the retention increased, hence, lower effective mobility as the pH was raised (Fig. 2). The chromatographic performance of the four packing materials differed somewhat, which will be commented in detail in Section 3.4. The general trends, however, were that the efficiency decreased while the symmetry factors were quite constant as the pH was raised. The resolutions were also relatively unaffected up to pH 6.0.

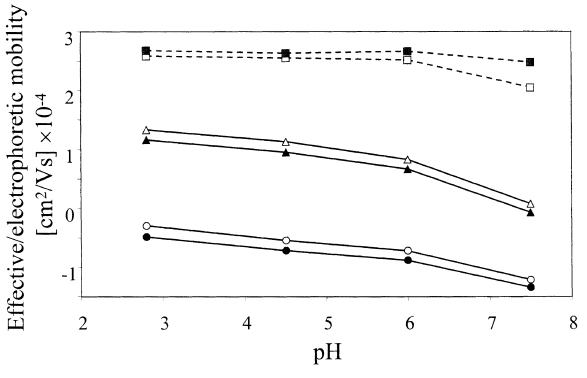


Fig. 2. Influence of pH on the effective mobility of M.AMI and NOR in CEC and the electrophoretic mobility in CZE. Conditions as in Fig. 1. Squares (■/□) for CZE, triangles (▲/△) for the XN500 stationary phase and circles (●/○) for XZ80. Filled symbols are the mobility of M.AMI and empty symbols for NOR.

3.2. Effect of ionic strength

In ion-exchange chromatography the ionic strength or rather the concentration of the counter ion, in this case the sodium ion, in the mobile phase is an important tool in controlling the retention. As the ionic strength increased the retention decreased as expected. The correlation between log k and log [concentration of sodium] was linear and e.g. the slope was -0.99 for M.AMI with XZ80 in the column, coincident with theory that the slope equals the magnitude of the charge of the solute [32]. Again comparing CZE with SCX systems, the μ_{ep} was consistent in CZE whereas the μ_{eff} on the CEC columns increased substantially as the concentration of the carrying electrolyte was raised. As an example the effective mobility of M.AMI increased from $-0.44 \cdot 10^{-4}$ to $1.76 \cdot 10^{-4}$ cm^2/V s on XN500 when the ionic strength increased from 4.2 to 20.8 m*M*, whereas the mobility difference in CZE was only $0.06 \cdot 10^{-4}$ cm^2/V s. In CEC, not only the retention but also the driving force, the EOF, is altered by the ionic strength. The EOF in CZE is proportional to

the zeta-potential, which decreases with increased buffer concentration, i.e. as the ionic strength is raised the EOF will be lower. This was also the case for two of the SCX materials (XZ80 and XN100), but the opposite for the other two stationary phases, which will be discussed further in Section 3.4. Also the EOFs on the CEC columns were mostly higher or of the same magnitude as in the CZE experiments. For the charged solutes the number of plates increased substantially with increased ionic strength, whereas, the increasing trend for the efficiency of the PED peak (uncharged solute) was more moderate (Fig. 3). There was a slight improvement of the symmetry as the buffer concentration was increased. As the interaction with the ion exchanger decreases when the concentration of the counter ion is higher, the sample zone is narrower. The effect on the resolution differed between the SCX materials and will be discussed further below.

3.3. Effect of acetonitrile content

ACN and other organic modifiers are used to modify the retention in chromatography. It is generally observed that the retention and often the resolution for structurally similar solutes are lowered as the content of ACN is increased. In CZE the addition of organic modifier has a large influence on EOF and μ_{ep} as the viscosity, zeta potential, pK_a and pH are altered [33], which all may affect the mobility and the resolution. For our test solutes the effective mobility increased in both CEC and CZE as the content of ACN was changed from 50 to 70% while the ionic strength was kept constant (Fig. 4). The effect on the mobility of the quaternary ammonium compound (M.AMI) was higher than on the secondary amine (NOR). The last eluting M.AMI came closer to NOR, hence the resolution decreased on the SCX columns. (In the CZE experiments M.AMI eluted before NOR which explains why the resolution increased the more ACN that was added.) As the concentration of ACN in the mobile phase increased the retention decreased for these rather hydrophobic analytes. The relationship between log k and log [%ACN] in our CEC systems was linear. As the interaction of the positively charged solutes with the stationary phase was lowered the efficiency and symmetry improved. The migration time for the EOF marker, PED, was prolonged as the concentration of ACN was raised on all SCX materials. This was neither seen in the CZE experiments, nor on a Spherisorb ODS1 column [18]. Whether the EOF increases [34] decreases [10] or even is almost constant [20] with the concentration of ACN has been a matter of confusion. This might be a consequence of the fact that it is hard to find an EOF marker that has no interaction with the stationary

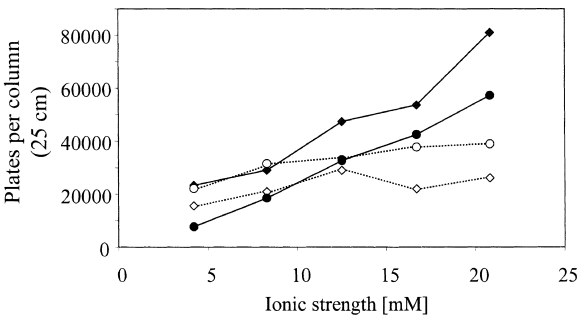


Fig. 3. Effect of the ionic strength on the efficiency for charged (M.AMI) and uncharged (PED) solutes. The mobile phase consisted of 3.3–16.7% buffer stock solution (pH 2.8), 36.7–23.3% water and 60% ACN. The ionic strength in the final mixtures was approximately 4.2 to 20.8 m*M*. Other conditions as in Fig. 1. Diamonds (◆/◇) for the XN100 material and circles (●/○) for the XZ80 material. Filled symbols with solid lines represent M.AMI and empty symbols and dashed lines represent PED, respectively.

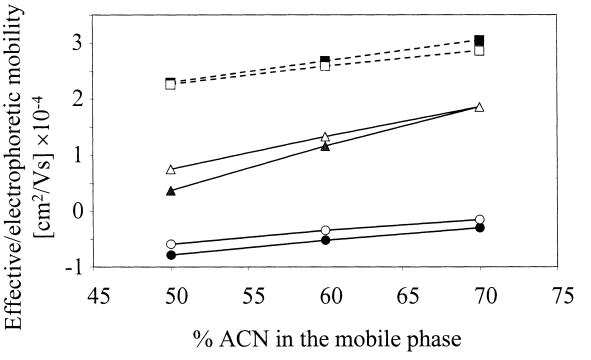


Fig. 4. Effect of the percentage of ACN on the effective mobility in CEC and electrophoretic mobility in CZE. The mobile phase consisted of 10% buffer stock solution (pH 2.8), 40–20% water and 50–70% ACN. The ionic strength was kept constant at 12.5 m*M*. Other conditions as in Fig. 1. Squares (■/□) for CZE, triangles (▲/△) for the XN500 stationary phase and circles (●/○) for XZ80. Filled symbols are the mobility of M.AMI and empty symbols for NOR.

phase, especially when dealing with this type of stationary phases and a high content of organic modifiers in the mobile phase, where the systems balance on the border between straight- and reversed-phase chromatography.

3.4. Different SCX materials

The properties of a stationary phase are reflected by the size of the retention factor, which usually are related to the specific area (square meter per gram adsorbent), provided that the density of the binding sites is equivalent for the different solid phases. The correlation between *k* and surface area was not convincing for the four cation exchangers. However, when plotting the data against pore size more general trends appeared. Whether this is a consequence of that the suppliers of Zorbax and Nucleosil use different methods for measuring the area or that they use different manufacturing procedures can not be distinguished by our studies. The only factor that correlated better to the nominal surface area than to pore size was the EOF. As pointed out above the different SCX materials exhibited different trends in performance as the composition of the mobile phase changed. The difference was often divided in two groups where the two wide-pore materials (XN500 and XZ800) differed from the particles with small pore width (XZ80 and XN100). As described above the efficiency declined when pH was raised, however, the resolution slightly increased between NOR and M.AMI on XN500 and XZ800 above pH 6. On the stationary phases with high specific area (XZ80 and XN100) the enhanced interaction with the binding sites resulted in a considerable loss in resolution at high pH. However, the resolution was more than sufficient on these two phases, except on XN100 at pH 7.5, whereas there was barely baseline separation on the wide-pore columns.

The pore size also influences the EOF by means of pore flow. Due to the double layer overlap EOF is much smaller in narrow pores compared to wide pores. Pore flow has been observed in particles with pores of 300 Å and wider [35]. This explains Fig. 1; the larger the pore size the higher the EOF. When increasing the ionic strength the thickness of the double layer decreases i.e. the zeta potential is reduced, hence, the EOF is lowered. This was the case for the XZ80 and XN100 materials (Fig. 5). However, in particles with pores wide enough to allow pore flow, such as XN500 and XZ800, the pattern was different. As the double layer became thinner the less was the overlap and a larger portion of the total flow could go through the particles instead of between them, hence, the EOF increased with the ionic strength. The resolution decreased on the wide-pore particles as the ionic strength was raised. As the concentration of the counter ion is increased the interaction with the stationary phase will be lower and the electrophoretic properties of

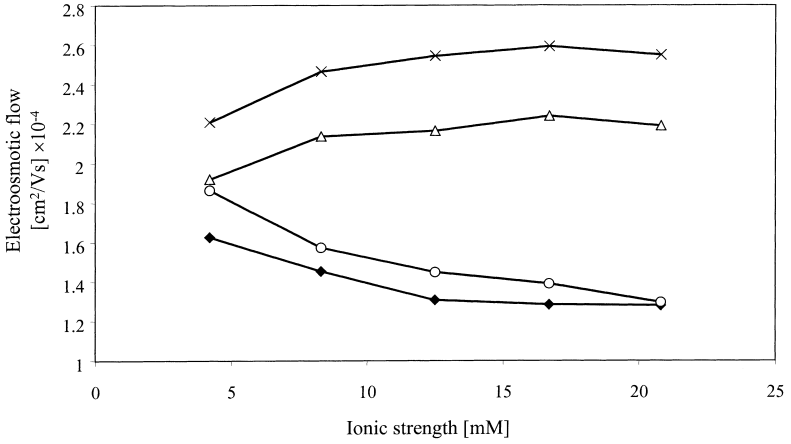


Fig. 5. Effect of ionic strength on the EOF differs depending on the pore size. Symbols for the different SCX materials: (×) XZ800, (△) XN500, (◆) XN100, (○) XZ80. Mobile phases as in Fig. 3, other conditions as in Fig. 1.

the analytes will dominate the retention. This in combination with the fact that the elution order in CZE is opposite to that in CEC resulted in loss of resolution. Impaired resolution could also be seen at the highest ionic strength (20.8 m*M*) on the particles with higher capacity (small pores). But when going from 4.2 to 16.7 m*M* the resolution improved as the efficiency increased.

To summarize the experiences with these SCX columns: the efficiency was 30 000–80 000 plates per column (25 cm) with the most commonly used mobile phase (pH 2.8, 60% ACN, ionic strength = 12.5 m*M*). This was in the same range as on Spherisorb ODS1 columns with addition of aliphatic amines to the mobile phase [18]. Note that the particle diameter of the SCX materials was 5 μm compared to 3-μm particles in the Spherisorb columns. The asymmetry was fully acceptable, ranging from 1 to 1.7 and rather unaffected by changes in the mobile phase and of the pore size. The retention factor was directly coupled to the pore size. XZ80 always had the highest *k* followed by XN100, XN500 and last XZ800. The resolution on this type of particle is lower than on Spherisorb ODS1. The best resolution was obtained on the XZ80 and XN100, but it was not possible to resolve as many tricyclic antidepressants and similar quaternary ammonium compounds on the SCX columns as in reversed phase (Fig. 6) [18]. These results indicate that applying smaller particles of small pore size would improve the resolution. On the small-pore particles the analytes elute after the EOF while the opposite was the case for the wide-pore materials illustrating the large difference in ion-exchange

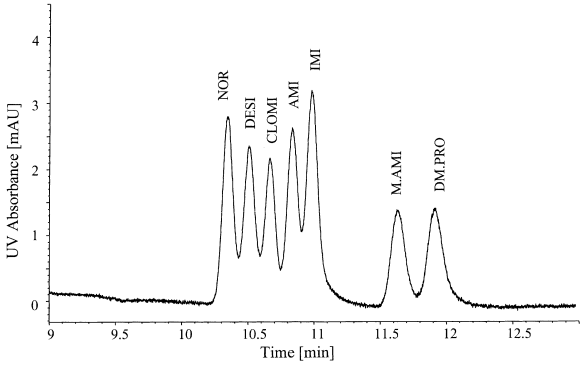


Fig. 6. Electrochromatogram showing the separation between seven tricyclic antidepressants and related quaternary ammonium compounds on a column with XN100 as stationary phase. Mobile phase: pH 2.8, 60% ACN, ionic strength 12.5 m*M*. Voltage 10 kV, current 11.4 μA. The electroosmotic marker, PED, would elute at 10.1 min. Solutes as in Table 1.

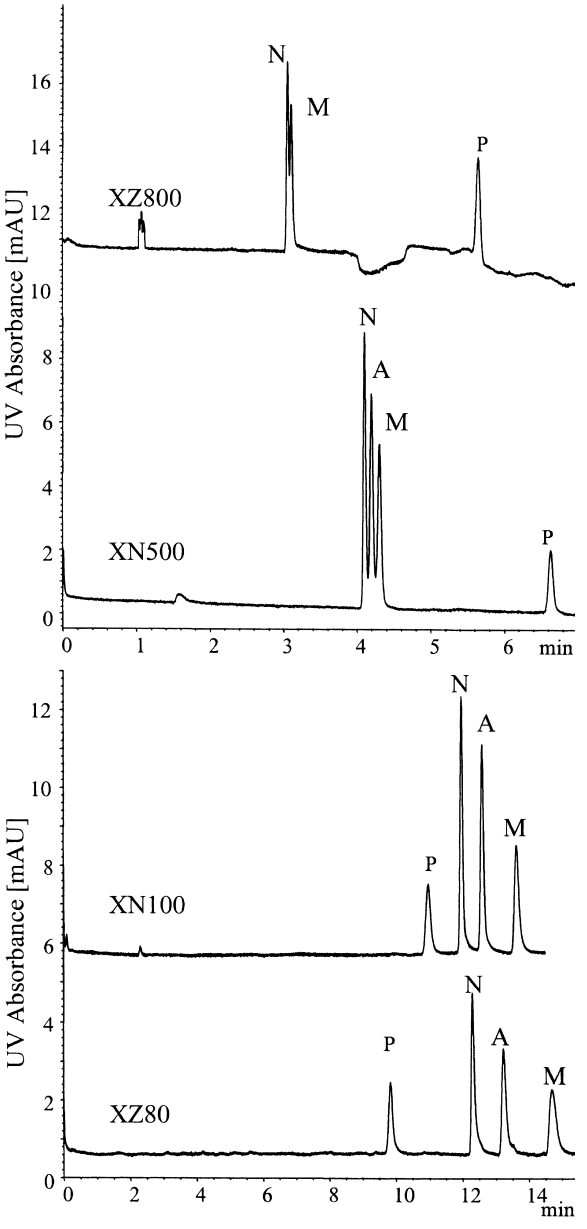


Fig. 7. Electrochromatograms from runs on columns with the four SCX stationary phases showing the difference in resolution between N, NOR; A, AMI and M, M.AMI. P, PED, the electroosmotic marker. Mobile phase: pH 2.8, 60% ACN, ionic strength 12.5 m*M*. Voltage 10 kV.

Table 3
Elution order in the different systems

System	SCX	ODS [18]	CZE
First	NOR_2	DESI	M.AMI
	$DESI_2$	NOR	
	$CLOMI_3$	IMI	
	AMI_3	AMI	AMI
	IMI_3	CLOMI	
	$DP.PRO_4$	DM.PRO	
	$M.AMI_4$	MA.AMI	
Last	$DM.PRO_4$	DP.PRO	NOR

The carrying electrolyte: pH 2.8, 60% ACN, ionic strength = 12.5 m*M*. Index 2, 3 and 4 indicates if the nitrogen is substituted with 2,3 or 4 alkyl groups.

capacity (Fig. 7). The elution order differs between reversed-phase, ion-exchange and CZE (Table 3). The analytes elute in groups according to their degree of substitution on the nitrogen. On the SCX columns the most hydrophobic solute in each group elutes first, while it is the opposite for reversed phase. In both CEC modes the secondary amines elute first and the quaternary ammonium compounds last, whereas in CZE these compounds have the highest mobility followed by the tertiary and finally the secondary amine. Stol et al. got very good resolution and high efficiency when analyzing polyaromatic hydrocarbons on columns with pore sizes up to 4000 Å [23]. We lost resolution as the pore size increased. This is probably due to that the elution order for our model compounds is opposite on the SCX material compared to an open capillary and hence, the electrophoretic and chromatographic migration properties counteract each other. As for the stability of chromatographic properties of these stationary phases strange effects on the peaks were very rare. When sharp or very broad peaks appeared the phenomenon was reproducible. A sharp M.AMI peak appeared on column XZ800 at the lowest ionic strength, but the efficiency was not extremely high, only about seven times higher than for the other two analytes (Fig. 8). With two of the mobile phases, at pH 4.5 and when the content of ACN was 50%, the M.AMI peak got really wide but still symmetric on the XN500 column compared to NOR and AMI. However, we have some preliminary results on 3-μm materials that are considerably more unstable, involving a non-reproducible peak sharpening effect with apparent efficiencies up to 4 million plates per column.

4. Concluding remarks

Our SCX particles exhibited a stable peak appearance in the electrochromatograms. The efficiency was comparable to that on reversed-phase materials, even better considering the influence of the particle

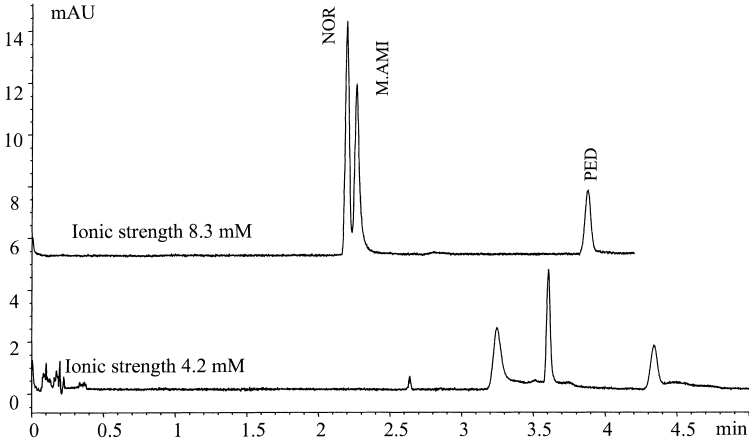


Fig. 8. Comparison of electrochromatograms. The lower electrochromatogram shows a stable peak compression of the M.AMI peak on a XZ800 column when using a mobile phase with the lowest ionic strength (4.2 m*M*), while in the upper electrochromatogram both analyte peaks have a similar appearance when the ionic strength was higher (8.3 m*M*). Other conditions as in Fig. 7.

diameter. The electrochromatographic data correlated better to pore size than to the denoted specific area. The two wide-pore materials (XN500 and XZ800) promoted pore flow, but the pore flow had no positive influence on the electrochromatographic performance due to too low capacity in combination with the larger influence of electrophoretic part of the separation, where the elution order is reversed compared to CEC. The electroosmotic flow was comparatively high and relatively independent of pH as can be expected when sulfonic acid ligands provide the negative charges on the particles. The two materials with small pores (XZ80 and XN100) provided the best separation.

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