



ELSEVIER

Journal of Chromatography A, 853 (1999) 45–54

JOURNAL OF
CHROMATOGRAPHY A

Capillary electrochromatography with macroporous particles

Remco Stol, Wim Th. Kok*, Hans Poppe

*Laboratory for Analytical Chemistry, Department of Chemical Engineering, University of Amsterdam, Nieuwe Achtergracht 166,
1018 WV Amsterdam, The Netherlands*

Abstract

The performance of macro-porous particles in capillary electrochromatography is studied. Three reversed-phase stationary phases with pore diameters between 500 Å and 4000 Å have been tested for separation efficiency and mobile phase velocity. With these stationary phases, a large portion of the total flow appears to be through the pores of particles, thereby increasing the separation efficiency through a further decrease of the flow inhomogeneity and through enhancement of the mass transfer kinetics.

The effects of pore size and mobile phase composition on the plate height and mobile phase velocity have been studied. With increasing buffer concentrations and larger pore diameters, higher mobile phase velocities and higher separation efficiencies have been obtained.

Columns packed with 7 µm particles containing pores with a diameter of 4000 Å generated up to 430 000 theoretical plates/m for retained compounds. Reduced plate heights as low as 0.34 have been observed, clearly demonstrating that a significant portion of the flow is through the pores. For the particles containing 4000 Å pores no minimum was observed in the H-u plot up to linear velocities of 3.3 mm/s, suggesting that the separation efficiency is dominated by axial diffusion. On relatively long (72 cm) columns, efficiencies of up to 230 000 theoretical plates/column have been obtained under non-optimal running conditions. On short (8.3 cm) columns fast separations could be performed with approximately 15 000 theoretical plates generated in less than 30 s. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Electrochromatography; Stationary phases, LC; Stationary phases, electrochromatography; Polynuclear aromatic hydrocarbons

1. Introduction

It is well known that the separation efficiency in capillary electrochromatography (CEC) can be substantially higher than in pressure-driven liquid chromatography (HPLC) [1]. In CEC the flow is driven by electroosmosis (EOF) and a nearly flat flow

profile is expected [2], resulting in strong decrease of the flow non-uniformity, with a corresponding lower contribution to the total plate height. Furthermore, since the EOF is in first approximation independent of the particle size, sub-micron sized particles may be used, ultimately leading to a separation technique with an separation efficiency solely limited by axial diffusion [3].

The tendency towards the use of sub-micron sized particles in CEC [4] is based on the van Deemter et al. [5] and the Kennedy and Knox [6] models for the

*Corresponding author. Tel.: +31-20-525-6539; fax: +31-20-525-6638.

E-mail address: wkok@its.chem.uva.nl (W.Th. Kok)

dispersion in a chromatographic column. In these models it is assumed that the liquid within the pores of the stationary phase is stagnant and that diffusion is the only form of mass transfer within the particles.

An alternative to the use of small particulate stationary phases in CEC might be the utilization of pore flow to enhance the separation efficiency. The effect of pore flow on mass flux kinetics in pressure driven liquid chromatography is well understood [7–9]. It enhances the mass transfer rates within the particles, resulting in a decrease of the theoretical plate height.

Based on the model devised by Rice and Whitehead [2] it has been assumed that in CEC complete double layer overlap occurs within the pores of the particles, so that pore flow in CEC would be strongly diminished and virtually absent. Recently, however, it has been suggested that pore flow is enhancing the separation efficiency in CEC [10] even with standard reversed-phase packing materials. Furthermore, during a study on electrically driven size-exclusion chromatography (ED-SEC) in our laboratory, it was found that under appropriate conditions the electroosmotic flow within the pores of the stationary phase particles can be significant [11]. In this study, pore flow was observed with bare silica stationary phases with pore sizes down to 300 Å. This was in contrast to the work of Li and Remcho [12], who observed pore flow only with stationary phases containing pores with diameters of 2000 Å and larger and only while using excessively high-ionic strength buffers. In ED-SEC the pore flow adversely affects the separation selectivity, while in CEC it might be an advantageous phenomenon to increase the separation efficiency.

In capillary electrochromatography the intra-particle mobile phase mobility is expected to be a function of double layer overlap within the pores and the structural geometry of the pore channel. When there is no significant double layer overlap within the pores of the stationary phase, the intra-particle linear flow velocity is the same as that in the inter-particle space. Under such conditions it can be expected that the mass transfer resistance contribution from the stationary phase to the total theoretical plate height (H) is strongly reduced. Also a further reduction of chromatographic dispersion due to flow non-uniformity can than be expected.

This report discusses the utilization of macroporous particles to increase the separation efficiency in CEC, as opposed to a reduction of the particle size. The stationary phases employed in this study have a particle size of 7 µm and differ in the pore diameter, which ranges from 500 Å to 4000 Å. The relations between the pore diameter, the ionic strength, the linear flow velocity and the efficiency are studied.

2. Experimental

2.1. Apparatus

All experiments were performed on a HP^{3D}CE system (Hewlett-Packard, Waldbronn, Germany), equipped with a diode array detector operated at 254 nm. The system was controlled using the standard Hewlett-Packard Chemstation software. This software was also used to calculate the separation efficiencies for the individual peaks in the chromatograms.

Samples were injected electrokinetically at 5 kV for 3 s. The column was thermostatted at 25°C throughout all the experiments. A helium gas bottle was connected to the CEC system and served as the external pressure source. A pressure of 10 bar was applied at both ends of the column during CEC operation.

The mercury porosity measurements were performed on a model Pascal 440 (CE instruments, Milan, Italy) according to the standard method DIN 66133.

2.2. Chemicals

The stationary phases employed in this study, Nucleosil 4000-7 C₁₈, Nucleosil 1000-7 C₁₈ and Nucleosil 500-7 C₁₈, were obtained from Machery–Nagel (Düren, Germany). HPLC grade acetonitrile was obtained from Acros Organics (Geel, Belgium). Other chemicals came from different standard suppliers. A stock solution of 1.0 mol l⁻¹ boric acid was prepared in sub-boiled water and adjusted to pH 8.3 with sodium hydroxide (Janssen Chimica, Beerse, Belgium). The mobile phase contained 80% (v/v) acetonitrile, except when specified otherwise in the

text. The mobile phases were prepared by mixing appropriate volumes of the boric acid stock solution, sub-boiled water and acetonitrile.

Stock solutions of polycyclic aromatic hydrocarbons (PAHs) were prepared in acetonitrile. Fresh sample solutions were prepared daily in acetonitrile–water (50:50, v/v) and were diluted to approximately 75 mg l^{-1} . All buffer solutions were filtered over a $0.45 \text{ }\mu\text{m}$ HVLP Durapore filter (Millipore, Etten Leur, Netherlands) and degassed by sonification prior to use.

2.3. Column preparation

Untreated fused-silica capillaries ($100 \text{ }\mu\text{m}$ inner diameter \times $375 \text{ }\mu\text{m}$ outer diameter, Polymicro Technologies, Phoenix, AZ, USA) were packed by a modified slurry packing technique. A temporary frit was made at one end of a piece of fused-silica capillary by tapping the capillary end into a pile of dry $5 \text{ }\mu\text{m}$ bare silica particles. The bare silica particles were sintered by heating with a small gas flame. A slurry (25 mg/ml in acetonitrile–water (80:20, v/v) containing 10 mmol l^{-1} borate buffer of pH 8.3) of the respective stationary phase was made in a small glass bottle which could be pressurized externally. The slurry was stirred slowly using a continuous magnetic stirrer. The open end of the capillary was placed at the bottom of the vial and the capillary was filled with the slurry using an air pressure of approximately 7 bar, which was delivered by an external air pump.

Next, the column was connected to a high pressure membrane pump and flushed with water to complete the packing procedure. The pressure was increased gradually in 10 min to a maximum of 250 bar. The water pressure was maintained at this level for at least 1 h before the pressure was allowed to decrease slowly to 120 bar. It was found that purging the column at a higher pressure than 250 bar resulted in a strong reduction of the separation efficiency when the column was operated in CEC mode. This was probably due to the collapse of the pores within the particles at these higher pressures.

At 120 bar water pressure the permanent in- and outlet frits were sintered from the packed bed by heating locally at 550°C for 20 s using a hot filament device. Subsequently, the pressure was released

slowly. The column was reconnected to the high pressure pump and the remainder of the particles was flushed out with water at a pressure of 100 bar from the inlet frit. A detection window was made by burning off the polyimide coating immediately adjacent to the outlet frit, using the same hot filament device also used to prepare the permanent in- and outlet frits.

The column was then flushed with the mobile phase for 15 min at 90 bar with an HPLC pump. The columns were preconditioned for CEC by applying an increasing voltage, up to 20 kV. During electrokinetic preconditioning of the column, voids appeared at the outlet frit. Probably this was due to resettling of the particles, leading to a more dense packing structure. Subsequently, all columns were operated in CEC mode for 1 h at 25 kV and a new, second outlet frit and detection window were prepared following the same method as described above.

The columns prepared according to this procedure were stable and over 200 injections could be performed on each column.

3. Results and discussion

3.1. Effect of pore diameter on the theoretical plate height

The pore diameters of the particles were determined by mercury porosimetry. As can be seen in Table 1, there is a large discrepancy between the nominal pore diameters of the particles and the actual measured pore diameters of the particles. However, the particles with a higher nominal pore diameter have a larger actual pore diameter.

Several columns were prepared from each of the reversed-phased particles. Their chromatographic behavior in CEC was studied with the separation of four PAHs and acetone, which was used as the electroosmotic flow (EOF) marker. Each separation was repeated five times on three different columns.

The effect of pore size on the separation efficiency was studied by evaluation of the H-u curves for each of the materials used. The electric field strength applied over the columns was varied between 5 and 30 kV in steps of 2.5 kV, resulting in linear velocities

Table 1

The nominal and the measured pore diameters from the particles as determined by mercury porosimetry

Nominal pore diameter (nm)	Pore diameter at 10% cumulative volume (nm)	Pore diameter at 50% cumulative volume (nm)	Pore diameter at 90% cumulative volume (nm)
50	15	75	160
100	75	160	450
400	110	240	610

between 0.5 and 3.3 mm/s on columns of 25 cm length. A plot of the theoretical plate height (H) of the fluorene peak against the linear velocity (u) of the mobile phase for the different materials is given in Fig. 1. The lowest plate height (2.35 μm) was obtained using the stationary phase with the 4000 \AA pores. Note that this plate height, obtained with 7 μm particles, is only 0.4 μm larger than those reported for columns prepared from 1.5 μm non-porous particles [13].

At linear velocities higher than 0.5 mm/s all materials generate reduced plate heights below unity and the separation efficiencies are increasing even further when higher electric field strengths are applied. For the stationary phase with the 4000 \AA

pores and a particle diameter of 7 μm a reduced plate height (h) as small as 0.34 is found. It is highly unlikely that the flat profile of the flow between the particles can account for this effect. Apparently, the flow through the particles is very significant and the stationary phase is more or less acting as if it is composed of several small particles.

The mass transfer is significantly enhanced and probably also the flow inhomogeneity across the packed bed is further reduced. Clearly, the use of reduced plate height as a measure of column quality or separation efficiency with these types of stationary phases bears little physical meaning in CEC.

With the 500 \AA and the 1000 \AA porous materials, a minimum in the H - u curves occurs at approximate-

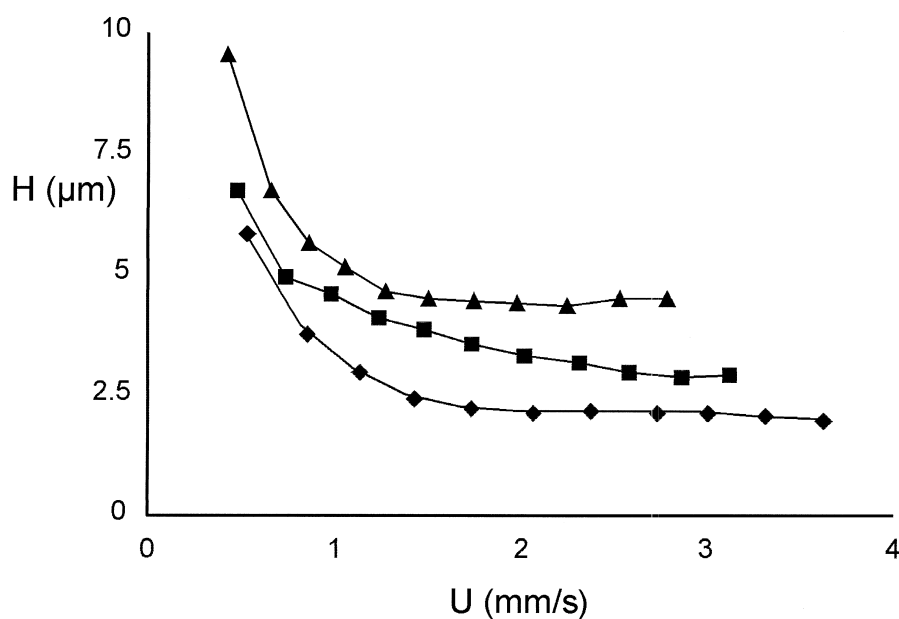


Fig. 1. The effect of pore size of the three different stationary phases on the theoretical plate height (H) of the fluorene peak against linear velocities (u) of the mobile phase. Columns were approximately 33 cm long (25 cm effective). Mobile phase: acetonitrile–water (80:20, v/v) containing 10 mmol l^{-1} borate, pH 8.3. (▲)=500 \AA , (■)=1000 \AA , (◆)=4000 \AA .

ly 2.5 and 3.0 mm/s respectively, indicating that with these packing materials at high linear velocities mass transfer resistance starts to dominate the total theoretical plate height. However, for the material with the 4000 Å pores, the plate height versus linear velocity curve is still decreasing even when the highest electric field strength is applied. This suggests that mass transfer resistance still gives a minor contribution to the total plate height, and that the separation efficiency is diffusion controlled. Due to the power limitations of our CEC system, it was not possible to apply higher electric field strengths to study the material at higher linear flow velocities.

The ability to preserve efficiency at high linear flow makes it possible to perform fast separations. A typical separation of some PAHs on the stationary phase with 4000 Å is shown in Fig. 2. Baseline resolution of these model compounds on a column of 23 cm length is obtained using a separation buffer consisting of an acetonitrile–water (80:20, v/v) mixture, containing 10 mmol l⁻¹, pH 8.3 borate. Due to the high linear velocity of 3.3 mm/s, complete separation is performed in less than 90 s, while the separation efficiency is 430 000 plates/m.

3.2. Effect of ionic strength on the theoretical plate height

The separation efficiency was also studied using mobile phases with different ionic strengths. It is expected that the use of lower ionic strength buffers will cause an increase in double layer overlap within the pores and a decrease of the separation efficiency. In Fig. 3, the effect of the buffer concentration on the separation efficiency is shown for the 4000 Å porous material. The H-u curves were determined for three different buffer concentrations using the same column.

When the column was operated using a mobile phase containing 1.0 mmol l⁻¹, pH 8.3 borate buffer, the efficiency for the fluorene peak decreased with approximately 20% as compared to the separation efficiency obtained using the mobile phase containing 10 mmol l⁻¹, pH 8.3 borate buffer. As a result of the lower ionic strength, the double layer thickness is increased, and double layer overlap within the pores is more pronounced so that intra-particle flow is reduced. When the buffer concentration is further decreased to 0.1 mmol l⁻¹, pH 8.3 borate, the effect

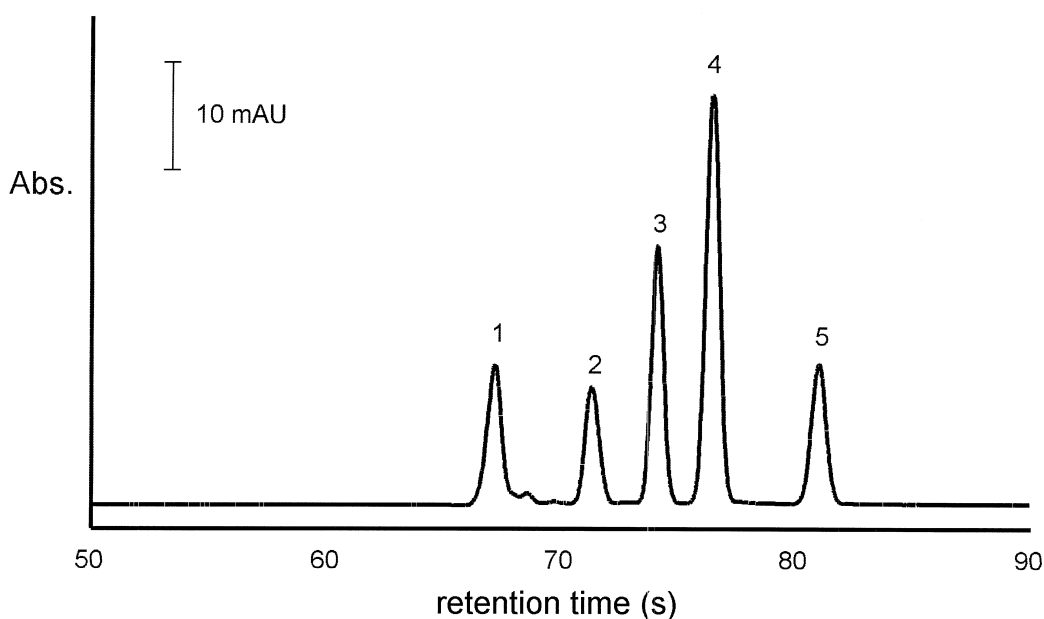


Fig. 2. The separation of four PAHs. Column: 31 cm (23 cm effective) nucleosil 4000-7 C₁₈. Mobile phase: acetonitrile–water (80:20, v/v) containing 10 mmol l⁻¹ borate, pH 8.3. Applied voltage=30 kV. Peaks: 1=acetone; 2=naphtalene; 3=fluorene; 4=anthracene and 5=pyrene.

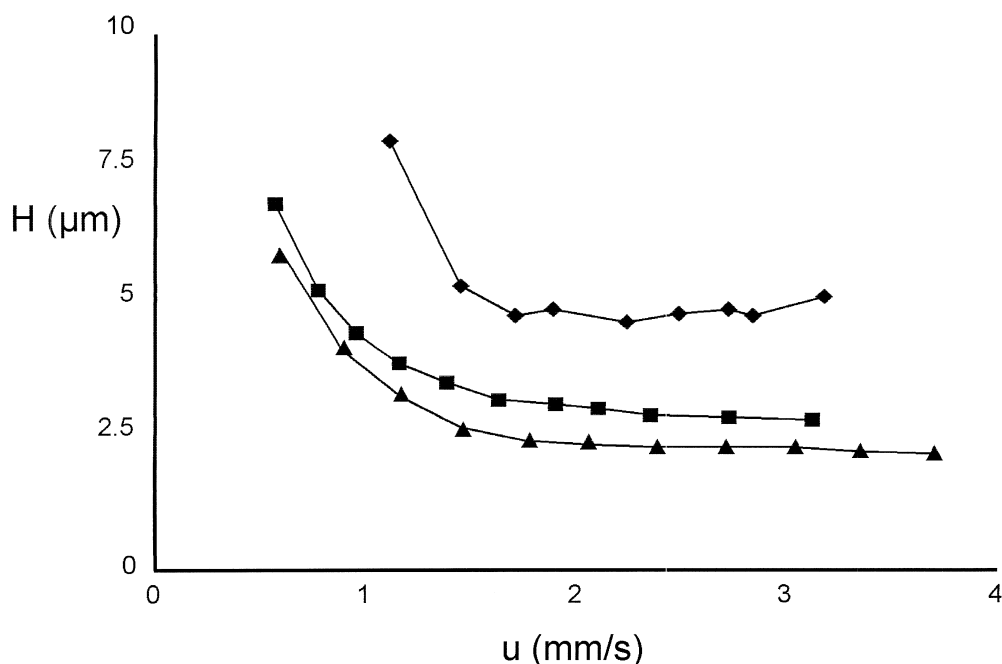


Fig. 3. The effect of the buffer concentration on the separation efficiency on Nucleosil 4000-7 C₁₈. Mobile phase: acetonitrile–water (80:20, v/v) and (◆)=0.1 mmol l⁻¹ borate, pH 8.3; (■)=1.0 mmol l⁻¹; (▲)=10 mmol l⁻¹.

of the double layer overlap becomes even more noticeable and the efficiency deteriorates further. However, even under these unfavorable conditions where significant double layer overlap within the pores is expected, reduced plate heights are still below unity. Thus under non-optimal conditions still a large fraction of the total flow is probably through the pores of the stationary phase.

Several other authors also found an increased separation efficiency when the ionic strength of the mobile phase was increased [9,14,15], while using stationary phases containing pores of much smaller pore diameters. Possibly, this is also an effect of pore flow caused then only through enhancement of the mass transfer, although it has been assumed previously that complete double layer overlap occurs in channels of such small diameter.

3.3. Effect of retention on efficiency

The effect of the mobile phase composition on the separation efficiency was studied by varying the acetonitrile content between 50 and 80% (v/v). This

resulted in retention factors for fluorene between 0.1 and 3. The influence of retention on efficiency can be seen in Fig. 4. As can be seen the plate height strongly increases with retention. This is typical for a mobile phase mass transfer contribution. This term in the van Deemter equation strongly increases with the retention factor k' in the range 1–10. Obviously, pore flow only decreases the stationary phase mass transfer contribution to peak broadening in addition to a reduction of the flow inhomogeneity. The stationary phase mass transfer term in the Van Deemter equation is most prominent for k' values around 1. Still, even at high retention the reduced plate heights are below 2. It may be expected that only a relatively moderate reduction of the particle size (e.g. to 3 μm) is enough to obtain high efficiencies also at higher retention factors.

3.4. Effect of pore diameter on the linear flow velocity

The effect of the pore diameter on the linear flow velocity can be seen in Fig. 1. The linear mobile

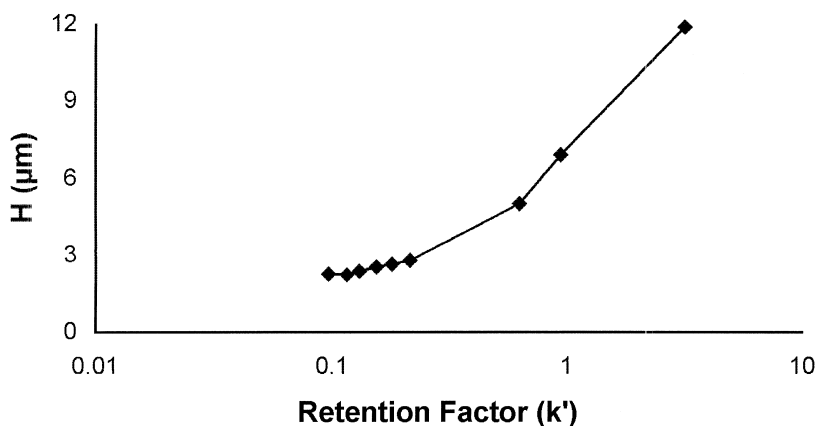


Fig. 4. Effect of the retention factor on the separation efficiency of fluorene. Column: 32 cm (24 cm effective) nucleosil 4000-7 C_{18} . Applied voltage=30 kV. The mobile phase organic modifier content was varied between 50 and 80%. The buffer concentration was kept constant at 10 mmol l^{-1} , pH 8.3 borate buffer.

phase velocity that can be obtained on stationary phases with large pore sizes is higher than those obtained on small pore diameters.

Double layer overlap inside pores of larger diameter is less prominent, which explains why the flow velocity through columns prepared from large pore containing stationary phases is higher.

3.5. Effect of ionic strength on the linear flow velocity

The linear velocity of the mobile phase through the column is also affected by the ionic strength of the separation buffer. In capillary electrochromatography, it is normally assumed that the use of separation buffers with low ionic strength results in a high linear velocity, because of the effect on the zeta potential. However, since here a large portion of the total flow may be through the pores, the use of high ionic strength buffers may result in an increase in pore flow and thus higher linear velocities. Both effects can be seen in Fig. 5. When increasing the buffer concentration from 0.1 to 1.0 mmol l^{-1} borate, pH 8.3, the linear velocity of the mobile phase through the CEC column is reduced by approximately 10%. The reduction of the EOF complies with current CEC understanding. However, a further increase in the buffer concentration to 10 mmol l^{-1} , pH 8.3 borate buffer, results in a higher linear velocity through the column. This effect is

explained by assuming that a large fraction of the total flow is through the pores of the stationary phase.

3.6. High-performance capillary electrochromatography

The ease of use and the attractive properties of wide-porous stationary phases in CEC is demonstrated by the ultra-fast separation of the PAHs on a short, 8.3 cm effective length column, packed with Nucleosil 4000-7 C_{18} (Fig. 6). The separation buffer was an acetonitrile–water (70:30, v/v) mixture, containing 10 mmol l^{-1} , pH 8.3 borate buffer and a voltage of 30 kV was applied. A separation efficiency of 15 000 theoretical plates was obtained in less than 30 s. With these short separation times the data sampling rate (maximum 20 Hz) of our CEC system was too slow to accurately determine the exact peak width, since only 5–6 data points are recorded for each peak. The reduced plate heights obtained on these short columns were higher than those obtained on the 25 cm effective length columns. Probably this is due to the strong influence of the in- and outlet frits on the observed theoretical plate height and by the slow data sampling rate.

It is relatively easy to prepare long capillary columns from these wide pore stationary phases of relatively large particle size. As an example a column of 72 cm in length was prepared from the

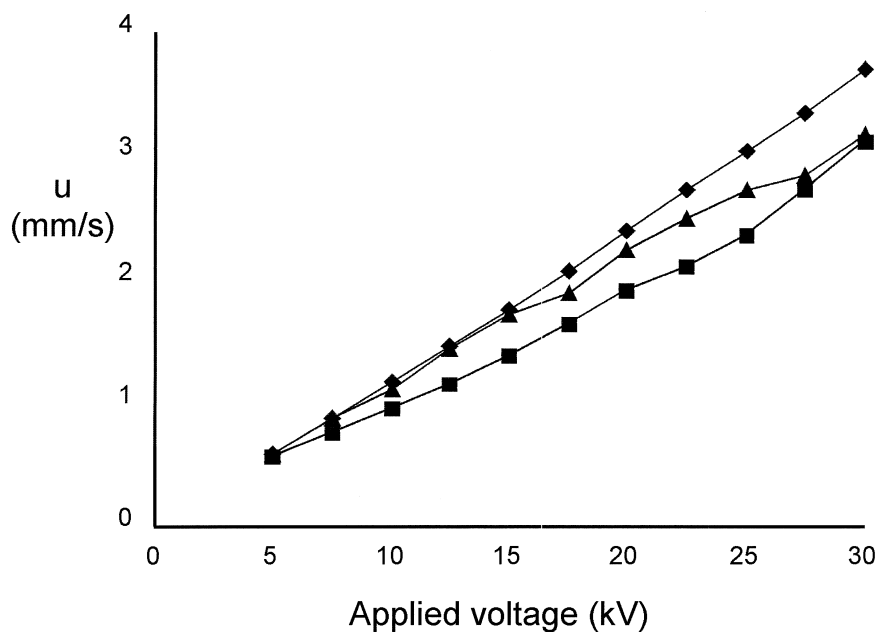


Fig. 5. The effect of buffer concentration on the linear velocities (u) of the mobile phase. The linear velocity was calculated using acetone as the EOF marker. Column: 31 cm (23 cm effective) Nucleosil 4000-7 C_{18} . Mobile phase: acetonitrile–water (80:20, v/v), containing (◆)=0.1 mmol l^{-1} , (■)=1.0 mmol l^{-1} , (▲)=10 mmol l^{-1} borate buffer, pH 8.3.

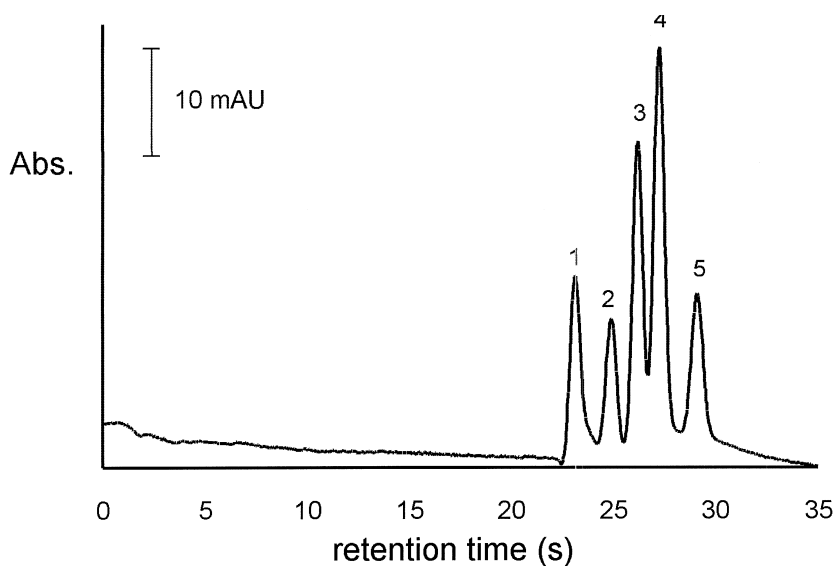


Fig. 6. Fast separation of PAHs. Column: 8.3 cm packed with Nucleosil 4000-7 C_{18} . Mobile phase: acetonitrile–water (70:30, v/v%) containing 10 mmol l^{-1} , pH 8.3 borate buffer. Applied voltage=30 kV. $N=15\,000$. Peaks: 1=acetone, 2=naphthalene, 3=fluorene, 4=anthracene and 5=pyrene.

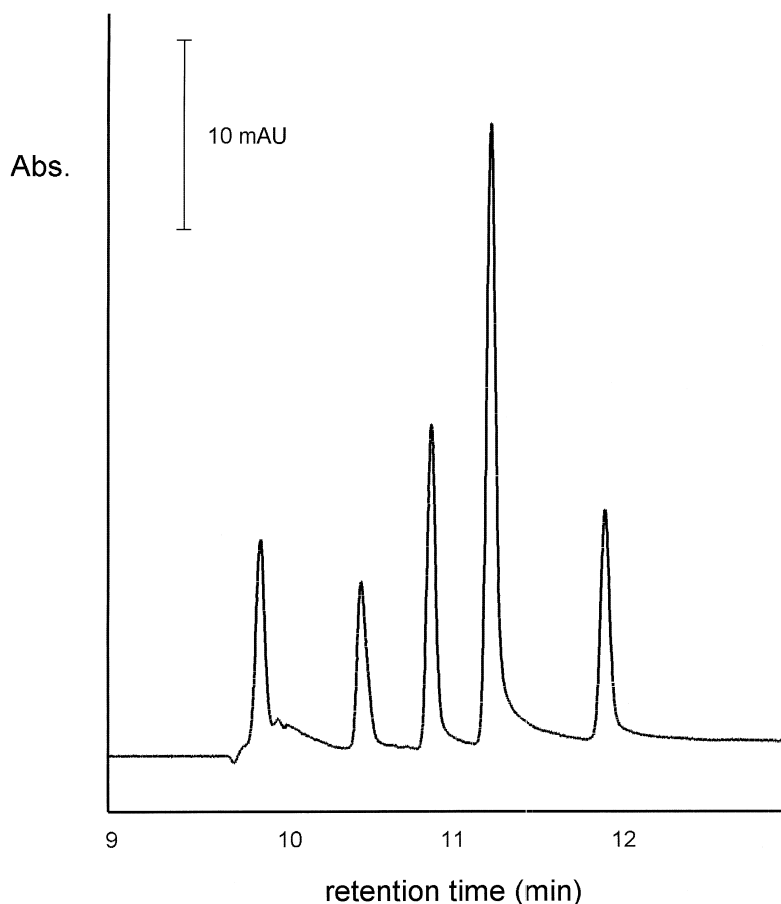


Fig. 7. High efficiency separation of PAHs. Up to 230 000 theoretical plates are generated on a column of 72 cm length, packed with Nucleosil 4000-7 C₁₈. Mobile phase: acetonitrile–water (80:20, v/v%) containing 10 mmol l⁻¹, pH 8.3 borate buffer. Applied voltage=30 kV. N=230 000. Peaks: 1=acetone, 2=naphtalene, 3=fluorene, 4=anthracene and 5=pyrene.

material containing 4000 Å pores. On this column high efficiencies were generated for the four PAHs (Fig. 7). Due to the upper power limitations of our CEC the maximum linear velocity that could be obtained was only 1.2 mm/s and separation took about 13 min. On this column 230 000 theoretical plates could be obtained. The reduced plate heights for fluorene on this column compared very well with those obtained on the 25 cm effective length columns and the use of higher field strengths will likely result in a strong increase of the separation efficiency.

4. Conclusion

It is demonstrated that in CEC, using columns

packed with wide-pore stationary phases, the flow through the pores is substantial. The pore flow appears to enhance the mass transfer rates in the stationary phase so that the observed theoretical plate heights are strongly reduced. Since the flow velocity through the particles is very high, it also may be expected that the flow across the column cross section is much more homogeneous, which will also result in a reduction of the observed plate height.

For fluorene theoretical plate heights of 2.35 μm are reported, corresponding to reduced plate heights as low as 0.34. This indicates that the use of reduced plate heights as a measure of column quality in CEC on wide-pore stationary phases can be questioned.

At high retention, the mass transfer resistance in the mobile phase dominates the separation efficiency.

To allow for efficient separation of compounds with high retention, the particle size should be decreased. However, the required reduction in particle diameter appears to be less than expected for stationary phase materials which do not support pore flow. The production of columns with relatively large particles is much easier than the production of columns from sub-micron particles [4] and therefore, to obtain high separation efficiencies, the use of wide-pore stationary phases in CEC might be a valuable alternative to the use of sub-micron sized particles.

Acknowledgements

The authors would like to thank M.C. Mittelmeier-Hazeleger from the University of Amsterdam for performing the mercury porosimetry measurements. The investigations were financially supported by the Netherlands Organization for Scientific Research (NOW) under grant 79.030.

References

- [1] J.H. Knox, *Chromatographia* 24 (1987) 135.
- [2] C.L. Rice, R. Whitehead, *J. Phys. Chem.* 69 (1965) 4017.
- [3] J.H. Knox, I.H. Grant, *Chromatographia* 23 (1991) 317.
- [4] S. Lüdtke, T. Adam, K.K. Ungr, *J. Chromatogr. A* 786 (1997) 229.
- [5] J.J. van Deemter, F.J. Zuiderweg, A. Klinkenberg, *Chem. Eng. Sci.* 6 (1956) 271.
- [6] G.J. Kennedy, J.H. Knox, *J. Chromatogr. Sci.* 10 (1972) 549.
- [7] M.E. van Kreveld, N. van den Hoed, *J. Chromatogr.* 149 (1978) 71.
- [8] A.E. Rodrigues, J.C. Lopes, Z.P. Lu, J.M. Loureiro, M.M. Dias, *J. Chromatogr.* 590 (1992) 93.
- [9] J.C. Giddings, *Dynamics of Chromatography, Part 1: Principles and Theory*, Marcel Dekker, New York, 1965.
- [10] R.M. Seifar, J.C. Kraak, W.Th. Kok, H. Poppe, *J. Chromatogr. A* 808 (1998) 71.
- [11] E. Venema, J.C. Kraak, H. Poppe, R. Tijssen, *Chromatographia*, in press.
- [12] D. Li, V.T. Remcho, *J. Microcol. Sep.* 9 (1997) 389.
- [13] R.M. Seifar, W.Th. Kok, J.C. Kraak, H. Poppe, *Chromatographia* 146 (1997) 131.
- [14] S.E. Van den Bosch, S. Heemstra, J.C. Kraak, H. Poppe, *J. Chromatogr. A* 755 (1996) 165.
- [15] Q. Wan, *J. Chromatogr. A* 782 (1997) 181.