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Capillary Electrochromatography with 1.5 µm ODS-Modified Non-Porous Silica Spheres

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Key Words

Capillary electrochromatography
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1.5 µm non-porous silica spheres
Sodium dodecylsulphate
Polycyclic aromatic hydrocarbons

Summary

The particle realization of electrochromatography on capillaries packed with 1.5 µm ODS-modified non-porous silica spheres is demonstrated. In order to realize stable separation conditions it is crucial to add sodium dodecylsulphate (SDS) to the mobile phase and to pressurize both buffer vials at 10 bar. The presence of SDS stabilizes the current and makes the electro-osmotic flow in the packing more uniform so that no air bubbles are generated at high field strengths. The capillary columns are extremely efficient and on a 24 cm long column about 120,000 plates can be generated (a reduced plate height of about 1.3). The columns are very stable and no loss in efficiency was found after using a column continuously for two months.

Introduction

The theory of chromatography predicts higher efficiencies and separation speeds when further decreasing the particle size below the commonly used 3–5 µm [1, 2]. However, in practice the application of smaller particles can not be fully exploited due to the pressure limitation; as a result only very short columns can be used [3]. In order to be able to utilize longer columns with sub-micron particles another way to propel the mobile phase is needed. An attractive alternative is to drive the mobile phase by means of electroendosmosis [4, 5]. As the electroosmotic flow profile is flat the band dispersion by convective mixing will be considerably smaller than in pressure driven systems, where the flow profile is parabolic. Further the electroosmotic flow depends only on the nature of the surface of the particles and is thus independent of the size of the channels. This means that in electrochromatography in principle reasonable flow rates can be generated on capillary columns length packed with sub-micron particles.

In the last years the practical problems encountered with capillary electrochromatography (CEC), e.g., bubble formation and difficulties with the preparation of the terminal frits, have been solved satisfactorily [6–10] and the applicability of the technique with 3–5 µm particles has been demonstrated now frequently [7, 16]. So far only a few number of papers have been appeared dealing with smaller particles. Knox and Grant [6] and Yamamoto et.al [17] demonstrated CEC with non-porous silica particles of 1.6 µm and Smith and Evans [7] used 1.8 µm porous Zorbax particles.

In this paper we report the results of an investigation to apply electrochromatography on capillaries packed with 1.5 µm ODS modified non-porous silica particles. Attention will be given to the preparation of the column, the effect of the addition of SDS on the performance and the effect of the composition of the mobile phase on the efficiency. Illustrative chromatograms will be shown.

Experimental

Apparatus

The experiments were performed with a HP^{3d} CE system (Hewlett Packard Waldbronn, Germany), equipped with an option to pressurize equally the buffer vials. The column was air thermostated at 25 °C. Samples were introduced electrokinetically at the anodic side. The 50 and 100 µm i.d., 375 µm o.d. fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA) and had a total length of 32 cm of which 24 cm was filled with the packing. An HPLC pump (Spectroflow, Kratos, Rotterdam, The Netherlands) adapted with a home made split device was used to flush the freshly packed capillaries at 150 bar. A high pressure

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(maximum 900 bar) membrane pump (Burdosa V140 Germany) was used to pack the capillaries.

Materials

The reversed phase particles were Chromspher-ODS 1.5 μm and were kindly donated by Chrompack (Middelburg, The Netherlands). Acetonitrile, acetone, isopropanol and hexane were purchased from ACROS (Geel, Belgium). Sodium tetraborate was obtained from BDH (Poole, England) and SDS from Sigma (Zwijndrecht, The Netherlands). The solutes were obtained from different commercial sources. Doubly distilled water was used in all experiments. All solvents and buffer solutions were filtered over a 0.45 μm HVLP Durapore filter (Millipore, Etten Leur, Netherlands). Prior to use all solvents were degassed with a stream of helium for 20 minutes.

Procedures

Column Preparation

In a previous paper we have described extensively the method to prepare columns with 5 μm particles for CEC [10]. In that work the frits were fabricated by sintering the packing locally by means of a heating filament [7, 9, 10]. The same technique was applied to prepare the columns with the 1.5 μm particles.

An in-line filter was mounted to the capillary and the other side to the slurry reservoir (a 150 × 1 mm i.d. stainless steel tube). The particles (0.03 g) were slurried in 2 mL of a mixture of hexane and isopropanol (1:1 v/v) and ultrasonificated for one hour to disintegrated aggregates if present. Next the slurry was pumped into the capillary at 600 bar using a mixture of hexane and isopropanole (9:1 v/v) as pressurizing liquid. Under these conditions it takes about four hours to pack a 24 cm column. After the bed had acquired the required length, the pressure was slowly (~1 minute) released. Next, the column was flushed with acetone and water, successively. While flushing the column with water at 600 bar, the terminal frit and the frit at the detection side were sintered by means of a heating filament. The frit at the detection side was fabricated at the end of the packing. The redundant piece of the capillary in front of the terminal frit was removed by cutting. Finally a window was made just after the frit at the detection side by means of the heating filament.

Chromatography

The mobile phase consisted of mixtures of water and acetonitrile containing sodium tetraborate at pH = 9.25 (0.8–1.6 mmol L^{-1}) and sodium dodecylsulphate (1–5 mmol L^{-1}). The effect of the pH of the mobile phase on the electroosmotic flow was measured with different buffers to cover a pH range of 2.5–9.25, i.e,. formate, acetate, phosphate and tetraborate. The pH of the buffer was measured in the aqueous phase before mixing with acetonitrile. All solutes were dissolved in the mobile phase. Prior to the start of the measurements the mobile phase was degassed with helium for 20 minutes. Flushing of the column with another mobile phase was performed electroosmotically. After terminating the measurements, both ends of the capillary were placed in water to avoid dissolution of the polyimide coating of the capillary by acetonitrile

Results and Discussion

In the beginning the measurements were performed with water-acetonitrile mixtures containing 0.8–1.6 mmol L^{-1} sodium tetraborate at pH = 9.25. With this mobile phase a stable current was found up to 20 kV, but at higher voltages usually the current dropped steeply to zero. Inspection of the packing revealed that at the detection side of the column the packing had become dry. This phenomenon has been often observed by others and is attributed to local differences of the electroosmotic flow in the packing and frits [6, 8, 10]. Pressurizing the buffer vials is recommended to avoid the bubble formation [6, 7, 13] and this was applied by us (10 bar) but appeared to be ineffective. When reversing the polarity of the voltage, the same drying of the packing was observed but now at the terminal side of the column. This finding makes it likely that the problems occur due to a difference in nature of the surface of the frits and the packing. Most probably, the ODS coating is partly or completely removed when sintering the packing. Because of this, the electroosmotic flow in the frits could be larger than in the part with coated particles and this may cause the problems. Another cause for the drying may be the extremely poor wettability of this particular packing. The 1.5 μm ODS-Chromspher particles appear to be very hydrophobic and are only wetted well with non-polar solvents.

The addition of the SDS to the mobile phase was found to be effective for obtaining a more uniform electroosmotic flow in the columns and to decrease the surface tension. It is known that this surfactant adsorbs on reversed phase packing and thus dynamically modifies the surface [18, 19]. The presence of SDS will increase the charge of the surface and affect the electroosmotic flow in the packing. Indeed the addition of SDS appeared to have a favorable effect on the stability of the current and measurements up to 30 kV (a field strength of 1200 $V \cdot cm^{-1}$) could be performed without drying out of the packing when both electrode vials are equally pressurized at 10 bar.

Therefore, in all further experiments SDS was present in the mobile phases. The SDS concentration was kept below the critical micelle concentration (cmc) in water (8 mmol. L^{-1}) [20]. In order to characterize the performance of the columns, the plate height of some test solutes as function of the mobile phase composition was determined and the effect of the SDS on the electroosmotic flow and retention was investigated.

Effect of the SDS on the Retention

SDS has been extensively used in micellar electrokinetic chromatography [21]. In order to establish whether the retention in the packed capillary is due to interactions with the reversed phase surface and not by a micellar mechanism, the migration behavior of some neutral solutes was measured with various mobile phases compositions in an open capillary. From these measurements it appeared that all neutral test solutes migrated with the electroosmotic flow and thus no retention via a micellar mechanism occurs. This could be expected since the SDS concentration was always kept below the cmc.

From the initial measurements at lower voltage the capacity factor of some test solutes could be determined for mobile phase containing only buffer. The capacity factors of the three solutes (naphthalene, phenanthrene, pyrene) with 60% acetonitrile in the absence of SDS were 0.19, 0.43, 0.73 respectively and in the presence of SDS 0.20, 0.43 and 0.71 respectively. This indicates that the presence of SDS hardly influences the capacity factors.

Electroosmotic Flow

Effect of the SDS Concentration

The effect of the SDS concentration on the electroosmotic flow was investigated with 60 % acetonitrile at 20 kV by varying the SDS concentration in the range 1–5 mmol. L^{-1}. The buffer concentration was kept constant (1.6 mmol L^{-1} sodium tetraborate pH = 9.25). Under these selected conditions very stable currents were obtained.

The electroosmotic flow is determined by the zeta potential which is determined by the surface charge on the packing and the ionic strength of the mobile phase. The zeta potential increases with decreasing ionic strength [22] and this will result in a larger electroosmotic flow. However, the opposite was found when SDS was added: the electroosmotic flow increases with increasing SDS concentration. The electroosmotic mobility was calculated from the migration time of an unretained solute (acetone). The electroosmotic mobility, μ_{eo} ($m^2\ V^{-1}s^{-1}$), found with 1, 3 and 5 mmol L^{-1} SDS were 1.5×10^{-8}, 1.8×10^{-8} and 1.9×10^{-8} respectively. This behavior of the electroosmotic flow indicates that the zeta potential (the surface charge) on the packing changes by adsorption of SDS molecules.

Effect of the pH

The effect of the pH on the electroosmotic flow was investigated with different buffers in 60 % acetonitrile and 5 mmol. L^{-1} SDS. The pH was adjusted in the aqueous phase prior to addition of the acetonitrile. The results are reflected in Figure 1. As can be seen, in the pH range 7–9.25 the electroosmotic velocity was nearly constant and decreases to about half the value at pH = 2.5. However, under these conditions still a good electroosmotic flow was generated.

Effect of the Acetonitrile Content

Figure 2 shows the electroosmotic mobility as function of the field strength with three different acetonitrile percentages and constant SDS concentration (5 mmol L^{-1}). The electroosmotic mobility appears to be constant up to 1200 $V \cdot cm^{-1}$. This indicates that no excessive heat was produced in the column. The electroosmotic flow increased with decreasing acetonitrile content. This behavior is opposite to that we previously found with acetonitrile-buffer solutions without SDS [10]. The difference has to be attributed to the modifica-

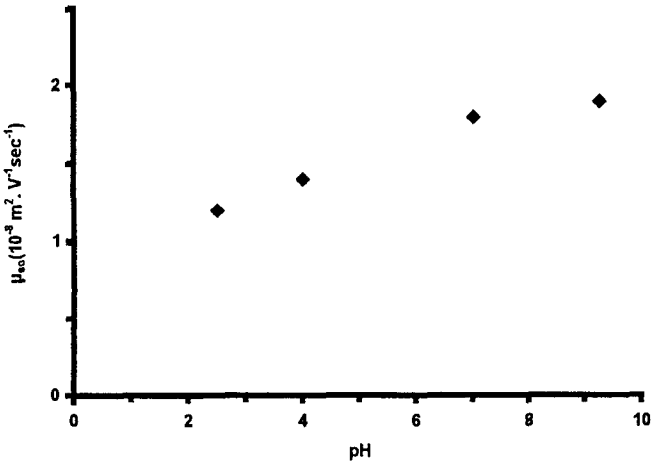

Figure 1
Effect of the pH on the electroosmotic mobility. Column: 32 cm × 100 µm i.d. Bed length: 23.8 cm. Packing: 1.5 µm ODS-Chromspher. Mobile phase: acetonitrile-water (3:2, v/v) + 5 mmol L^{-1} SDS + 1.6 mmol L^{-1} of various buffers. pH 2.5 formate; pH 4.0, acetate; pH 7.0, phosphate; pH 9.25, tetraborate.

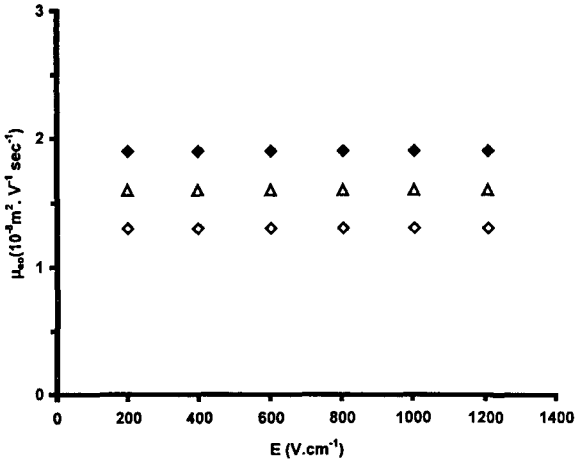

Figure 2
Effect of the acetonitrile content on the electroosmotic mobility. Column: 31.3 cm × 50 µm i.d. Bed length: 23.1 cm. Mobile phase: acetonitrile-water + 5 mmol L^{-1} SDS + 1.6 mmol L^{-1} sodium tetraborate pH 9.25. Acetonitrile: 60 % (◆), 70 % (△), 80 % (◇).

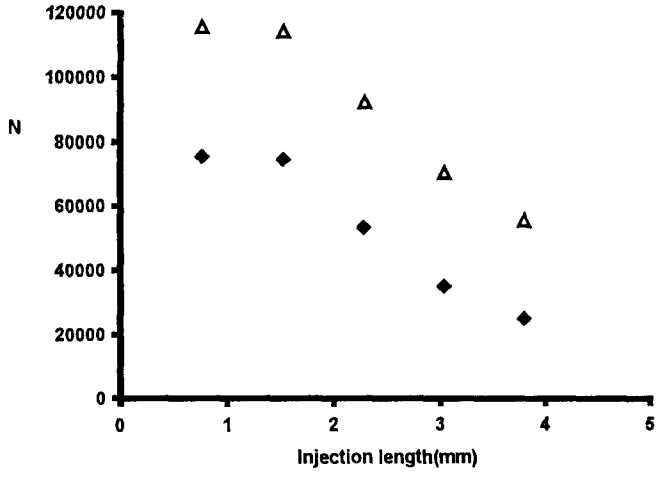

Figure 3
Effect of the injection length on the plate number. Column: as in Figure 1. Mobile phase: acetonitrile-water (3:2, v/v) + 5 mmol L^{-1} SDS + 1.6 mmol L^{-1} sodium tetraborate pH 9.25. Solutes: acetone (◆) and pyrene (△). Run voltage: 30 kV, injection voltage: 10 kV.

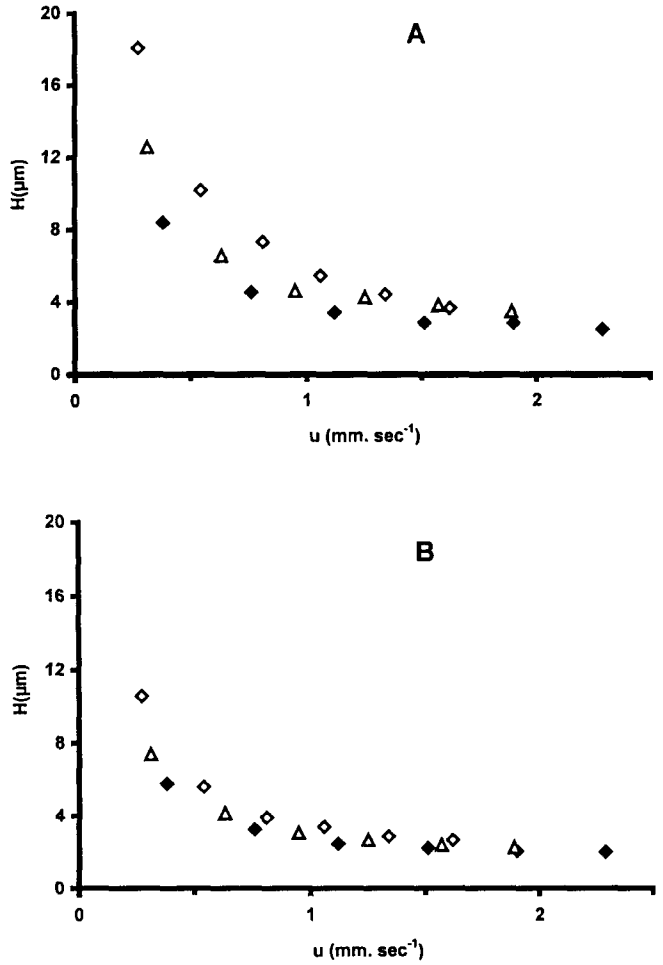

Figure 4
H-u curves for acetone (A) and pyrene (B). Experimental conditions: as in Figure 2. Acetonitrile content: 60 % (◆), 70 % (△), 80 % (◇). Injection: 1 s, 10 kV.

tion of the surface by SDS. The adsorption of SDS increases with decreasing acetonitrile [18, 19] and thus the charge on the surface of the particles increases with decreasing acetonitrile content. As discussed before this will result in higher electroosmotic flows. As can be seen in Figure 2, with 60 % acetonitrile and 5 mmol L^{-1} SDS a linear velocity of about 2.3 mm s^{-1} can be realized, a value comparable to the values commonly used in HPLC.

Column Efficiency

Effect of the Injection Volume

The dispersion in columns filled with 1.5 µm particles will be very small and precautions have to be taken to avoid loss in efficiency by external peak broadening in particular the injection volume may be critical. The effect of extra peak broadening on the efficiency will decrease with increasing retention of the solutes. In order to determine the effect of the injection volume, the plate number of an unretained solute (acetone) and retained solute (pyrene, $k' = 0.71$) at a run voltage of 30 kV was determined as function of the injection length (injection voltage 10 kV). The results are given in Figure 3. As can be seen the injection length has a strong effect on the plate number and has to be below 2 mm to preserve the efficiency.

Effect of the Solute Concentration

In order to assess the loadability of the present columns, the effect of the concentration of a retained solute on the plate number at constant voltage and injection time was investigated. The plate number was constant up to about 10^{-3} mol L^{-1} and then gradually decreased with increasing concentration. The decrease of the plate number was accompanied with an increasing peak-asymmetry. Yamamoto et al. [17] observed about four times smaller plate height for the unretained solute (thiourea) than obtained with retained solutes on 1.6 µm ODS modified non-porous particles. They attributed this dramatic decrease in efficiency to the limited loadability of the packing.

H versus u Plots

The efficiency of the capillary columns was established by constructing plate height versus linear velocity plots. Figure 4 shows a typical H versus u plot of acetone (unretained) and pyrene as found with different acetonitrile contents and constant SDS concentration (5 mmol L^{-1}), using a 50 µm i.d. packed capillary. As can be seen, the plots show the same behavior of the plate height for both compounds. The plate heights decrease steeply with increasing linear velocity and then become more or less constant at higher velocities. At low linear velocities the plate height depends on the acetonitrile content but

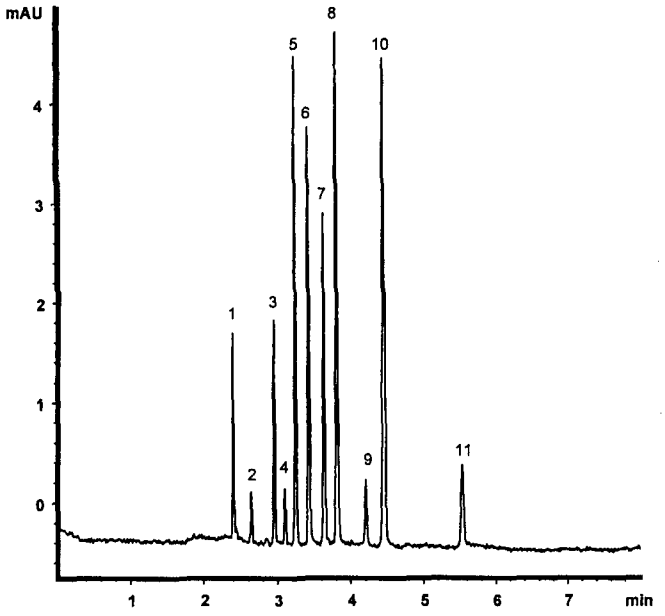

Figure 5
CEC chromatogram of a mixture of polycyclic aromatic hydrocarbons. Experimental conditions: as in Figure 3. Solutes: **1** = acetone; **2** = benzene; **3** = naphthalene; **4** = acenaphthylene; **5** = acenaphthene; **6** = fluorene; **7** = phenanthrene; **8** = anthracene; **9** = fluoranthene; **10** = Pyrene; **11** = Chrysene. Run voltage: 20 kV.

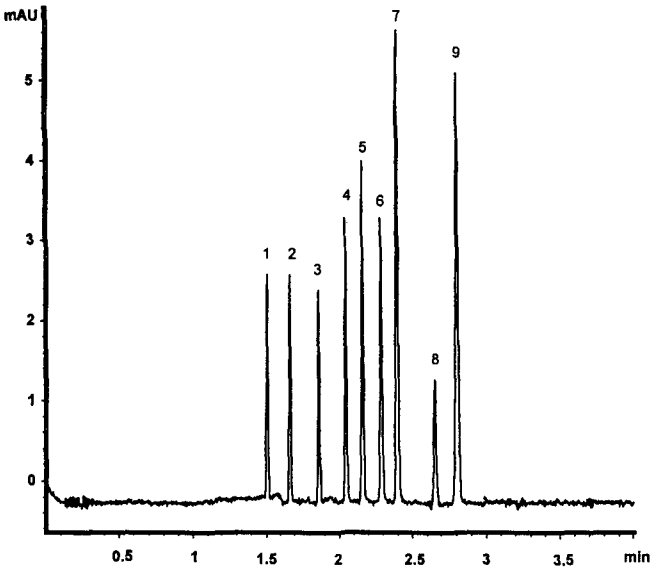

Figure 6
CEC chromatogram of a mixture of alkylbenzenes. Experimental conditions: as in Figure 3. Solutes: **1** = acetone **2** = benzene; **3** = toluene; **4** = ethylbenzene; **5** = propylbenzene; **6** = butylbenzene; **7** = impurity; **8** = pentylbenzene; **9** = hexylbenzene.

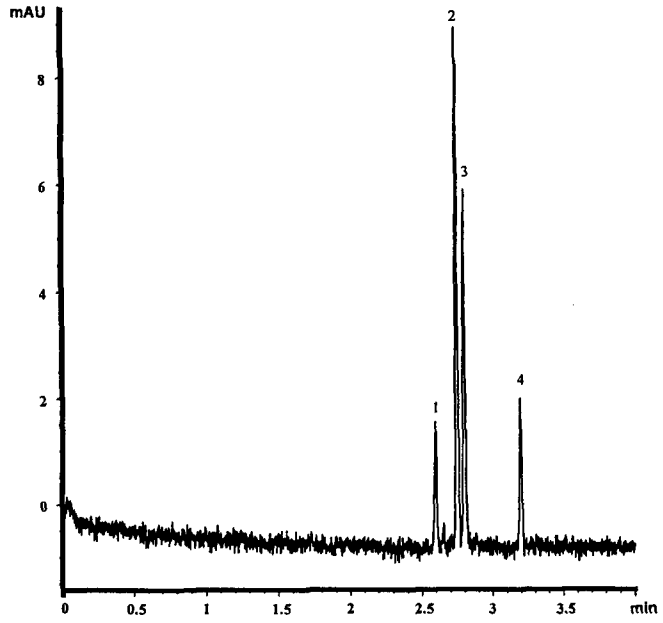

Figure 7
CEC chromatogram of a mixture of steroids. Experimental conditions: as in Figure 3 Run voltage: 20 kV. Solutes: **1** = hydrocortisone; **2** = testosterone; **3** = 17-α-methyltestosterone; **4** = progesterone.

at higher velocities the plots tend to coincide. At low linear velocity the plate height is largely dominated by axial molecular diffusion in the mobile phase. The diffusion coefficient depends on the viscosity of the mobile phase, which becomes smaller with increasing acetonitrile content. This largely explains the difference in plate height when changing the acetonitrile content. With acetone the plate heights are larger than that obtained with pyrene. This difference can be attributed to a difference in diffusion coefficient of these solutes (which is larger for the smaller molecule acetone) and to external peak broadening by the injection and detection. In the last case the effect on the efficiency is the largest for the unretained solute.

It was noticed that the plate heights of the test solutes with 3 mmol L^{-1} SDS were the same as those obtained with 5 mmol L^{-1}, but with 1 mmol L^{-1} SDS slightly larger plate heights (about 5 %) were found.

In order to determine whether the internal diameter of the capillary affects the efficiency, the performance of 100 μm i.d. capillary packed with the 1.5 μm particles was investigated. The efficiency on the 100 μm i.d. capillary appeared to be slightly better than obtained with the 50 μm i.d. capillary column. At a linear velocity of 2.3 mm s^{-1} for pyrene (k' = 0.71) about 120,000 theoretical plates are generated on the 24 cm column (a reduced plate height of about 1.3). It can be noticed that in order to realize such a linear velocity under pressure, about 1900 bar is required. The excellent performance of the capillary packed with 1.5 μm particles is illustrated in Figures 5–7, showing the fast and efficient separation of a mixture of polycyclic aromatic hydrocarbons, alkylbenzenes and steroids respectively. The efficiency with the alkylbenzenes is similar to that found with pyrene (about 120,000 plates) which with the steroids even higher plate numbers (about 150,000) were found.

Conclusions

It has been demonstrated that capillary electrochromatography is feasible with 1.5 μm ODS modified non-porous silica particles when SDS is added to the mobile phase. The SDS adsorbs on the packing and creates a more uniform charge on the surface by which local differences in the electroosmotic flow, responsible for bubble formation in the packing, are aligned. SDS concentrations in the range of 1–5 mmol L^{-1} are sufficient to create a stable system allowing electroosmotic flows up to 2.3 mm s^{-1}. These SDS concentrations are below the critical micelle concentration of SDS (8 mmol L^{-1}).

The capillaries packed with 1.5 μm ODS non-porous particles appeared to be extremely efficient and on a 24 cm long column about 120,000 plates were generated (about 500,000 plates m^{-1}). The columns are quite stable and have been used for more than two months without loss in efficiency.

Future work is now devoted to the application of even smaller porous and non-porous particles down to 0.5 μm. Also attention will be given to the search for other surface modifiers than SDS since surfactants usually prohibit the use of mass spectrometric detection, an attractive feature of CEC.

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