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## Experiences with packed capillary electrochromatography at ambient pressure

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### Abstract

The practical execution of packed capillary electrochromatography is evaluated. The most serious problem is the generation of air bubbles in the system. This occurs especially in frits which were fabricated by glueing particles differing from the packing particles. No air bubbles were generated with frits which were prepared by locally sintering a narrow band of the reversed-phase packing by means of a heating filament, while flushing the column with water. In this way electrochromatography can be performed simply without pressurizing the buffer vials, providing the mobile phase is frequently degassed with helium. The performance under electrically driven conditions appears to be excellent and for 5  $\mu\text{m}$  reversed-phase particles reduced plate heights down to 1.8 were achieved. Compared to pressure driven conditions, 25–40% smaller plate heights were obtained under electrically driven conditions.

*Keywords:* Air bubbles; Electrochromatography; Capillary columns; Packed capillary columns; Packing retainers; Frits; Phenols; Polynuclear aromatic hydrocarbons

### 1. Introduction

Packed capillary electrochromatography is a common capillary liquid chromatographic technique with the difference that the mobile phase is propelled by electroosmosis. By applying a potential gradient across both ends of the capillary, an electroosmotic flow is generated in the electrical double layer present at the liquid–solid interface. Electrically driven systems have some attractive advantages compared to pressure driven systems. The flow has a plug profile and the flow velocity is independent of the channel width, providing there is no electrical double layer overlap. This behaviour predicts less

convective broadening in liquid chromatography and thus higher efficiencies can be achieved compared to pressure driven LC, where the flow profile is parabolic. Moreover, since the flow velocity is independent of the channel width, an acceptable flow-rate can be generated in the interparticle space on columns of common length packed with very small particles. It was Pretorius [1] who first demonstrated in 1974 the application of electroosmosis as an alternative for propelling the mobile phase. Unfortunately, his work got little attention because at that time pressure driven liquid chromatography was being rapidly explored. It was Jorgenson and Lukacs [2] who renewed attention to electrochromatography in 1981, following their important paper about capillary electrophoresis. Some years later the fea-

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tures of capillary electrochromatography (CEC) were extensively discussed by Knox and Grant [3,4]. Since then scientists showed an increasing interest in capillary electrochromatography presumably also because in theory the technique looks very simple [5–15]. However, in practice this appears not to be the case, mainly because of air-bubble formation in the system. In order to suppress bubble formation, Knox and Grant [4] advocated to pressurize the whole system. Recently several authors [10,12,14,15] have succeeded in avoiding bubble formation by pressurizing both buffer vials. Others applied an additional pressure (flow) at the inlet side of the capillary to circumvent bubble formation [7,8,13]. However, both expedients will alter unfavourably the experimental simplicity of the method; a technique whereby electrochromatography can be carried out on a common CE instrument would be highly preferable.

In this paper we present our experiences with packed capillary electrochromatography without pressurization. Ample attention will be given to the role of packing retainer and on the preparation of the

column. The performance of packed capillaries under electrically and pressure driven conditions will be compared.

## 2. Experimental

### 2.1. Set up

The experimental set-up is schematically represented in Fig. 1. It consisted of a high-voltage power supply (Model HCN 35-35000, FUG, Rosenheim, Germany); a laboratory-made injection timer; a UV-detector (Linear Instruments, Reno, USA); a platinum electrode to connect the power supply with the glass buffer or sample vial and a stainless steel buffer reservoir acting as ground electrode. The reservoir has three connection ports: one to mount the capillary, a second to flush the reservoir and a third is permanently connected to a HPLC pump (Spectroflow 400, Kratos, Rotterdam, Netherlands). Between the pump and stainless steel reservoir a split was installed to reduce the flow-rate from ml/min to

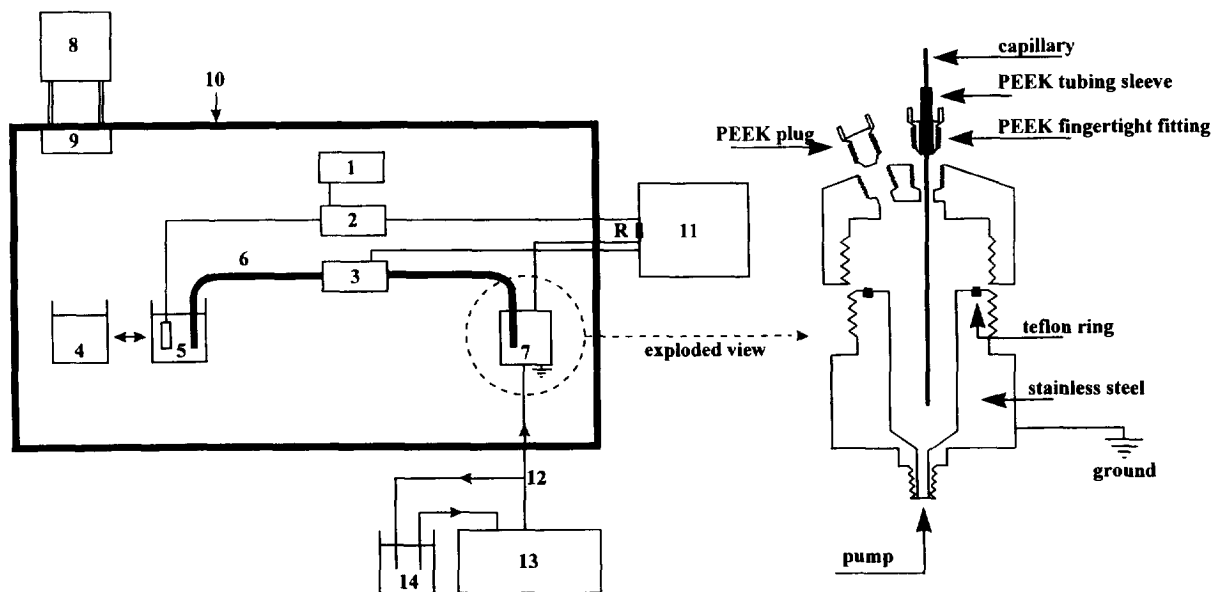


Fig. 1. Schematic representation of the CEC system: 1=timer; 2=high-voltage supply; 3=UV detector; 4=glass sample vial; 5=glass buffer vial; 6=packed capillary; 7=stainless steel buffer vial; 8=water bath; 9=fan; 10=safety box; 11=recorder; 12=split; 13=LC pump; 14=buffer reservoir.

$\mu\text{l}/\text{min}$ . In order to rinse the column at higher pressures (up to 100 bar), the flushing port is closed with a dummy male PEEK connector, and the pump is switched on. After rinsing the column, the pump is switched off, the flushing port is opened in order to release the pressure and the system is ready for electrochromatography. It must be noticed that the reservoir and pump have to be connected to the same ground or the plug of the pump has to be disconnected before switching on of the high voltage.

The fused-silica capillaries were  $100\ \mu\text{m}$  I.D.  $\times$   $375\ \mu\text{m}$  O.D. (Polymicro Technologies, Phoenix, AZ, USA) and had a total length of about 50 cm, of which 20–25 cm was filled with reversed-phase packing. The whole set-up was placed in a Plexiglass safety box; opening of this box automatically shuts off the high voltage. The box can be thermostatted by means of air convection via a fan, incorporated in the wall connected to a thermostated waterbath.

Samples were introduced electrokinetically at the anodic side by applying voltage for several seconds. The current and the detector signal were monitored with a double-pen recorder at the ground end.

## 2.2. Materials and chemicals

The reversed-phase particles were Hypersil-ODS 5  $\mu\text{m}$  (Shandon, Astmoor, UK). Acetonitrile was obtained from ACROS (Geel, Belgium) and water was doubly distilled. Sodium tetraborate was purchased from BDH (Poole, UK). All solvents were filtered over a  $0.45\text{-}\mu\text{m}$  HVLP Durapore filter (Millipore, Etten Leur, Netherlands) and extensively degassed before use with a stream of helium.

## 3. Procedures

### 3.1. Preparation of the column

The preparation of the column is adapted from the method as described by Smith et al. [10] and involves the following steps (see Fig. 2). The downstream end of a 60 cm long fused-silica capillary, was fitted with a commercially available in-line filter containing a metal frit (Swagelok Canada, Niagara Falls, Canada), inserting the capillary end

before in a 3 cm long piece of 0.4 mm I.D. PEEK tubing. The other end of the capillary was connected to the slurry reservoir (a  $60\times 3$  mm I.D. stainless tube, fitted with 1/4 to 1/16 Swagelok reducing connectors) similarly using a piece of 0.4  $\mu\text{m}$  I.D. PEEK tube. The packing slurry was prepared by adding 20 mg of the reversed-phase particles to 1–2 ml acetone and vibrating this mixture with a mechanical mixer for 2 min. After filling of the slurry reservoir, the slurry was pumped into the capillary using acetone as displacer solvent at a pressure of 500 bar. In order to keep the pressure at 500 bar, the flow-rate had to be decreased during the packing. When the packing is completed, the column is flushed for another 30 min at 500 bar. Then the pump is switched off and the pressure was allowed to drop to zero (at least 1 h) before disconnecting the column from the slurry reservoir.

The column is then is connected to a HPLC pump and flushed with water at about 300 bar. While flushing with water a frit is fabricated in the middle of the packed capillary by means of a home made heating device (see Fig. 3). The heating filament consisted of a resistance ribbon in which a  $450\text{-}\mu\text{m}$  hole was drilled. The temperature of the filament can be adjusted by the voltage across the ribbon. A rough indication of the temperature of the filament can be read from a sensor mounted very close to the ribbon. The packed capillary is threaded through the hole in the ribbon until the middle of the capillary and a narrow band of the packing is locally sintered (8 s, approximately  $450^\circ\text{C}$ ) into a frit by applying voltage. Next the low dead volume in-line filter is removed and the packing is flushed away at the downstream side. Then the other end of the capillary is connected to the pump and the particles remaining at the other side are also flushed away, leaving an empty column with a frit in the middle. Subsequently the capillary is connected to the slurry reservoir and packed again in the same way as described before. After flushing the column with water the second frit is fabricated in the same way. The redundant piece of capillary in front of the second frit is removed by cutting. Finally, a detection window is made by burning off the polyimide protective layer just after the middle frit. After mounting the column in the CE system, the column is flushed extensively with the degassed mobile phase, via the stainless steel ground

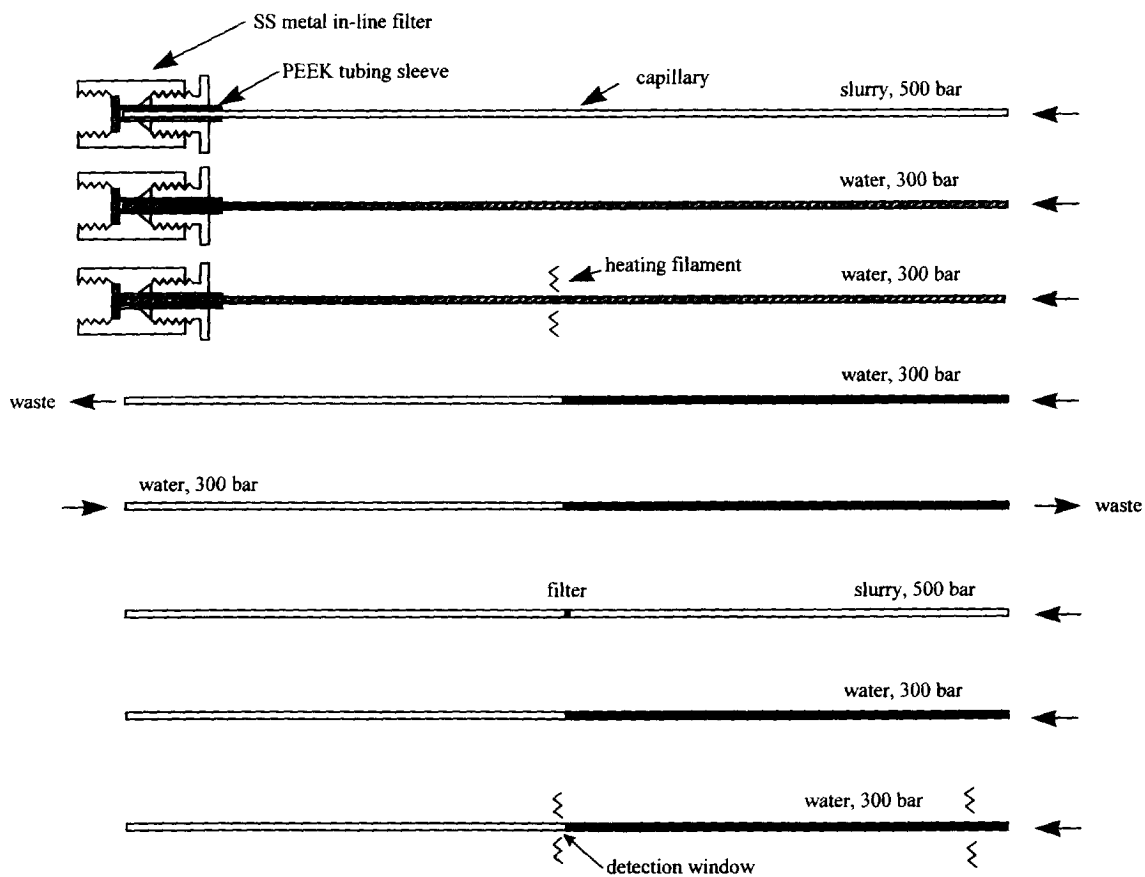


Fig. 2. Successive steps of the column packing and the fabrication of the retaining frits.

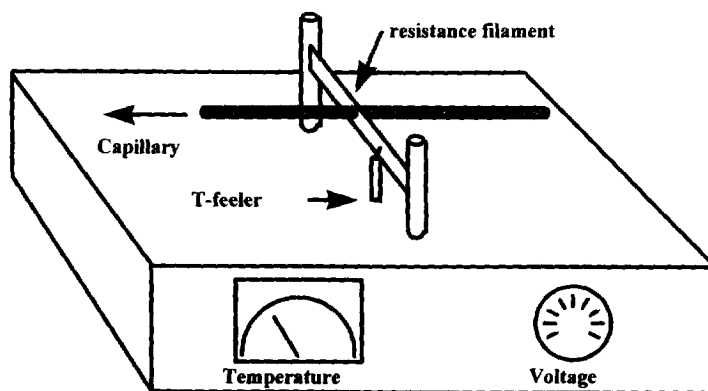


Fig. 3. Schematic representation of the heating filament.

reservoir, using a high-pressure pump as described before.

### 3.2. Chromatography

The mobile phase for the electrically and pressure driven liquid chromatography consisted of mixtures of acetonitrile and water containing 1–4 mmol/l of sodium tetraborate pH 9.2. The mobile phases were extensively flushed with helium before use. The helium flushing was repeated sometimes during the course of the day. It was found that the outside coating of the fused-silica capillary was slowly attacked in time by acetonitrile and this makes the capillary brittle. To minimize the contact with acetonitrile, the buffer vials were replaced by water after terminating the measurements. The test solutes were always dissolved in the mobile phase.

## 4. Results and discussion

In order to judge the applicability of CEC, several aspects were investigated such as the role of the packing retainer, the packing of the column and the effects of the buffer on the electroosmotic flow, the current and the efficiency were investigated. The performance under electrically and pressure driven conditions were compared.

### 4.1. Packing retainers

In order to avoid packing particles migrating of the capillary, the packing has to be locked between two packing retainers. Ideally the retainer should have no effect on the performance of the CEC system. Different ways to retain the packing were investigated by us: (i) a frit prepared by glueing of silica particles; (ii) mounting a filter in a straight PEEK connector; and (iii) a frit prepared by sintering of particles of the packing. The retainers were evaluated by comparison of the pressure drop at a given flow-rate of the pump, before and after mounting of the retainer and the behaviour of the current when applying an electric field. Also their strength was tested at higher pressures. Arbitrary a retainer was indicated as good when the pressure drop, at a

flow-rate of 10  $\mu\text{l}/\text{min}$ , has increased  $<5$  bar compared to that of the open capillary.

#### 4.1.1. Frits of glued particles

Three ways to glue unmodified silica particles into a frit were tested i.e.: 1.5  $\mu\text{m}$  non-porous silica particles and polyethoxysiloxane (PES), 10  $\mu\text{m}$  porous silica particles and waterglass, 10  $\mu\text{m}$  porous silica particles and lead borate glass powder. In all methods a small zone of the particles is created by pressing the end of the capillary in a pile of the wetted or mixed particles put on a slide.

The PES method [16] involves the wetting of the particles with PES, drying of the zone and then exposing the zone to ammonia vapour by which the particles are glued together via porous silica bridges. The waterglass method has been applied by others [4,17] and briefly involves the wetting of a small zone of the particles with waterglass, drying this zone and then glueing the particles by heating the zone by means of the heating filament as described in Fig. 2. In the lead borate glass technique, the silica particles are mixed with the lead borate glass powder in various weight ratios and the particles are glued by heating the zone at a fixed time at approximately 500°C by means of the heating filament.

The permeability of the prepared frits appeared to differ significantly. The PES frits are the most permeable but are rather fragile. The permeability of the lead borate frits increases with decreasing amount of lead borate glass powder. The waterglass frits were the worse. Examination under the microscope reveals that usually no regular bed of glued particles is formed in the heated zone. The temperature and the exposing time seem to be very critical. Too low a temperature results in an incomplete frit, while with a too high temperature the frit is often not very permeable.

All prepared frits were also tested under an electric field by monitoring the current in the time at different voltages. The behaviour of the current was found to be the same for all frits. With 40 kV/m and using acetonitrile–water (4:1, v/v) containing 4 mmol/l sodium tetraborate as mobile phase, initially the current has a value between 5 and 10  $\mu\text{A}$ , but rapidly decayed (in 10–180 s) to a very small value ( $<0.1$   $\mu\text{A}$ ) or even became zero. The decay was the smallest with the PES frit and the steepest with the

waterglass frits. At smaller lower voltages, the decay of the current last longer (1–4 min). The decay of the current appeared to be caused by bubble formation in the frits as could be established under a microscope.

From these results it must be concluded that the glued frits, as prepared by us, are not suitable as packing retainers in CEC. Moreover, the application of glued frits has the disadvantage that the column has to be coupled to the detector via a teflon tube which can cause additional peakbroadening.

#### 4.1.2. Retainer mounted in a PEEK lock connector

In analogy to the metal low dead volume in-line filter connectors as used in HPLC, a PEEK connector was modified so that a filter can be mounted between two fused-silica capillaries. For that purpose the original PEEK connector was drilled through to a diameter equal to the outer diameter of PEEK tubing. By means of two small pieces of PEEK tubing, a filter with a diameter equal to the outer diameter of the PEEK tube, can be mounted between two fused-silica capillaries. The filters can be punched out of filter material. The following filter materials were tested: paper filters, HVLP and PTFE filters (Millipore), metal screen and glass frits drilled from G4 (10–16  $\mu\text{m}$  pores) and G5 (1–1.6  $\mu\text{m}$  pores) filters (Jena). The filters were evaluated with respect to permeability and the behaviour of the current under electric field.

The HVLP and PTFE filters exhibited a very poor permeability and the current declined very quickly to a small value. These filters are therefore unsuitable as retainers. Paper filters show a very good permeability and the current stays at a good value. However, the mechanical strength of the paper filters is very poor and they even fell apart after some time. This make them unsuitable as retainers. A metal screen with 2  $\mu\text{m}$  pores showed a good permeability and mechanical strength. However, no stable current was found. The permeability and mechanical strength of the G4 and G5 glass frits appeared to be good. However, the current decreased rapidly after switching on the voltage. The decay of the current is steeper with the G5 filter than with the G4 filter. In order to determine whether the decay of the current is due to self-heating in the frit, the PEEK coupling was cooled with ice. Under these conditions the

decay of the current was significantly less steep. This indicates the occurrence of excessive heat production in the frit and as a result bubbles may be formed due to boiling of the solvent.

#### 4.1.3. Frit of sintered particles

From the experiments with the lead borate glass it was found that the permeability of the frit increases with decreasing amount of lead borate glass powder. Therefore attempts were made to sinter the dry silica particles in absence of lead borate glass using the heating filament. Usually the particles appears to burst away when heating the zone. However, sometimes the particle zone stays intact and a permeable sintered frit is obtained. This observation indicates that it must be possible to sinter particles into a frit providing the right conditions are created to avoid bursting of the particles when heating the zone. It was Smith et al. [10] who demonstrated recently the clever solution, i.e., flushing the column with water under pressure during installation of the frits by locally sintering of a small zone of the (reversed-phase) packing. Apart from small differences, we successfully adapted their method to prepare frits by a heating filament in a pre-packed capillary. In contrast to columns whereby the frits were made from particles differing from the packing particles, no bubble formation occurred with the columns in which the frit is sintered from the packing.

Therefore in all further experiments this way to prepare the frit was used.

#### 4.2. Packing and stability of the column

Initially the columns were packed using *n*-propanol as slurry liquid and methanol as driving solvent. However, under these conditions the column efficiency was found to be less than expected according to theory. The reduced plate heights ranged from 3 to 4. Moreover, it appeared that the efficiency declined rapidly in time because of the occurrence of a hole at the detection side of the column. Since the frits were still intact, it looked that the hole in the packing in that part of the column is caused by a rearrangement of the particles when applying voltage. This might occur when the packing in that part is loosely packed because the flow-rate during the packing is decreasing rapidly or the

packing structure is disturbed when fabricating the frit. In order to create a larger flow-rate during the packing and thus a more dense packing, acetone was used as slurry and driving solvent. Under these conditions very efficient and more stable columns were obtained. The success rate was about 25% and the long term stability of the columns about 2 weeks. After that time again a small hole appears in the packing at the detection side. In an attempt to avoid that hole due to the loose packing structure, in a packed column first a frit at the detection side was made. Then the column was disconnected from the packing filter and the packing was washed away from the two sides of the frit. Then the column was connected to the slurry reservoir and packed on the frit. Finally the second frit, at the injection side, was fabricated. The columns prepared in this way appeared to be very efficient and extremely stable. The success rate was almost 100%. Although the preparation of the column is more laborious, in our opinion this looks so far the best manner to make efficient and stable columns for CEC. Fig. 4 shows a typical chromatogram as obtained on a column fabricated in this way.

#### 4.3. Effect of the injection zone

In order to determine the effect of the injection zone on the efficiency, the injection time was varied at a fixed injection voltage of 10 kV. The total amount of injected solute was kept the same to avoid additional peak broadening due to mass overloading. The injection plug length is the product of the injection time and the electroosmotic flow. Fig. 5 shows the plate numbers of three solutes, acetone ( $k'=0$ ), naphthalene ( $k'=0.64$ ) and pyrene ( $k'=1.75$ ) as determined at 40 kV/m. As can be seen the injection plug length has a large effect on the efficiency. The effect is the largest for the unretained solute and decreases with increasing  $k'$ . Fig. 5 shows that, on the used  $250 \times 100 \mu\text{m}$  I.D. column, the length of the injection plug should not exceed 1 mm, to maintain the high efficiency.

#### 4.4. Effect of the buffer concentration

The effects of the buffer concentration on the current, electroosmotic flow and plate height as

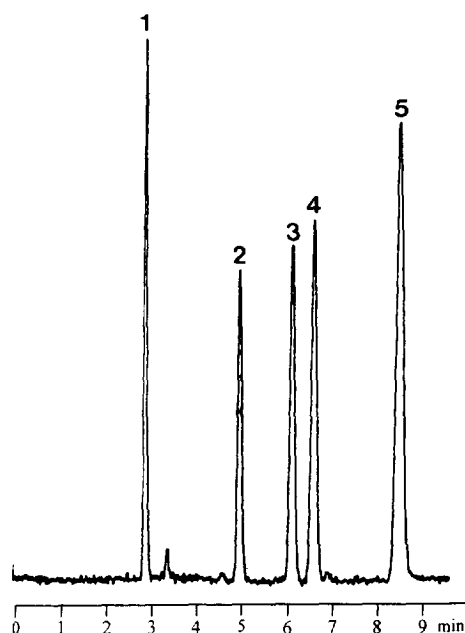


Fig. 4. Typical electrochromatogram of a test mixture of poly-aromatic hydrocarbons. Experimental conditions: capillary, 50 cm  $\times$  100  $\mu\text{m}$  I.D.; packed bed length, 25 cm; packing, 5  $\mu\text{m}$  Hypersil-ODS; mobile phase, acetonitrile–water (4:1, v/v)+4 mmol/l sodium tetraborate (STB); injection, 3 s, 5 kV; run voltage, 20 kV. Solutes: (1) acetone; (2) naphthalene; (3) fluorene; (4) phenanthrene; (5) pyrene.

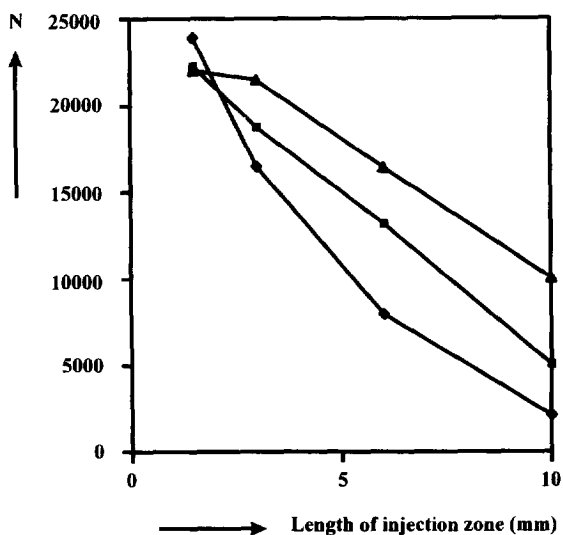


Fig. 5. Effect of the length of the injection plug on the plate number. Conditions as in Fig. 4. Capillary: 48 cm  $\times$  100  $\mu\text{m}$  I.D.; packed bed length: 22 cm. Injection: 2–20 s, 5 kV; acetone (■); naphthalene (□); pyrene (▲).

function of the voltage was investigated with acetonitrile–water (4:1, v/v) containing 1–4 mmol of sodium tetraborate. Acetone was used as neutral marker.

#### 4.4.1. Current

The effects of the buffer concentration on the current was investigated in open and packed capillaries of the same diameters. Fig. 6 shows the plots of the current as function of the applied field at three different sodium tetraborate concentrations. As can be seen for the 2 and 4 mmol/l sodium tetraborate, the current of the open tube is always larger than in the packed column. However, for the 1 mmol/l sodium tetraborate sometimes a larger current is found for the packed column. This behaviour has to be attributed to the experimental error in the mea-

surement inherent to the rather small currents at lower electrical fields. From the good linearity of the plot with 2 and 4 mmol/l buffer it can be concluded that no excessive heat is produced in the packed capillary. The slopes represents also the conductivity of the contents of the open capillary and that of the packed capillary. The ratio of  $i_{\text{packed}}/i_{\text{open}}$  is the so called obstruction factor,  $\gamma$ . For a packed bed of particles the inter particle porosity is usually 0.4; the obstruction factor is expected to be even smaller by a factor of about  $\sqrt{2}$  because of the tortuosity of the current paths. However, for the 4 mmol/l and 2 mmol/l significantly higher obstruction factors have been observed i.e., 0.7 and 0.6, respectively. These higher values indicate that electrical conduction takes place for a substantial part within the particles. A similar phenomenon has been observed for lateral

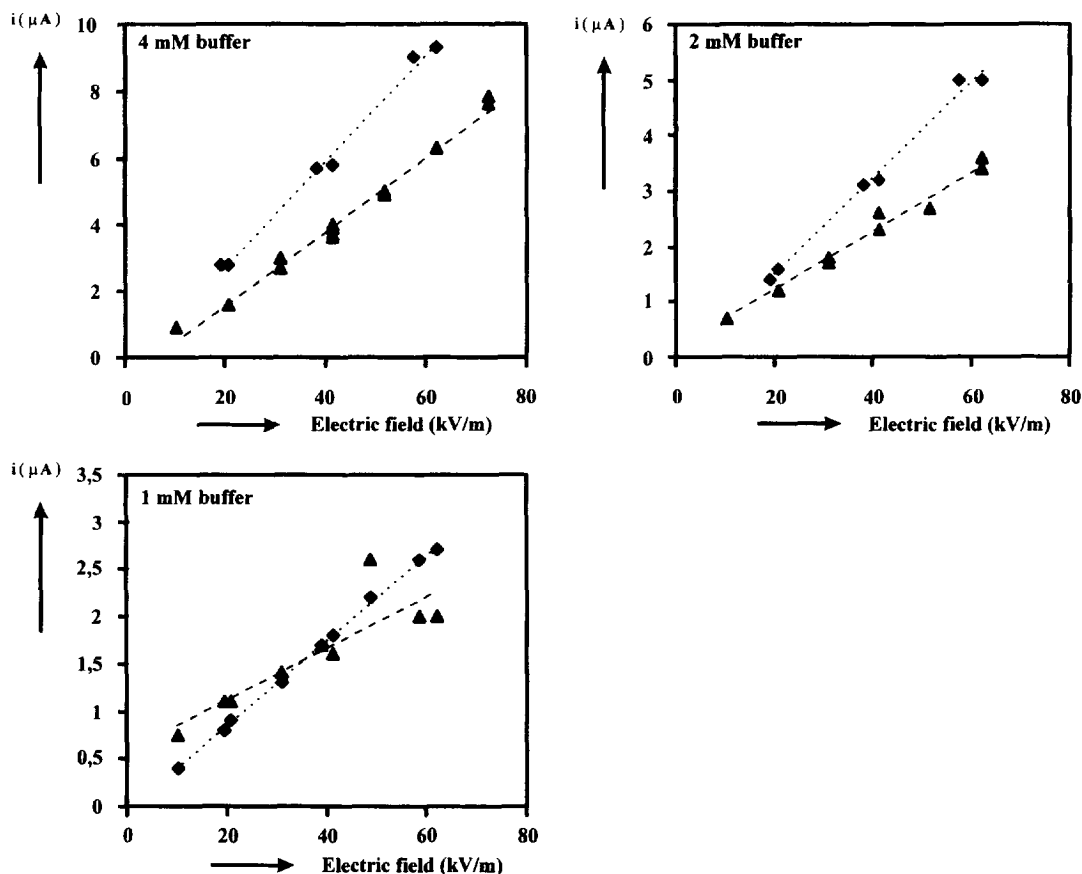


Fig. 6. Plot of the current versus applied voltage at three buffer concentrations with an open capillary ( $\diamond$ ) and a packed capillary ( $\blacktriangle$ ). Mobile phase as in Fig. 4. Buffer concentration: 1–4 mmol/l. Capillary: 49 cm  $\times$  100  $\mu\text{m}$  I.D.; packed bed length: 24 cm.



diffusion in pressure driven LC by Knox and Scott [18] and by Crombeen et al. [19]. On the basis of this finding, it is likely that electrical conduction occurs through the micro pores of the particles.

#### 4.4.2. Electroosmotic flow

Fig. 7 shows the effect of the buffer concentration on the electroosmotic flow as function of the applied field. As can be seen the electroosmotic flow is larger when decreasing the buffer concentration from 4 to 2 mmol/l. However, the change observed when changing from 2 to 1 mmol/l is insignificant. The interpretation of these data is at present unclear and probably complicated. Normally, a decrease of the flow with increasing ionic strength is expected theoretically and found experimentally, as a result of the decrease of the Debye length, i.e. contraction of the diffuse double layer. However, in our case also double layer overlap [3] plays a role. Double layer overlap is probably unimportant in the interstices, having diameters of around 1  $\mu\text{m}$ , i.e., many times the Debye length (5–10 nm). However, the electroosmotic flow within the particles, in their pore space, must be seriously affected by this phenomena, as the pore diameter (ca. 10 nm) is comparable to the Debye length. At present we are not in a position to

give even a qualitative interpretation to the observed trends. We note, however, that a possible electroosmotic flow in the particles deserves further study, as it would enhance mass transfer and thus increase efficiency in the same manner as occurs in perfusion chromatography.

#### 4.4.3. Efficiency

The effect of the buffer concentration on the efficiency was determined by constructing  $H-u$  plots for acetone ( $k'=0$ ) and pyrene ( $k'=1.75$ ) as measured at three buffer concentrations (1, 2 and 4 mmol/l). The results are given in Fig. 8. As can be seen for both solutes the lowest plate heights are obtained at the highest buffer concentration (4 mmol/l). The  $H-u$  curves obtained with 1 and 2 mmol/l largely coincide. A tentative speculative explanation of this behaviour by considering double layer overlap can be given. At the lowest concentrations, the electroosmotic flow is restricted to the interparticle space, as the double layers overlap seriously in the particle pore space. At the highest concentration, electroosmotic flow within in the particle starts to develop. This increase mass transfer and increases efficiency (note that the “unretained”

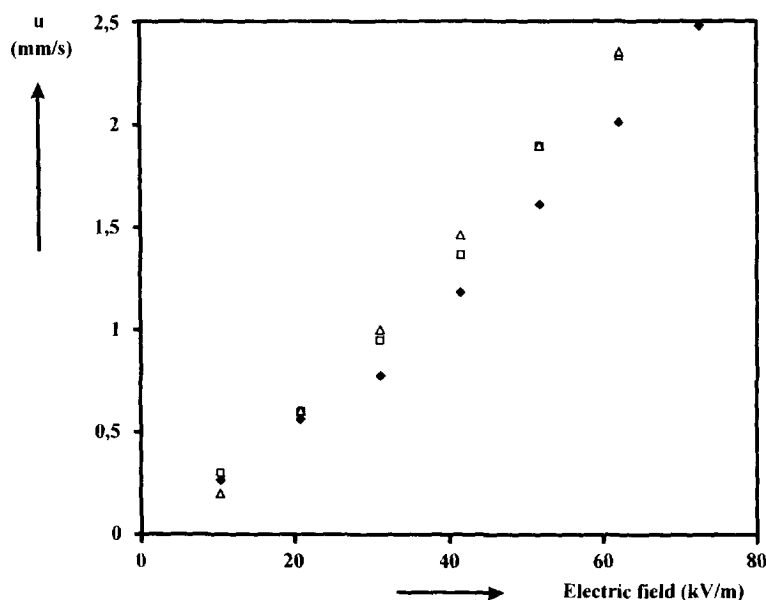


Fig. 7. Plot of the electroosmotic flow versus applied voltage at three buffer concentrations. Conditions as in Fig. 6. Run voltage: 5–30 kV; 4 mM buffer (◇); 2 mM buffer (□); 1 mM buffer (▲).

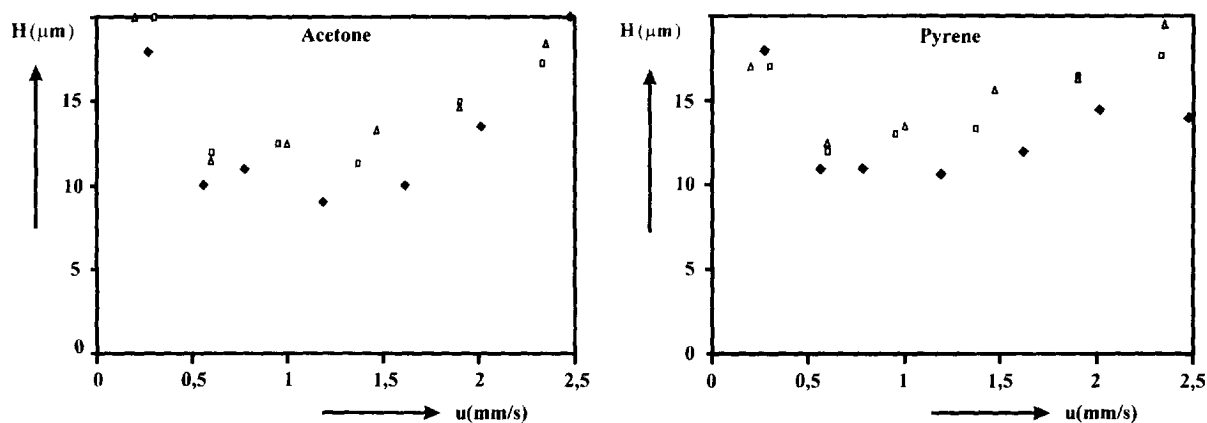


Fig. 8.  $H$ - $u$  plots measured with acetone ( $k'=0$ ) and pyrene ( $k'=1.75$ ) at different buffer concentrations: 4 mM buffer (◇); 2 mM buffer (□); 1 mM buffer (▲). Conditions as in Fig. 6.

solute, acetone, has to be distributed also between the particle and flow channels).

An alternative explanation on the basis of the disruption of plug flow in the interstices, due to double layer overlap could be given. However, this is unlikely, as the interstices have a pore diameter much larger than the Debye length.

#### 4.5. Effect of the mobile phase composition

The effects of the mobile phase composition on the same column was investigated with different acetonitrile percentages (20–90%, v/v) while keeping the sodium tetraborate concentration at 4 mmol/l

1. The electroosmotic flow and the time course of the current were measured at 20 kV. The current increases with decreasing acetonitrile percentage from about 2  $\mu\text{A}$  with 90% acetonitrile to 13  $\mu\text{A}$  with 20% acetonitrile. It took about 30 min before the current became constant; after that it stayed constant for several hours, except with 90% where it took one hour to reach a constant current. The effect of the addition of organic solvents on the electroosmotic flow in open tubes has been investigated by Kenndler et al. [20]. For acetonitrile they found that the electroosmotic flow decreases with increasing acetonitrile content. In packed capillaries, however, a reverse effect is found as can be seen in Fig. 9. The

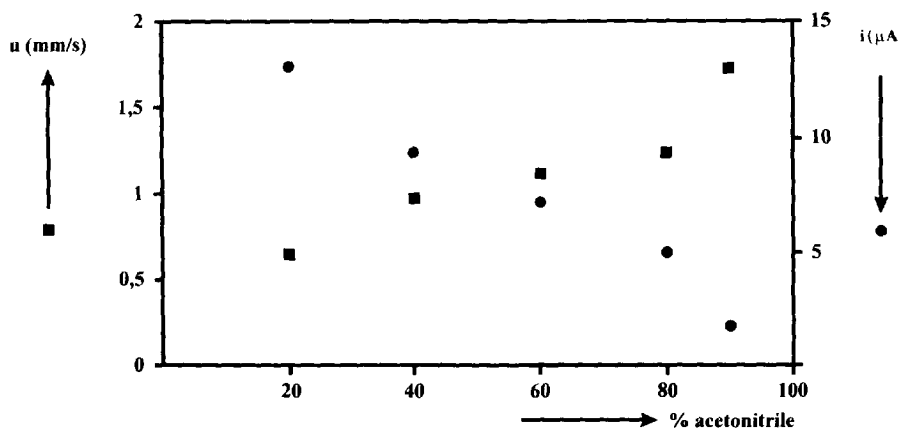


Fig. 9. Effect of the percentage of acetonitrile in the mobile phase on the electroosmotic flow and current. Capillary: 51 cm  $\times$  100  $\mu\text{m}$  I.D.; packed bed length: 25 cm; buffer: 4 mmol/l STB; run voltage: 20 kV; unretained markers: acetone and caffeine.

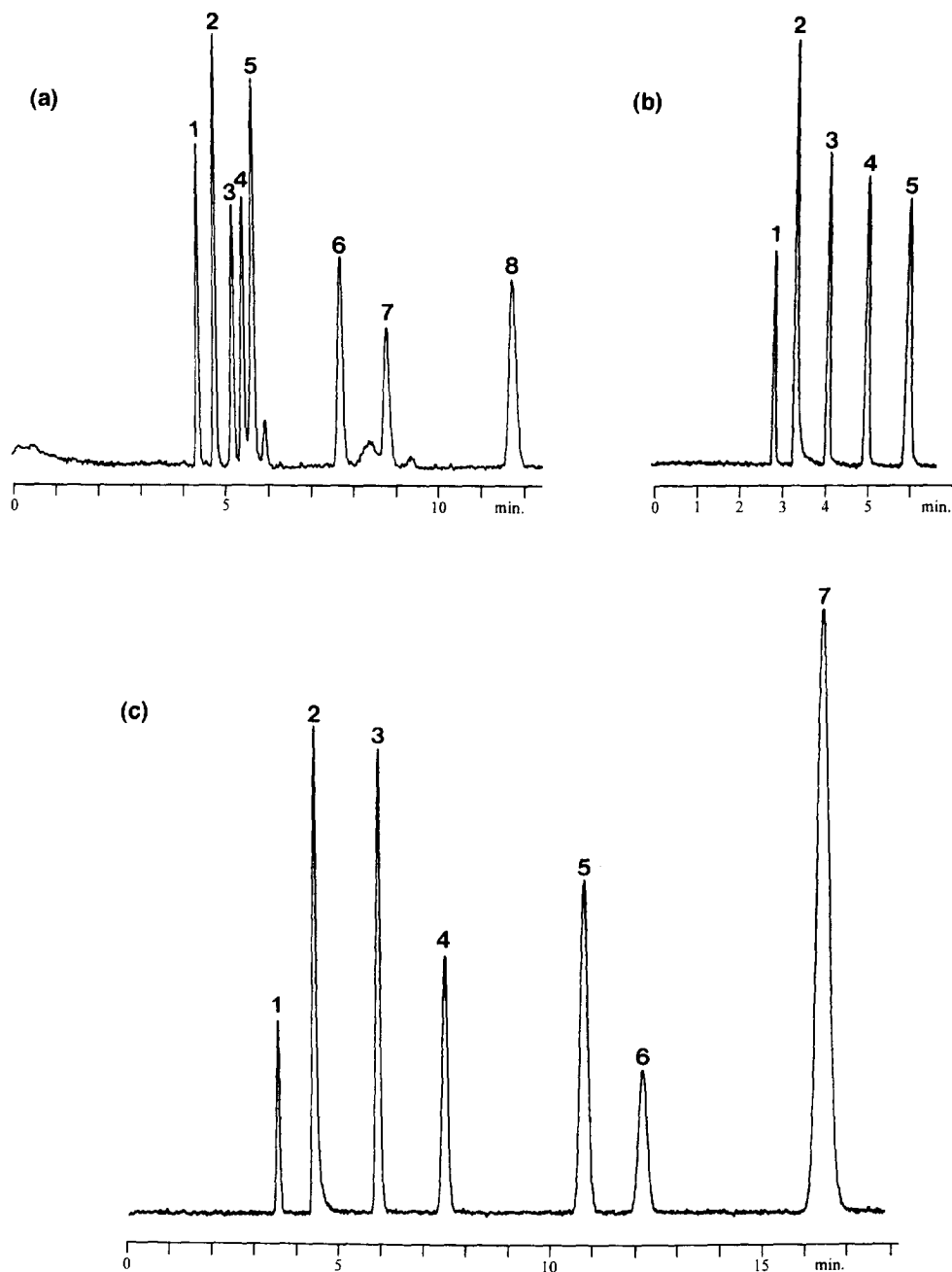


Fig. 10. Some electrochromatograms of test mixtures obtained with different mobile phase compositions. Conditions as in Fig. 9. (a) Separation of a mixture of phenols using 80% of acetonitrile. (1) Phenol; (2) 3,5-dimethylphenol; (3) 2,3,5-trimethylphenol; (4) *p*-*tert*-butylphenol; (5) 4-*sec*-butylphenol; (6) 3,5 di-*tert*-butylphenol; (7) 4-*tert*-octylphenol; (8) 2,6 di-*tert*-butylphenol. (b) Separation of a test mixture using 60% acetonitrile. (1) Caffeine; (2) pyridine; (3) coumarin; (4) 2,6-dimethylaniline; (5) acridine. (c) Separation of a test mixture using 40% of acetonitrile. (1) Caffeine; (2) pyridine; (3) aniline; (4) coumarin; (5) 2,6-dimethylaniline; (6) indole; (7) acridine.

electroosmotic flow increases with increasing acetonitrile content. The different behaviour might be partly due to the difference in type and concentration of the buffer but must most probably be attributed to the specific nature of the ODS chemical modified surface of the particles. The performance with different acetonitrile contents is excellent as can be seen from Fig. 10, showing the chromatograms obtained with some test solutes using 40, 60 and 80% acetonitrile. In all cases the peak asymmetry is excellent and ranges between 1.00–1.04.

#### 4.6. Electrical versus pressure driven

In order to compare the performance under electrically and pressure driven conditions the plate height was measured on the same column and mobile phase [acetonitrile–water+4 mmol/l sodium tetraborate (4:1, v/v)] using phenanthrene ( $k'=1.14$ ) and pyrene ( $k'=1.78$ ) as test solutes. In the case of electrically driven operation, the samples were injected by electromigration and with pressure driven operation by split injection using a split ratio of about 25. Fig. 11 shows the  $H-u$  curves of phenanthrene (A) and pyrene (B) under electrically and pressure driven conditions. At small linear velocities the plate heights coincide. This can be expected since the plate height under these conditions is predominated by axial molecular diffusion. At larger velocities significantly smaller plate heights are obtained on the electrically driven column. The plate height in the

minimum of the curve is about  $9 \mu\text{m}$  for phenanthrene and  $10 \mu\text{m}$  for pyrene, corresponding to a reduced plate height of 1.8 and 2, respectively. On the pressure driven column, a reduced plate height of about 2.6 and 2.8, respectively is found. Moreover, on the pressure driven column the plate height increases steeply with increasing linear velocity. This in contrast to the electrically driven column where the  $H-u$  curves are rather flat. From Fig. 11 it can be concluded that the plate height on the electrically driven column is about 25–40% smaller than on the pressure driven column. This is in agreement with the theoretical expectations [4].

## 5. Conclusions

Our experiences with electrochromatography can be summarized as follows:

1. With suitable precautions and column preparation technique it appears possible to carry out electrically driven chromatography without pressurization. This constitutes an important advantage as injection of sample can be experimentally as simple as in regular capillary electrophoresis.
2. The air bubble generation arises especially in frits prepared by glueing particles which differ from the packing particles. These glued frits are therefore not recommended to lock the packing for electrochromatography.

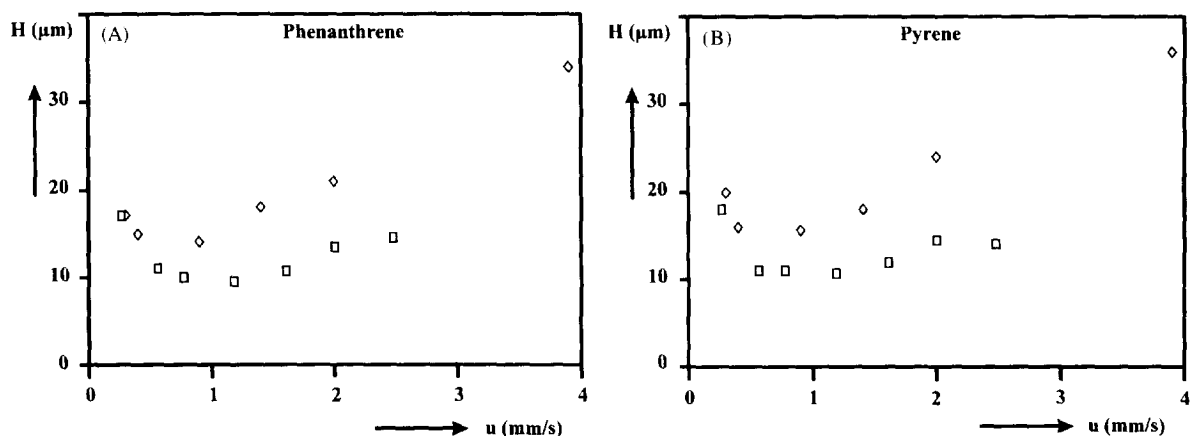


Fig. 11.  $H-u$  curves obtained on the same column with phenanthrene (A) and pyrene (B) under electrically ( $\square$ ) and pressure driven ( $\diamond$ ) conditions. Phase system as in Fig. 4. Capillary:  $48 \text{ cm} \times 100 \mu\text{m}$  I.D.; packed bed length: 26 cm; injection: electrically driven: 3 s, 5 kV; pressure driven: split injection.

3. So far the best way to retain the packing is to make a frit by sintering locally the packing itself with a heating filament while flushing the column under high pressure with water, as described by Smith and Evans [10] or as adapted in this paper.
4. It appears to be of paramount importance to flush the columns extensively and frequently with the helium degassed mobile phase to retain the stability of the system.
5. The use of a stainless steel buffer reservoir as ground electrode, permanently connected to the HPLC pump, is very convenient and diminishes the risk of breaking the column. Recently Rebscher and Pyell [21] reported a similar approach to use a metal T piece as ground reservoir, connected to the capillary and the LC pump.
6. The efficiency of the electrically driven columns is excellent and reduced plate heights of about 1.8 are found. In comparison, under pressure driven conditions, the same columns exhibited a reduced plate height of about 2.6. This is in agreement with the theoretical expectations.

The main conclusion of the present investigation is that packed capillary electrochromatography is practically applicable. To further exploit the technique smaller particles must be applied. Attention should also be given to influencing the nature of the surface by dynamically adsorbing surfactants to the surface. Finally the application of CEC to charged species is worth investigation.

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