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Preparation of continuous beds for electrochromatography and reversed-phase liquid chromatography of low-molecular-mass compounds

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Abstract

High-performance capillary columns for electrochromatography and reversed-phase liquid chromatography are often prepared from silica beads. However, the synthesis of the beads involves many expensive and complicated steps and the packing of reproducible, stable and uniform beds requires great experience. In this paper we propose a simpler and more cost-effective approach, not based on preformed beads but on continuous polymer beds synthesized in situ in the chromatographic tube. The continuous bed columns were prepared by polymerizing two different monomer solutions in two steps directly in the capillary, followed by effective derivatization with hydrophobic ligands (C_{18}). Electroendosmosis was created by embedding a long, charged polymer (dextran sulfate). The continuous beds, as synthesized previously for the separation of proteins by reversed-phase chromatography, showed low resolution for the separation of low-molecular-mass compounds owing to a relatively low ligand density. This disadvantage was overcome by a new method designed to increase the ligand density and, in addition, to achieve a more rigid gel matrix. For rapid removal of the Joule-heat generated in electrochromatography, it is mandatory to employ very narrow columns. Continuous polymer bed columns with an inner diameter of 25 μm or less are easy to prepare. The potential of this type of capillary column is demonstrated by the separation of non-charged polycyclic aromatic hydrocarbons at an efficiency of 120 000 plates/m for a retained solute using electro-driven buffer flow. The performance is thus comparable to that of electrochromatography columns of 40–50 μm I.D. packed with 3–5 μm silica beads. In accordance with theoretical considerations only a somewhat higher plate height was obtained when the same continuous bed column was eluted with a pressure-driven flow. The columns have the distinct advantage over conventional capillary columns packed with beads that air bubbles seldom form and spoil a run, partly due to the absence of a supporting frit.

Keywords: Electrochromatography; Capillary columns; Continuous beds; Electroendosmosis; Stationary phases, LC; Polynuclear aromatic hydrocarbons

1. Introduction

Conventional columns for capillary chromatography are prepared by packing a tube with uniform

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beads. The time-consuming and expensive beading process as well as the very troublesome packing of the columns are obvious drawbacks. The continuous beds have the advantage that they can be prepared directly in the chromatographic tube, thus eliminating both the synthesis of beads and the packing procedure. When the column diameter is larger than 1–2 mm the bed can alternatively be prepared in a beaker and then packed into the column. Another advantage is that the continuous bed matrix is synthesized from water-soluble monomers to minimize non-specific hydrophobic interactions between the matrix and the substances to be separated. Furthermore, the synthesis complies with the general recommendations to decrease the consumption of organic solvents in chemical laboratories.

In a series of papers we have shown that the continuous beds have excellent chromatographic properties and can with advantage be used for enzyme reactors [1], ion-exchange [2,3], hydrophobic-interaction [4], chiral-recognition [5], affinity [6] and reversed-phase liquid chromatography [7,8] and for chromatofocusing [9]. In the reversed-phase mode they were employed for the separation of proteins and larger peptides. Another type of continuous polymer bed has recently been prepared from non-water-soluble monomers (styrene–divinylbenzene) for reversed-phase [10] and hydrophobic-interaction chromatography [11]. Electrochromatography has also been performed in polyacrylamide gels [12].

This paper deals with a method for the synthesis of a continuous bed for the separation of low-molecular-mass compounds by capillary reversed-phase liquid chromatography and capillary electrochromatography (CEC). The greatest practical difficulty was to find experimental conditions that gave sufficiently high ligand density. This problem was particularly pronounced for the electrochromatographic beds, where the requirement for high ligand density must be combined with a need for strong electroendosmosis, i.e., a high zeta potential of the bed surface. Superimposed on all these demands was a desire for low to moderate back pressure for the elution and washing of the columns.

2. Materials

Fused-silica capillaries (25 μm I.D.×375 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Electrophoresis-grade reagents [piperazine diacrylamide, ammonium persulfate, N,N,N′,N′-tetramethylethylenediamine (TEMED)] and ammonium sulfate (HPLC-grade) were obtained from Bio-Rad Labs. (Richmond, CA, USA); 2-hydroxyethyl methacrylate, allyl glycidyl ether, 1,2-epoxyoctadecane (C_{18}) and dehydrated activated molecular sieves, 3A, 1/8, from Aldrich-Chemie (Steinheim, Germany); dextran sulfate, sodium salt (M_r 500 000), methacrylamide and borontrifluoride, 43% solution (BF_3) from Fluka (Buchs, Switzerland). 3-Methacryloyloxypropyl trimethoxysilane (Bind-Silane, A-174) was from Pharmacia BioTech (Uppsala, Sweden) and chromatography-grade acetonitrile from Merck (Darmstadt, Germany). Double-distilled water was used in all experiments.

Sample solutions were prepared from five polycyclic aromatic hydrocarbons (naphtalene, 2-methylnaphtalene, fluorene, phenanthrene and anthracene).

3. Experimental

3.1. Equipment and procedures

The electrochromatographic runs were performed with a laboratory set-up, resembling that used for capillary zone electrophoresis. It was composed of a separate high-voltage power supply, an anode/injection unit, a horizontally aligned capillary column, a UV detector and a grounded cathode. A schematic diagram is shown in Fig. 1.

The power supply (0–30 kV, Glassman, Whitehouse Stadium, NJ, USA) was connected to the anode/injection unit (U) housing an anode electrolyte vessel (A) and an adjacent sample injection cavity (C), both fitted with built-in platinum electrodes (E). A pocket (P) in the anode vessel assured that the end of the capillary was held below the buffer level. To prevent evaporation of the electrolyte solution, a transparent lid (L) with a silicone gasket was placed on top of the anode/injection unit. This unit could be moved in the horizontal plane to accommodate different column lengths. Close to its outlet end the capillary column passed through the UV detector (Bio-Rad, HPE 100 electrophoresis system, modified to accept long straight capillaries

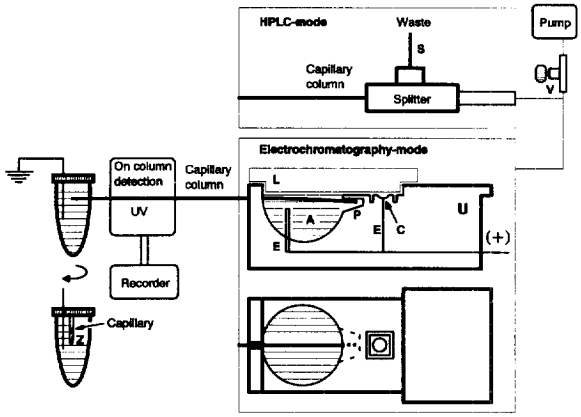

Fig. 1. Schematic diagram of the set-up for electrochromatography and reversed-phase chromatography. The anode/injection unit (U) is shown in cross-section and from above.

and with a re-designed detection cartridge). The hot air produced by the deuterium lamp was removed by a fan to get a more stable recorder baseline. The capillary was introduced into the cathode vessel through a slit (Z), so narrow that the buffer was prevented by surface tension from running out through the slit (alternatively silicon grease can be employed).

Plastic materials often contain different UV-absorbing additives, for instance, softening agents, which may be extracted into the mobile phase, particularly in the presence of organic solvents. Disturbances from such additives were minimized by manufacturing the electrolyte vessels from the highly inert material Robalon (Leripa, Rohrbach, Austria). Voltage and current were measured by a multimeter constructed by Mr. Per-Axel Lidström of this department. Modifications of the HPE 100 capillary electrophoresis apparatus were made by Mr. Curt Lindh, also of this department.

The electrochromatographic experiments were performed as follows.

The mobile phase was passed twice through a 0.20-μm organic filter (Sartorius, Minisart RC 25) followed by extensive purging with nitrogen. Immediately, the capillary was equilibrated with this solution at a relatively low pressure (<100 bar) using an HPLC pump (Series 1350, Bio-Rad). Polyether ether ketone (PEEK) tubing, fingertights, ferrules and stainless steel units (all from Upchurch Scientific, Oak Harbor, WA, USA) were used to connect the columns to the pump. An additional stainless steel "T"-unit, fitted with modified fingertights, and positioned between the HPLC pump and the column, served as an adjustable pressure valve (V). Following equilibration for 30 min the pressure over the column was slowly decreased to atmospheric level and the inlet end was disconnected from the pump and moved to the adjacent anode vessel (A), keeping the capillary end immersed in buffer at all times to avoid formation of air bubbles.

A voltage of 20 kV (about 0.4 kV/cm) was applied over the column for a few minutes in order to obtain a stable baseline and a constant current. The sample was then injected electroendosmotically by placing the capillary inlet end in the injection cavity (C) containing a droplet of the sample and applying 10 kV (about 0.18 kV/cm) for 4–7 s. The running voltages and other experimental data are given in the legends to the respective figures.

The samples were also eluted under pressure using the same 25-μm I.D. column. To adjust the volumetric flow to the low values required in capillary chromatography, a "splitting-capillary" (S) was connected to the HPLC-pump. Injection of the sample was performed by electroendosmosis in a way similar to that described above for the electrochromatographic run.

3.2. Pretreatment of columns

3.2.1. Activation of the capillary wall

The principle

Prior to the polymerization of the monomer solution in the capillary, the inner walls were treated with 3-methacryloyloxypropyl trimethoxysilane. The methoxy groups in this compound react readily with the silanol groups at the surface of the silica wall, leaving the methacryloyl end to react later with the acrylic groups present in the monomer solution (see Step 1, Section 3.4.1 below). In this way the continuous bed became covalently bound to the capillary wall.

Practical performance

By means of a water aspirator the capillary was treated for 30 min with 0.2 *M* sodium hydroxide and for 30 min with 0.2 *M* hydrochloric acid, and finally rinsed with distilled water. The capillary was then

filled with a solution of 3-methacryloyloxypropyl trimethoxysilane in acetone (30%, v/v) and left for 24 h, washed with acetone to remove excess 3-methacryloyloxypropyl trimethoxysilane and then with water.

3.3. The design of the bed—guiding studies

To help the reader to understand the strategy behind the synthesis of the continuous beds some background information is given below.

A straightforward approach to synthesize a continuous bed for electrochromatography is to include allyl glycidylether and/or hydroxyethyl methacrylate in the monomer solution, along with a charged compound, such as acrylic acid or vinyl sulfonic acid, in order to obtain a strong electroendosmotic flow and, following polymerization, to pump into the column a solution of a hydrophobic epoxide, such as 1,2-epoxyoctadecane, for reaction with the epoxide and/or hydroxyl groups in the matrix in the presence of BF_3 as catalyst. Great effort was devoted to the synthesis of a bed of this type using a single polymerisation step combined with a subsequent ligand coupling.

It turned out that high concentrations of hydroxyethyl methacrylate and particularly allyl glycidyl ether were needed as ligand-binding monomers along with a high concentration of a charged monomer to create a sufficiently high zeta potential on the bed surface following the introduction of hydrophobic ligands. Therefore, high concentrations of all of these monomers were required. However, when the total monomer concentration T was increased (above T= 35%, w/v) the bed started to loose its mechanical rigidity, resulting in a very high flow resistance. This problem could partly be overcome by decreasing the amount of initiator and accelerator (e.g., ammonium persulfate and TEMED) in order to increase the length of the polymer chains. Further reduction of the flow resistance was obtained by increasing the concentration of ammonium sulfate. (The ammonium sulfate in the monomer mixture serves to increase the hydrophobic interactions between the polymer chains formed and thereby creates channels in the gel matrix through which the mobile phase can pass.)

The electroendosmotic flow in these beds was very high before the derivatization with 1,2-epoxyoctadecane but was very low afterwards, probably owing to shielding of the charged groups by the C_{18} ligands. However, following the introduction of these non-polar ligands the flow resistance became so high that the washing of the bed took an unacceptably long time. The high flow resistance may be due to an increase in the viscosity caused by the formation of C_{18} polymers via reactions between the epoxide groups in the 1,2-epoxy octadecane molecules. An electrochromatographic separation of polyaromatic hydrocarbons on these beds is shown in Figure 9 in Ref. [13].

Besides varying the monomer composition, we also tried to immobilize a number of different polymers in the gel matrix, including polymers derivatized with C_{18} groups. Among these experiments, the only successful ones were those where the zeta potential of the bed was increased by immobilization of an OH-rich, charged polymer (dextran sulfate).

Immobilization can be accomplished by embedding of the polymer, which therefore should have a high-molecular-mass to decrease the risk of leakage, and/or by covalent linkage between the hydroxyl groups in the polymer and the epoxide groups in the matrix, originating from the allyl glycidyl ether. In an effort to lower the flow resistance a rigid matrix was first synthesized according to a method similar to the standard approach [14,15] we have employed for a series of different chromatographic columns based on continuous beds (see Section 1). This matrix (see Step 1, Section 3.4.1) was then used for immobilization of dextran sulfate and epoxide groups (see Step 2, Section 3.4.2). C_{18} groups were then introduced with BF_3 as catalyst and toluene as solvent (see Step 3, Section 3.4.3). In an attempt to decrease the formation of C_{18} polymers (which increases the viscosity and thereby the flow resistance) we utilized ultrasonication during the coupling reaction. Upon exchanging the widely used ether for toluene as solvent for 1,2-epoxyoctadecane the formation of polymers was suppressed still more since the reaction between epoxide groups is slower in toluene.

Another advantage is that the solubility of 1,2-epoxyoctadecane is high in toluene. Under these conditions the C_{18}-ligand density could be increased considera-

bly. Furthermore the performances of two halves of the same column were almost identical which indicates that the density of ligands was evenly distributed along the length of the column.

3.4. In situ preparation of the continuous bed

A column of a continuous polymer bed with immobilized dextran sulfate to create electroendosmotic flow and with hydroxyethyl and epoxide groups derivatized with C_{18} chains for reversed-phase separation was prepared as follows in three steps, directly in the capillary.

3.4.1. Step 1. A first polymerization to create a rigid matrix

Hydroxyethyl methacrylate (93 μl) and piperazine diacrylamide (78 mg) were dissolved in 1.0 ml of potassium phosphate buffer (pH 7.0, 80 m*M*). With stirring, ammonium sulfate (75 mg) was added and after a few minutes 12 μl of a 10% (w/v) aqueous solution of ammonium persulfate. Following degassing with nitrogen for 6 min and addition of 12 μl of TEMED (5%, v/v) the capillary was completely filled with this monomer solution using a water aspirator (3–4 void volumes). Both ends were plugged with desiccator grease to prevent air from entering the capillary. The column was left horizontally overnight for complete polymerization. Salt residues were then carefully removed by pumping with distilled water (more than 15 void volumes).

3.4.2. Step 2. A second polymerization to immobilize dextran sulfate, the electroendosmosis-creating substance

Allyl glycidyl ether (100 μl), hydroxyethyl methacrylate (210 μl), piperazine diacrylamide (140 mg) and 400 μl of a 8% (w/v) aqueous solution of dextran sulfate (sodium salt) were dissolved in 1.0 ml of 80 m*M* potassium phosphate (pH 7.0). Ammonium sulfate (48 mg) and 12 μl of a 10% (w/v) aqueous solution of ammonium persulfate were added. After degassing with nitrogen for 6 min, 12 μl of the TEMED solution was added.

This degassed reaction mixture was then immediately pumped into the capillary column (2–3 void volumes). The capillary must be completely filled with the monomer mixture before the polymerization starts, i.e., within 15 min. As in step 1 the polymerization in the horizontal capillary proceeded overnight with both ends plugged with grease.

Stainless steel loops connected to the HPLC pump were used for different solutions, including the monomer mixtures.

A window for on-line detection was made in the polymer-filled capillary by burning off a 1–2 mm section of the polyimide coating with the aid of an electrically heated tungsten wire while pumping cold water continuously through the column. The heat from the wire breaks covalent bonds at the inner wall and creates a small water-filled gap (≈1 mm, 0.5 nl). The detection window prepared in this way has about the same UV transmission as a window in an empty capillary. It is important to wash with cold water to avoid decomposition of some of the adjacent bed. The advantage of having the same stationary phase on each side of the detection window is discussed in Section 4.1.

For reversed-phase liquid chromatography, but not for electrochromatography, the dextran sulfate in the monomer mixture can be exchanged for 400 μl 4% (w/v) dextran (M_r 500 000).

3.4.3. Step 3. Covalent linkage of C_{18} to the continuous bed

For this derivatization we utilized the BF_3-catalyzed reaction between the hydroxy and epoxide groups in the bed and the epoxide groups in 1,2-epoxyoctadecane, as follows.

The columns were washed sequentially with distilled water (10–15 void volumes) to remove salt residues, acetone (3–4 void volumes) and toluene (4–6 void volumes). Boron trifluoride–diethyletherate (43%, see Section 2) was diluted 1:4 in toluene and then slowly pumped through the water-free continuous bed for 40 min. In the meantime, 1.2 g of 1,2-epoxyoctadecane (m.p. 37°C) was dissolved in 0.6 ml of water-saturated toluene in an oven at 50°C. This C_{18} solution was then filled into a stainless steel loop and pumped very slowly through the capillary column, keeping both the steel loop and the capillary immersed in a ultrasonic bath at 65°C. After sonication for 1 h the column was washed with toluene (5–6 void volumes) to remove unreacted C_{18}, with acetonitrile (3–4 void volumes) to remove the toluene and finally with the mobile phase 5 m*M*

borate buffer (pH 8.7) containing 62% (v/v) acetonitrile. All washing and treatment of the capillary described above were performed at pressures below 150 bar. The toluene and acetone were anhydrous, unless otherwise stated, and stored in the presence of dehydrated activated molecular sieves. Before use the column was equilibrated with the mobile phase for at least 24 h.

4. Results and discussion

4.1. Suppression of the formation of air bubbles

Many electrochromatographic experiments are spoiled by bubble formation [16]. We never had this problem with the columns described herein. One reason may be that the inside diameter of the columns was small (25 μm), which means that the Joule-heat was rapidly dissipated. Another reason could be that our beds are attached covalently to the capillary wall and, therefore, do not require supporting frits which generate a strong Joule heating due to their high electrical resistance and thereby form air bubbles. The polymer bed is the same on both sides of the detection window (see Step 2, Section 3.4.2) and thereby also the electroendosmotic flow, which is important, since a difference in flow causes local circulation of the liquid in the capillary with attendant loss in resolution [17–20].

We also observed that the filtration of the mobile phase was a necessary complement to the N_2 degassing. Filtration and immersion of the mobile phase in an ultrasonic bath was not sufficient.

Dust particles (or gas bubbles) stuck at one end of the capillary can easily be removed by cutting the capillary (compare with packed-silica columns, where supporting frits are required).

4.2. Dependence of plate height on linear velocity

In order to compare the performance of CEC with that of capillary chromatography the effect of linear velocity on plate height was investigated. A sample of acetone (for UV spectroscopy) was eluted with both electroendosmosis- and pressure-driven flow using the same column under otherwise identical conditions. As shown in Fig. 2 electrochromatography was slightly more efficient than capillary chromatography. Observe that the plate height is virtually independent of flow velocities above 0.5 mm/s for both elution modes. Accordingly very short analysis times can be obtained without significant loss in resolution. The difference in efficiency is further demonstrated in Fig. 3 where the expanded peaks for acetone using the same column have been superimposed. A plot of electroendosmotic velocity vs. applied field strength is shown in Fig. 4. The relationship between the electroendosmotic flow and electric field is linear up to about 2 kV/cm—an extremely high field strength—which suggests that the Joule heat was rapidly dissipated from this 25-μm I.D. column.

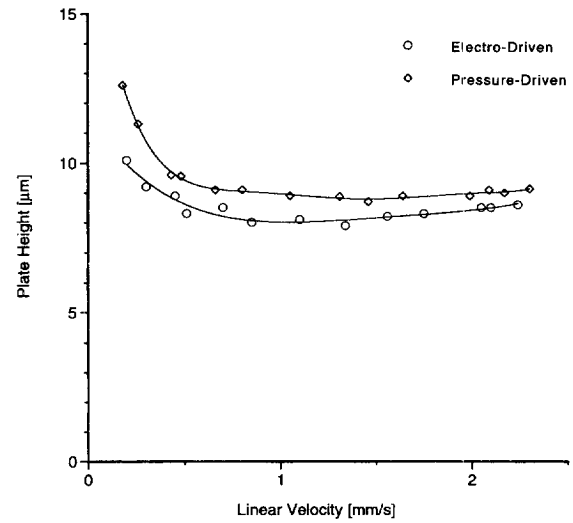

Fig. 2. Dependence of plate height on linear velocity for both electroendosmosis- and pressure-driven flow. Capillary column: 125 mm (effective length 95 mm)×25 μm I.D.×375 μm O.D. Mobile phase: 5 m*M* sodium borate buffer (pH 8.7) containing 62% (v/v) acetonitrile. Sampling: 1 kV for 4 s. Unretained marker: acetone.

4.3. Pressure elution vs. electroendosmotic elution

It has been stated that electroendosmosis in CEC gives a perfect plug flow and therefore causes less zone deformation than that caused by the hydrodynamic flow created by pumping the mobile phase through the same column. The following discussion

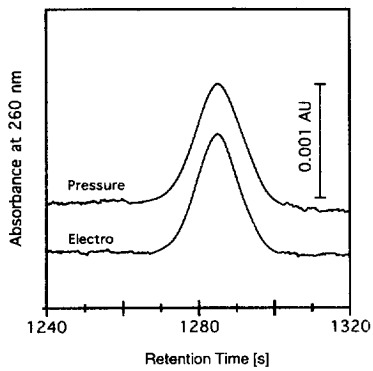

Fig. 3. Expanded acetone peaks obtained from pressure-driven (H=9.4 μm) and electroendosmosis-driven (H=8.4 μm) chromatography having identical elution times in the same column. Capillary column: 550 mm (effective length 525 mm)×25 μm I.D.×375 μm O.D. Applied voltage: 26 kV. Other conditions as in Fig. 2.

shows that this statement is not true under all experimental conditions.

The electroendosmotic velocity, u_{eo}, is determined by the expression

$$u_{eo} = \mu_{eo} \cdot E \quad (1)$$

where E is the field strength and μ_{eo} is the electroendosmotic mobility governed by the relation

$$\mu_{eo} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\eta} \quad (2)$$

where ε_0=the permittivity of vacuum, ε_r=the relative permittivity of the mobile phase, ζ=the zeta potential and η=the viscosity of the mobile phase. Eq. (1) shows that constant electroendosmotic flow at any point in a packed column can be obtained only if the product $\mu_{eo} \cdot E$ has the same value everywhere in the column, i.e., in practice, every bead should have the same zeta potential in any point (Eq. (2)) and the field strength should be the same in any region where there is a solute molecule. These requirements might be impossible to fulfill in practice. For instance, dust particles can easily be trapped or adsorbed in the column and also solutes may be adsorbed and even precipitate, causing a change in the charge (the zeta potential) of some parts of the surface of the beads. Another contribution to the distortion of the plug flow originates from the fact that the channels in a packed or continuous bed are curved, i.e., the electrical field strength—and thereby the electroendosmotic velocity (Eq. (1))—at the "outer lane" is somewhat lower than that at the "inner lane" (compare the situation in a coiled capillary where the field strength varies over the cross-section [13,21,22]).

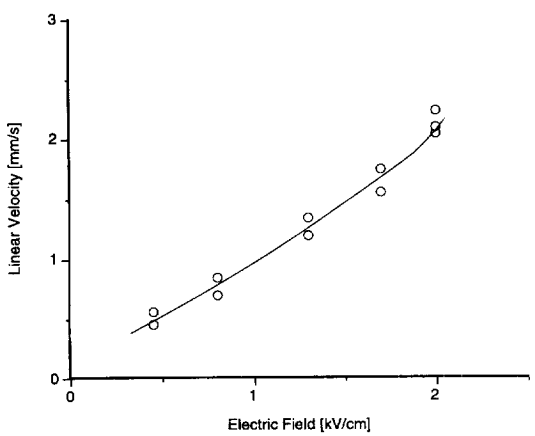

Fig. 4. Effect of field strength on electroendosmotic flow velocity. Conditions as in Fig. 2.

These variations in the parameters ζ and E will cause variations in the electroendosmotic velocity, u_{eo}. Since the net flow through each cross section of the capillary column must be the same, variations in u_{eo} will give rise to a circulation of the mobile phase, i.e., a zone deformation [17–20]. It should also be recalled that the electroendosmotic velocity is reduced in regions where the beads are so close to each other (or so small in size) that the double layers overlap [23,24]. These local electroendosmotic flows which impair the resolution have been discussed and verified experimentally in capillary free zone electrophoresis [19,25]. Moreover, there are indications that electroendosmosis does not create a perfect plug flow even in an open capillary [26].

From the above discussion one can conclude that there should not always be a great difference in resolution of solutes separated by hydrodynamically or electroendosmotically driven chromatography, i.e., reversed-phase liquid chromatography or electrochromatography, respectively, which is illustrated in Figs. 2, 3 and 5a and 5b.

The resolution in capillary chromatography can be increased, theoretically, by decreasing the diameter of the particles making up the bed, but at the expense

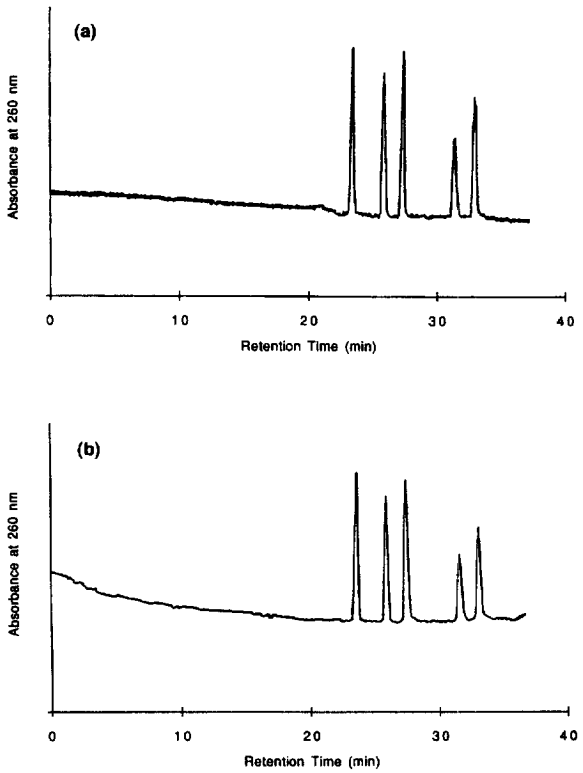

Fig. 5. Chromatograms obtained by electrochromatography and reversed-phase liquid chromatography on a continuous bed with C_{18} ligands and immobilized dextran sulfate. Capillary column: 550 mm (effective length 525 mm)×25 μm I.D.×375 μm O.D. The sample consisted of (1) naphtalene; (2) 2-methylnaphtalene; (3) fluorene; (4) phenanthrene; (5) anthracene. Mobile phase: 5 m*M* borate buffer (pH 8.7) containing 62% (v/v) acetonitrile. (a): Separation with electroendosmotic elution. Applied voltage: 26 kV (N = 120 000/m measured on the fluorene peak). (b): Separation with pressure-driven elution (N = 105 000/m). Pressure: 52 bar.

of an increase in back pressure. Unfortunately, in practice, the packing often becomes heterogeneous when the diameter of the beds is below 50 μm I.D. However, in CEC the quality of the packing is less important than in chromatography (which easily can be demonstrated with colored analytes). The limit of the particle diameter in CEC is set practically by the overlap of the double layers. The small difference in plate height between the chromatographic and the electrochromatographic experiments (Fig. 2) indicates that the continuous beds fulfill the requirement of uniform packing.

"It appears that until the packing of small diameter (50 μm I.D.) capillaries can be made more reliable and simple, the technology will not advance properly as the increased experimental difficulties do not currently justify using CEC in preference to HPLC." [27]. This problem has largely been overcome by the introduction of the continuous beds, since these can be prepared directly in the capillary. Columns with inner diameters as small as 10 μm and with a fairly homogeneous polymer bed can be synthesized easily (Fig. 6). In this connection it should be recalled that a decrease in the diameter of the capillary may decrease the plate height [28,29].

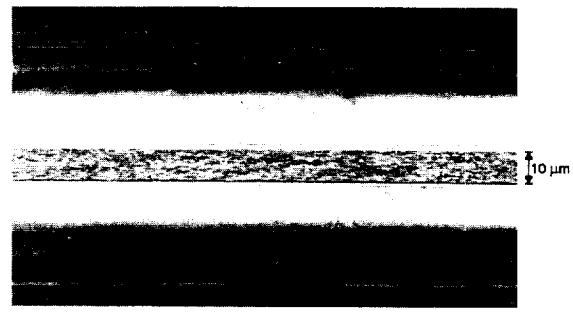

Fig. 6. Photomicrograph of a continuous polymer bed. Capillary column, 10 μm I.D.×78 μm O.D.

The electroendosmotic velocity, u_{eo}, decreased upon an increase in the concentration of acetonitrile. This effect can be explained by Eq. (2), which shows that the electroendosmotic mobility is reduced when the relative permittivity, ε_r, decreases. The decrease was about 30% for an increase in acetonitrile concentration from 0% to 60% (v/v) (Fig. 7), which is similar to the effect observed in packed-silica columns [30] (ε_r for acetonitrile and water is 40 and 80, respectively). The linear electroendosmotic velocity, u_{eo}, was determined by measuring the migration time of an unretained peak (acetone).

5. Conclusions

Since the monomers are polymerized directly in the capillary, a continuous bed is inexpensive and relatively easy to prepare, even in very narrow-bore capillaries with dimensions well below 25 μm. At such small inner diameters the Joule-heating is virtually negligible, which minimizes the risk of

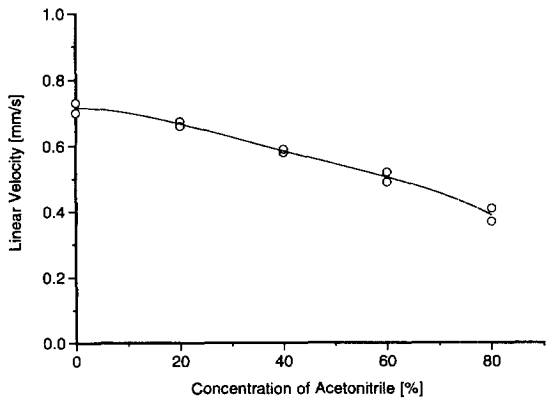

Fig. 7. Electroendosmotic velocity as a function of the concentration (%, v/v) of acetonitrile in the mobile phase. Capillary column, 660 mm (effective length 630 mm)×25 μm I.D.×375 μm O.D. filled with a C_{18}-derivatized continuous bed. Applied voltage: 30 kV. Mobile phase: 5 m*M* borate buffer (pH 8.7)–acetonitrile.

bubble formation. Supporting frits are not required because the continuous bed is covalently bound to the wall of the capillary. The synthesis of the beds is based on the use of water-soluble monomers and minute amounts of organic solvents (for attachments of non-polar groups) and is thus favorable from an environmental point of view.

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