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Electroosmosis- and Pressure-Driven Chromatography in Chips Using Continuous Beds

Christer Ericson,[†] Johan Holm,[‡] Thomas Ericson,[‡] and Stellan Hjertén*,[†]

Department of Biochemistry, Biomedical Center, University of Uppsala, P.O. Box 576, S-751 23 Uppsala, Sweden, and Acreo AB, Electrum 236, S-164 40, Stockholm-Kista, Sweden

The application range of microchips can be extended to any mode of chromatography by filling the narrow channels with continuous polymer beds, exemplified by electrochromatography and ion-exchange chromatography. "Wall effects" are eliminated by anchoring the bed to the wall of the channel, an arrangement which has the additional advantage that no frits to support the bed are required. The design of the equipment is based on a quartz chip with all auxiliary pieces (for example, electrode vessels and fluid transfer fittings) placed in a rack, which permits a flexibility of great importance for automation. The same resolution and van Deemter plots were obtained in experiments performed in fused-silica capillaries and in chips for both low-molecular-weight (alkyl phenones, antidepressants) and high-molecular-weight substances (proteins). A sample of uracil, phenol, and benzyl alcohol was separated by electrochromatography in less than 20 s.

It is a relatively straightforward procedure to transfer *carrier-free* zone electrophoresis and *open-tubular* chromatography experiments in capillaries to analogous runs in the narrow channels in chips. Many such studies have been reported.[1−10] However, to achieve uniform packing of such channels with beads for chromatographic experiments seems to be an impossible task, particularly if the channels are not straight but are curved, for instance, into a serpentine configuration. The difficulty of affixing frits to support the bed is obvious. These practical obstacles are pointed out in the only paper dealing with packing a chip channel.[11] The main objective of our paper was, therefore, to show that the problems outlined can be eliminated if the packed bed is replaced by a continuous bed polymerized in situ in the chip channels.[12−22] The preparation of the continuous bed is relatively easy, since a monomer solution with low viscosity is simply pressed into the channels. Accordingly, there is no chromatographic restriction as to the width of the channel or its configuration. The continuous bed, which can be regarded as a polymer rod consisting of covalently linked 0.1−0.4 μm particles, is anchored to the channel wall. No supporting frit is, therefore, required. The potential of the micromethods presented is illustrated by the separation of low-molecular-weight neutral and basic compounds and acidic proteins, using both pressure- and electroosmosis-driven chromatography. The importance of extending HPLC to the chip format is apparent if we bear in mind that chromatography is perhaps the most used (micro)method in separation science. As alternatives to the method described herein, there are other in situ techniques that might also be employed for microchip chromatography.[23−37]

* To whom correspondence should be addressed: (tel) +46 18 471 4461; (fax) +46 18 55 21 39; (e-mail) stellan.hjerten@biokem.uu.se.

[†] University of Uppsala.

[‡] Acreo AB.

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The majority of microflow systems are fabricated in silicon or ordinary glass wafers. The commonly used manufacturing method involves the etching of channels in a glass or a silicon substrate and subsequent glass-wafer bonding to form an enclosed "capillary" channel. However, in this study we used channels fabricated in quartz substrates to obtain high UV transmission at short wavelengths. Compared with that of fused-silica capillaries, the surface of the quartz chips is virtually flat, which reduces the loss of UV-light intensity. The fabrication method requires only one mask layer and few processing steps and makes it possible to produce channels close to the surface of the wafer which facilitates liquid handling in many chemical analysis systems, including attachments of various fluid-transfer fittings.[38,39]

EXPERIMENTAL SECTION

Materials. Piperazine diacrylamide (PDA), *N*,*N*,*N*′,*N*′-tetramethylethylendiamine (TEMED), ammonium persulfate (electrophoresis-purity reagents), ammonium sulfate (HPLC-grade), and a protein sample consisting of myoglobin (horse), conalbumin, ovalbumin (chicken), and trypsin inhibitor (soyabean) were from Bio-Rad (Hercules, CA). Methacrylamide (MA), vinyl sulfonic acid (VSA), and 3-methacryloyloxypropyl trimethoxysilane (Bind-Silane A 174) were obtained from Fluka (Buchs, Switzerland). Dimethyl diallylammonium chloride (DDA) was from Polysciences, Inc. (Warrington, PA) and *N*-isopropylacrylamide (IPA) from Tokyo Chemical Industries (Tokyo, Japan). Fused-silica capillaries (20- and 50-μm i.d. × 365-μm o.d) were purchased from Polymicro Technologies, Inc. (Phoenix, AZ). The mobile phase was passed through a filter of 0.20-μm pore size (Minisart RC 25, from Sartorius, Göttingen, Germany) and then purged with nitrogen to expel dissolved oxygen. Two antidepressant drugs, nortriptyline and amitriptyline, and the related compound methyl amitriptyline were kind gifts from the Department of Analytical Pharmaceutical Chemistry at the Biomedical Center. Aniline, acetophenone, propiophenone, and butyrophenone were purchased from Sigma (St. Louis, MO). 2,6-Dihydroxyacetophenone and 2,5-dihydroxypropiophenone were from Aldrich (Milwaukee, WI).

Microchip Separation Device. The quartz chips were fabricated by Acreo AB (Stockholm, Sweden) using a one-step photolithographic procedure[38,39] (Figure 1). A layer of polycrystalline silicon (poly-Si) was deposited on the quartz substrate. This layer was then patterned with rows of holes according to the desired column geometry followed by dry etching to make the holes reach the quartz substrate and wet etching with hydrofluoric acid to create the channels. Following oxidation of the poly-Si to make it transparent, a layer of SiO_2 was deposited using low-pressure chemical vapor deposition (LPCVD), which sealed the holes and left the underlying channel system with a roof of about

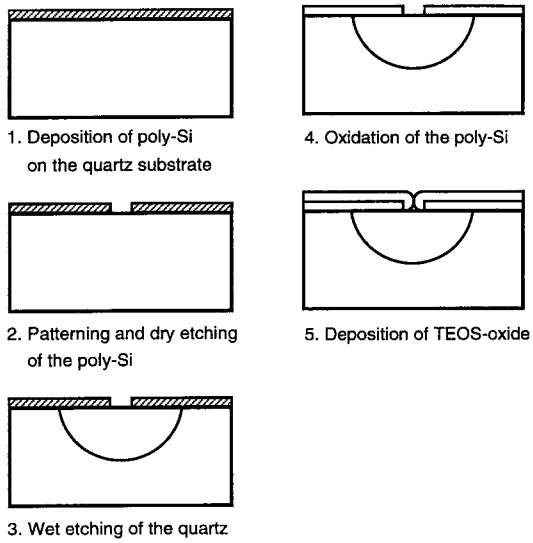

Figure 1. Process for the fabrication of channels in the quartz substrate. Poly-Si, polycrystalline silicon. The TEOS-oxide (tetraethyl orthosilicate) is deposited by LPCVD.

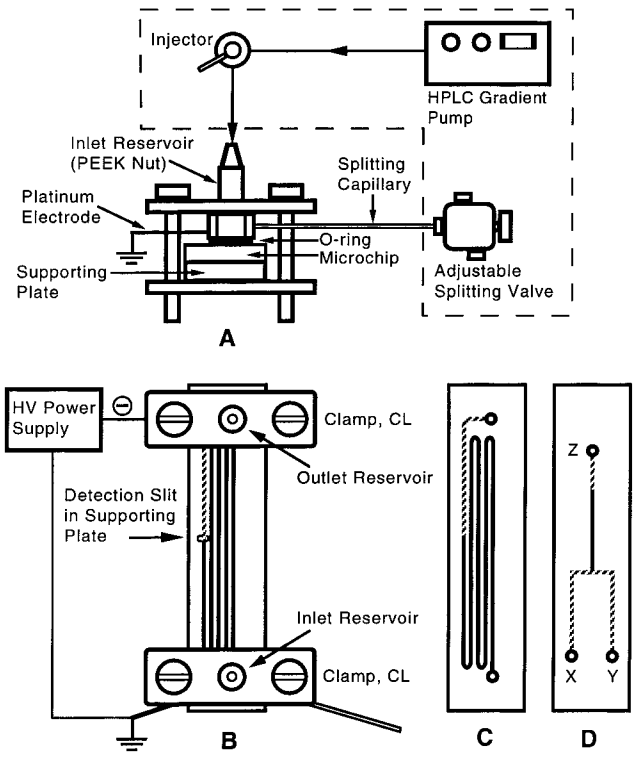

Figure 2. Setup for microchip electrochromatography and microchip chromatography with gradient-elution capability. The screw clamps and the reservoirs are shown in side view (A) and top view (B). The geometries of the serpentine and the "h"-shaped columns in the quartz chip are depicted in C and D, respectively. Channels without a continuous bed are drawn with dashed lines.

4 μm. The etched channels had semicircular cross-sections with 40-μm (i.d.) width at the roof. For the electrochromatographic separations, we used a serpentine column geometry with a total enclosed column length of 30.6 cm from inlet to outlet reservoir (28.1-cm effective bed length) and a "h"-shaped column geometry (1.8 cm effective bed length)(Figure 2C,D). For anion-exchange chromatography, we used a straight column design with an enclosed column length of 6.2 cm (4.5-cm effective bed length).

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The chip was mounted on a supporting plate of black Plexiglass with a slit for UV detection (Figure 2A,B). The two screw clamps (CL) contained electrode reservoirs fitted with platinum electrodes. These electrode reservoirs were constructed from PEEK nuts and ferrules from Rheodyne (Cotati, CA) and could therefore be connected to ordinary fluid-transfer fittings (PEEK units, nuts, and ferrules from Upchurch, Oak Harbor, WA). The electrode of the inlet reservoir was grounded, and the reservoir was supplied with a splitting capillary connected to an adjustable microsplitter valve (P460S), also from Upchurch. Soft O-rings, 0.8-mm in thickness and 5-mm in diameter, were used as gaskets. This design permitted hydrostatic pressures to be applied for liquid chromatography and sample solutions to be introduced both hydrostatically and electrokinetically.

The setup used for chromatography in the gradient mode and isocratic electrochromatography consisted of an HPLC pump with an LC controller (models 2152 and 2150, respectively, from LKB, Stockholm, Sweden) which was connected to an extensively modified CE apparatus (model HPE 100 from Bio-Rad Laboratories, Hercules, CA). For instance, the detection cartridge was designed to fix a horizontally aligned microchip device. To reduce stray light and current leakage between adjacent column sections of the serpentine-shaped column and to avoid mechanical damage to the channel roof, the entire surface of the chip was coated with a dull, black epoxy paint with the exception of a small zone for UV detection. A stainless steel six-port rotary valve from Rheodyne was used for sample injection. A power supply with positive ground was from Glassman (0−30 kV, Whitehouse Stadium, NJ). Current and voltage were measured with a multimeter designed by Mr. Per-Axel Lidström of this Department.

Electrochromatographic Procedures. The HPLC pump with the LC controller was connected to the grounded electrolyte reservoir and set to deliver an isocratic flow at zero pressure by keeping the splitting valve fully open (for EOF-driven gradient elution of proteins, see ref 21). Fresh eluent was thus supplied continuously to the reservoir, and when voltage was applied, the eluent was driven through the microchip column solely by electroosmosis. However, for equilibration of the column the electroosmotic flow was combined with a hydrostatic pressure of 4−6 bar regulated by means of the adjustable splitting valve. The injection valve was used to transfer the sample to the inlet reservoir. The flow was stopped for electrokinetic injection, and the reservoir was then flushed with the mobile phase before a run was started.

Electrochromatography in ordinary capillary columns was carried out in a similar way, except that a reconstructed microvalve (P451) from Upchurch was used instead of the inlet reservoir in Figure 2.[21]

Chromatographic Procedures. A mixture of proteins was separated on an anion-exchange column by pressure-driven gradient elution. For this purpose, the HPLC pump and the LC controller were set to deliver a gradient running from 0 to 0.4 M sodium chloride in 20 mM sodium phosphate, pH 7.8. The adjustable splitting valve was used to achieve the low flow velocities required in microchip chromatography. The sample was introduced into the inlet reservoir using the six-port rotary valve and then injected by applying pressure to the reservoir.

Table 1. Composition of Monomer Solutions for Preparing Microchip Columns

	PDA (g)	MA (g)	IPA (g)	VSA (μL)	DDA (μL)	$(NH_4)_2SO_4$ (g)	buffer[a] (mL)
$C_3^-(1)$[b]	0.30	0.14	0.18	8		0.01	1.7
$C_3^-(2)$[b]	0.30	0.14	0.26	70		0.01	1.7
AIEC[c]	0.35	0.26			44	0.18	2.5

[a] Sodium phosphate (50 mM, pH 7.0). [b] Column for electrochromatography (derivatized with C_3 and SO_3^-). [c] Column for anion−exchange chromatography. PDA, Piperazine diacrylamide. MA, Methacrylamide. IPA, Isopropyl acrylamide. VSA, Vinylsulfonic acid. DDA, Diallyl dimethylammonium chloride.

In Situ Preparation of Chromatographic Columns. Three types of continuous polymer beds were prepared directly in the channels of the microchip: (1) a bed derivatized with sulfonic acid groups (SO_3^-, for the generation of electroosmosis, also at low pH) and isopropyl groups (C_3, for weak reversed-phase separation); (2) a bed similar to bed no. 1 except that the concentration of C_3 groups and SO_3^- groups was higher; (3) a bed derivatized with ammonium groups and used for pressure-driven anion-exchange chromatography. The first bed was used for both reversed-phase electrochromatography of alkyl phenones and for isocratic separation of basic antidepressants by mixed-mode electrochromatography based on ion-exchange and aromatic adsorption chromatography.[20]

Surface Activation of the Inner Wall of the Microchip. To attach the continuous bed covalently to the chip channel, the wall was pretreated in the following way.

By means of a water aspirator connected to one of the electrode reservoirs, the channel of the chip was washed with acetone (>5 void volumes) and then treated with 0.2 M sodium hydroxide for 30 min and 0.2 M hydrochloric acid for 30 min and finally flushed with water (>5 void volumes). The inner wall was activated with methacryloyl groups by filling the channel with a solution of [3-(methacryloyloxy)propyl]trimethoxysilane in acetone (30% v/v) and leaving the chip in an oven at 150 °C for 1 h. The channel was then flushed with acetone and water. During the course of the subsequent polymerization (see below), the free methacryloyl groups reacted with the monomers, thus linking the bed covalently to the wall.

Preparation of the Continuous Polymer Bed in the Microchip. Table 1 shows the compositions of the monomer solutions used to synthesize the continuous beds employed for electrochromatography and for anion-exchange chromatography. Following degassing with nitrogen and addition of 12 μL of TEMED(5%, v/v (aq)), and 12 μL of ammonium persulfate (10%, w/v (aq)), a small volume of the monomer mixture (20−30 μL) was transferred to the inlet reservoir. The channel of the microchip was then filled completely with the monomer solution by pressurizing the reservoir with nitrogen (<0.5 bar). The monomer solution should be introduced slowly into the column in order to avoid pressure drops at the turns of the serpentine-shaped column and attendant void formation. A window in the bed for UV detection was then created as follows. The outlet reservoir was filled with a degassed aqueous solution containing poly(ethylene glycol) (PEG, MW 8000, 10% w/v), ammonium persulfate (20% w/v), and TEMED (10% v/v) and then pressurized with nitrogen

for a few seconds in order to substitute about 2 cm of the monomer mixture at the outlet end of the column for PEG solution. Subsequently, the monomer solution in the inlet reservoir was replaced by PEG solution, and both reservoirs were covered with PEEK plugs and the chip was left in a horizontal position overnight for complete polymerization. A similar technique was used to polymerize a bed in the separation channel of the chip with h-shaped geometry (Figure 2 D). In this case, the monomer solution in the injection channel (X−Y in Figure 2 D) was replaced by the PEG solution before the polymerization proceeded. By keeping reservoir Z closed, the monomer solution in the actual separation channel was prevented from being flushed out.

The above, viscous PEG solution served to create sharper and more homogeneous ends of the polymer bed by accelerating the polymerization rate and decreasing the convection at the water/monomer interface. UV detection was performed through the slit in the supporting plate at a spot close to the end of the polymer bed. Detection could also be done directly through the somewhat UV-transmitting continuous bed, but the sensitivity became about 10-fold lower. Before use, the C_3^- columns were equilibrated with the mobile phase by a combination of electroosmotic flow and hydrodynamic flow generated by a pressure of about 6 bar. The pressure was adjusted with the splitting valve. During the equilibration the PEG solution at the outlet end of the column was flushed out and replaced by the mobile phase. The anion-exchange column was equilibrated only by pressure-generated flow.

The continuous beds were also polymerized in conventional fused silica capillaries (20- and 50-μm i.d.) using an in-situ procedure similar to that described above for the chip.[22] Prior to activating the capillary walls with methacryloyl groups, a window was made in the capillary by burning off a 1−2 mm section of the polyimide coating.

RESULTS AND DISCUSSION

The Design of the Chip Setup. In this study we have mainly focused on the polymeric stationary phase and its use in chips. We also wanted to make direct comparisons between these beds and those polymerized in conventional fused-silica capillaries, which means that injection, detection, etc., should be similar in both cases. Therefore, in several of our experiments, we have chosen to employ cartridge-type (monomodular) chips containing only the immobilized polymer bed and located the electrode reservoirs and other necessary accessories on a separate frame rather than fixing them permanently to the chip. This approach has, in addition, the advantage that it permits great flexibility in the design of the chip, thereby broadening the application range and facilitating and lowering the cost of possible future automation.

A Comparison Between Chip and Capillary Electrochromatography. The performance of a chip column and a conventional capillary column may be different since some parameters which can affect the synthesis of the beds are different (different shapes of the cross sections, different concentrations of OH^- on the surfaces, different thermal conductivity of the surrounding medium leading to different polymerization rates, etc). Therefore, we investigated the effect of electroosmotic velocity on the plate height for both types of columns (Figure 3). For this purpose we prepared the C_3^- bed (no. 1 in Table 1) in a *straight* chip channel of 40-μm i.d.(width) and in a fused-silica capillary of 20-μm i.d.,

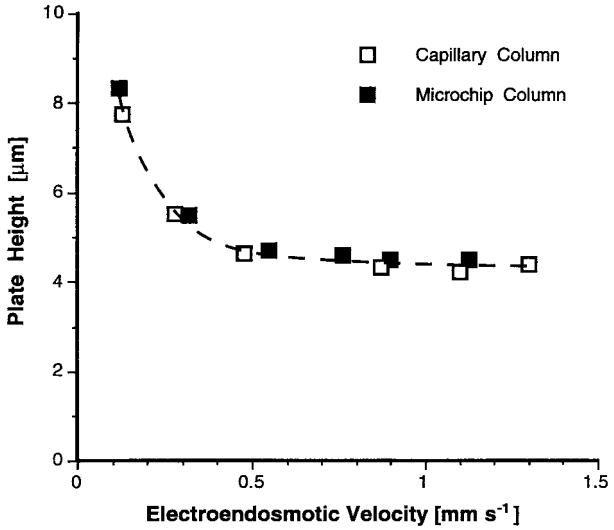

Figure 3. Plate height vs linear velocity for a chip column and a fused silica capillary filled with a continuous bed derivatized with C_3 and SO_3^- ligands. Straight chip column (semicircular cross section), 6.2 cm (4.5-cm effective length) × 40-μm i.d.(width); capillary column, 6.2 cm (4.5-cm effective length) × 20-μm i.d.; both types of columns have approximately the same cross-sectional area; mobile phase, 5 mM sodium phosphate, pH 2.5, containing 20% acetonitrile (v/v); the flow rate through the inlet reservoir was, in both modes, 50 μL/min; UV detection at 260 nm; electrokinetic injection, 100 V, 2 s.; unretained marker, acetone (dissolved in the mobile phase, 20% v/v).

both having an effective length of 4.5 cm and about the same cross-sectional area. A sample of acetone, a neutral and unretained marker, was eluted by electroosmosis on both columns using a 5 mM phosphate buffer containing 20% acetonitrile (v/v). To inject a very narrow sample plug into the *chip* column it is important that the bed is in close contact with the sample (no void between them) and that the interface between the bed and the mobile phase at the inlet end is sharp, which was achieved by having the viscous PEG solutions (containing the initiator) at the ends of the chip channel during the polymerization (see the section titled Preparation of the Continuous Polymer Bed in the Microchip). Sample injection into the *capillary* column, on the other hand, is relatively straightforward. The inlet end of the capillary can be cut off sharply leaving an even surface of continuous bed in direct contact with the sample solution.[21] As shown in Figure 3, no significant differences in plate height were observed between the chip column and the capillary column, which indicates that a uniform polymer bed was formed in both cases and that the sample injection technique used for the chip column was satisfactory. Notice that the plate height is virtually independent of the flow rate above 0.5 mm/s, which means that the analysis times can be made short without sacrificing the efficiency, provided that the C term in the van Deemter equation does not affect the zone width significantly. The "wall effects" are minimized, since the bed is covalently attached to the inner wall (Figures 4A, and 5). Figure 4A,B shows the "joined-particle-matrix" character of the continuous bed which has a high degree of homogeneity, also in the region close to the inner wall. The C_3^- beds used in this study was synthesized with very small flow channels in order to attain high efficiency. The attendant high flow resistance renders this bed more suitable for electroosmotic than hydrodynamic elution. However, a bed with a lower flow resistance can be prepared for

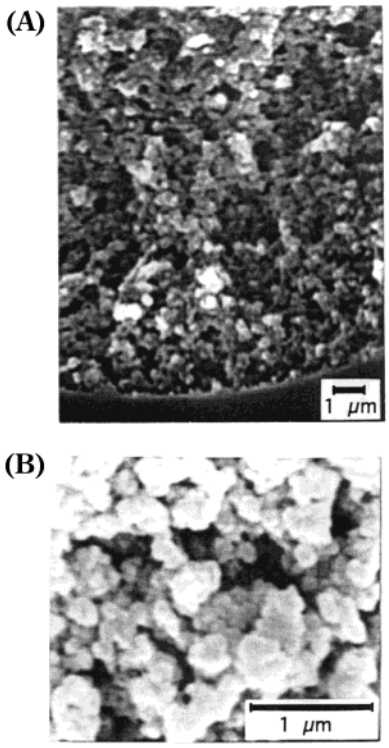

Figure 4. Macrostructure of the continuous bed prepared for microchip electrochromatography. The bed is covalently attached to the wall of the channel (A) and is made up of linked 0.1−0.4 μm polymer particles (B) (SEM).

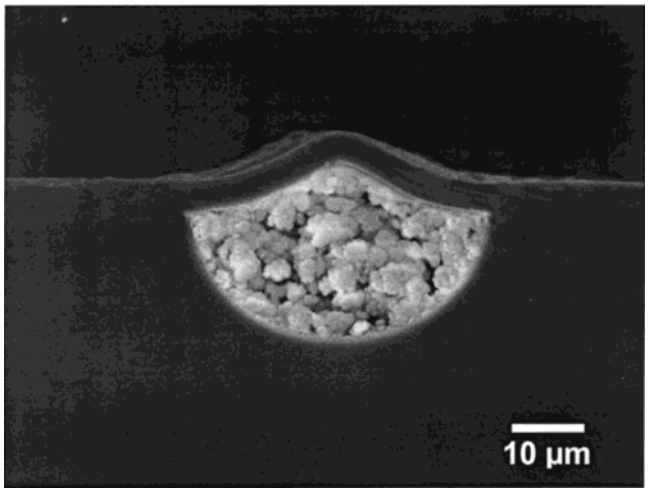

Figure 5. Continuous bed polymerized in situ in the chip channel. This bed is synthesized for anion-exchange chromatography and has, compared with the electrochromatographic bed in Figure 4, relatively large aggregates of polymer particles to ensure low flow resistance (SEM).

pressure-driven elution simply by increasing the amount of ammonium sulfate from 0.01 g (in Table 1) to 0.08 g or more.[12,22]

Electrochromatographic Separation of Alkyl Phenones. The chip setup and the C_3^- column (no. 1) were evaluated using a test sample consisting of neutral alkyl phenones. The result is illustrated by the electrochromatogram in Figure 6. The sample was injected electrokinetically at 1 kV for 1 s, and the inlet reservoir was purged continuously with the eluent containing 5 mM sodium phosphate, pH 2.5, in 30% acetonitrile at a flow rate of 50 μL/min. Acetone was used as the EOF marker. At pH 2.5

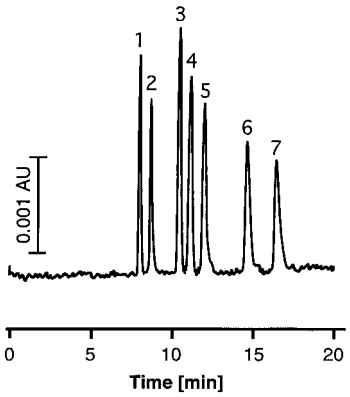

Figure 6. Electrochromatography of alkyl phenones in a chip column filled with a continuous bed derivatized with C_3 and SO_3^- ligands. Serpentine column geometry, 30.6 cm (28.1-cm effective length); mobile phase, 5 mM sodium phosphate, pH 2.5, containing 30% acetonitrile (v/v); flow rate of the mobile phase through the inlet reservoir, 50 μL/min; applied voltage, 16 kV (500 V/cm); UV detection at 240 nm; electrokinetic injection, 1 kV, 1 s; sample concentration 0.30−0.60 mg/mL. Sample: 1, acetone; 2, aniline; 3, acetophenone; 4, propiophenone; 5, butyrophenone; 6, 2,6-dihydroxyacetophenone; 7, 2,5-dihydroxypropiophenone. Plates/m ≈ 300 000 (acetone).

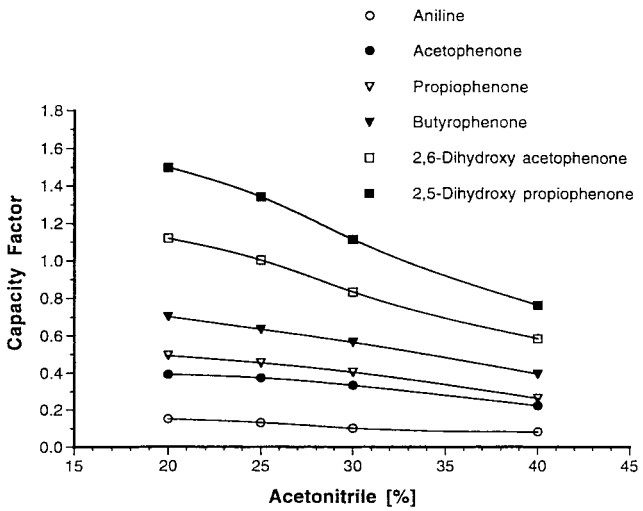

Figure 7. Dependence of capacity factor (k') on the acetonitrile concentration (%, v/v) in the mobile phase upon reversed-phase chromatography on the continuous bed derivatized with C_3 and SO_3^- ligands. For sample identification and experimental conditions, see Figure 6.

all of the solutes were uncharged. To shed some light on the separation mode, the effect of the acetonitrile concentration on the capacity factor was studied. Figure 7 shows that the capacity factors decreased for all the compounds when the acetonitrile concentration in the mobile phase increased from 20 to 40%, indicating that the separation was based on reversed-phase chromatography. However, some separation, albeit with less resolution, was also observed when a sample of acetone and acetophenone was eluted (at acetonitrile concentrations below 35%) on a bed lacking the C_3 ligand, which means that another separation mechanism was also operative. The nature of this interaction was not investigated in this study, but it has previously been reported that low-molecular-weight aromatic compounds interact with polyacrylamide-based separation media in both

electrophoresis[40] and chromatography.[20,41] Such interactions are sometimes referred to as "aromatic adsorption". As observed with other continuous polymer media, including those synthesized for CEC, the velocity of the electroosmotic flow decreased by about 5% when the acetonitrile concentration increased from 0 to 40% (not shown herein).[14,34] In both electrophoresis and chromatography, the efficiency decreases if the separation column is bent or coiled,[6,42] mainly due to differences in path lengths between the "outer" and "inner lanes", which corresponds to an increase in the A term in the van Deemter equation. (Most commercial HPCE instruments are, for practical reasons, designed to accommodate bent capillaries only). To a certain degree, this attendant zone broadening also occurs in a serpentine-shaped column. This contribution to the plate height can be minimized by decreasing the width of the channel, the angle of the turns, and the number of turns.[6,42] We estimated this zone broadening by comparing the performance of the microchip column with that of a straight capillary column having the same effective length (28.1 cm) under otherwise identical conditions. The contribution of the *serpentine* column geometry to the total plate height ranged from 5 to 10%, depending on the analyte and the linear velocity of the eluent. There are also other plausible causes for this zone deformation, such as slower heat dissipation (the supporting plate almost completely covered the chip on one side and the epoxy paint on the other). However, no significant increase in plate height was found when a microchip with a *straight* column was used under similar experimental conditions (see previous section).

Generally, field strengths above 700 V/cm were avoided because of the risk of electrical shock through the thin roof of SiO_2 deposited on the channels and the small distance between adjacent channels in the serpentine-shaped column. At 500 V/cm the electroosmotic velocity was 0.7 mm/s (mobile phase, 20% acetonitrile in 5 mM phosphate buffer).

Electrochromatographic Separation of Antidepressants. The C_3^- column (no. 1) was used also for an isocratic separation of three tricyclic antidepressants (Figure 8). The bed permitted electroosmotic flow at low pH also and has both cation-exchange and adsorptive (reversed-phase) properties. The sample was injected electrokinetically at 1 kV for 4−6 s. The same mobile phase was employed as in the separation of the alkyl phenones, except that the concentration of acetonitrile was higher (70% (v/v)). The antidepressant drugs, nortriptyline and amitriptyline, and the related quaternary ammonium compound, methyl amitriptyline, are secondary, tertiary, and quaternary amines, respectively, and differ structurally by one methyl group. The separation pattern is influenced by both electrostatic and other interactions as well as differences in electrophoretic mobility. The peak asymmetries were calculated to be 1.15 for methyl amitriptyline and 1.25 for nortriptyline.

Pressure Driven Anion-Exchange Chromatography. A continuous bed for anion-exchange chromatography was prepared in a straight chip column of 40-μm i.d.(width) with an effective length of 4.5 cm (Figure 5). Four proteins with isoelectric points ranging from 4.5 to 6.9 were separated by gradient elution. The chromatogram is depicted in Figure 9. The sample was injected by applying a pressure of 3 bar for 10 s and then washed into the column with the starting buffer for another 10 s. During the run, the pressure in the inlet reservoir was 22 bar and the composition of the mobile phase in the reservoir was changed from 20 mM sodium phosphate buffer, pH 7.8, to 0.4 M sodium chloride in

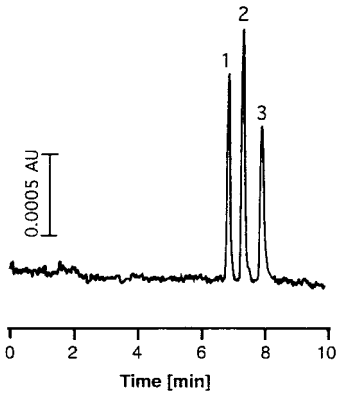

Figure 8. Electrochromatogram of two antidepressant drugs (amitriptyline, nortriptyline) and a related quaternary ammonium compound (methyl amitriptyline) obtained by isocratic elution. Chip column as in Figure 6; mobile phase, 5 mM sodium phosphate, pH 2.5, containing 70% acetonitrile (v/v); flow rate of the mobile phase through the inlet reservoir, 50 μL/min; applied voltage, 12 kV (400 V/cm); electrokinetic injection, 1 kV, 5 s; UV detection at 239 nm; sample concentration, 0.10−0.15 mg/mL. Sample: 1, methyl amitriptyline; 2, amitriptyline; 3, nortriptyline.

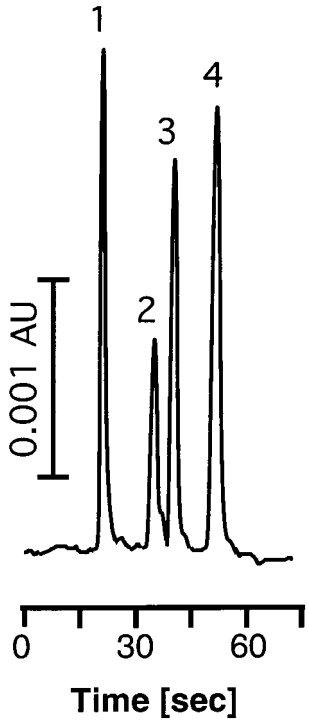

Figure 9. Fast anion-exchange chromatography of four standard proteins in a chip column filled with a continuous bed derivatized with ammonium groups. Straight column design, 6.2 cm (4.5-cm effective length) × 40-μm i.d.(width); mobile phase, (A), 20 mM sodium phosphate, pH 7.8, (B) 20 mM sodium phosphate, pH 7.8, containing 0.4 M sodium chloride; linear gradient 0−100% B in 1 min; flow rate through the inlet reservoir, 0.1 mL/min; flow rate through the chip column, 0.4 μL /min; pressure, 22 bar; injection by pressure, 3 bar, 20 s. UV detection at 230 nm; sample concentration, 0.5−1.5 mg/mL. Sample: 1, myoglobin (horse); 2, conalbumin; 3, ovalbumin (chicken); 4, trypsin inhibitor (soybean).

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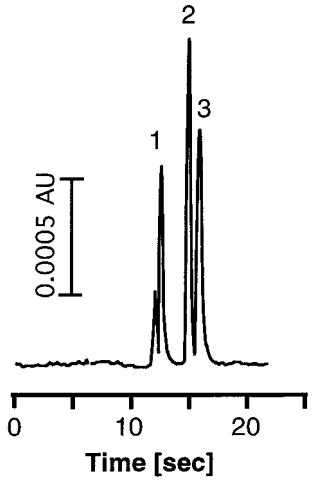

Figure 10. Fast electrochromatography on chips using a C_3^- bed with high EOF velocity. Straight column (18-mm effective length) × 40-μm i.d. (width); mobile phase, 5 mM sodium phosphate, pH 7.4, containing 15% acetonitrile (v/v); applied voltage, 2.4 kV (500 V/cm); EOF velocity, 2.1 mm/s. UV detection at 254 nm; electrokinetic injection, 100 V, 2 s. Sample: 1, uracil; 2, phenol; 3. benzyl alcohol. Separation efficency of uracil was 350 000 plates/m (650 plates/sec).

the 20 mM phosphate buffer, pH 7.8, over a period of 1 min. For pressure-driven gradient elution we also applied 1, 3, and 6 bar, resulting in elution times of 24, 8, and 4 min, respectively. The chromatograms showed the same resolution as that in Figure 9. These results are in accord with the finding that the resolution in continuous beds is virtually independent of the flow rate.[12,43] The bed was designed to be operated at moderate pressures and had therefore relatively large channels compared with the electrochromatographic column (it is easy to adapt the back pressure to any particular chromatographic mode by changing the composition of the bed). The resolution was still high due to the zone-sharpening effect of the gradient.[12,22] In this experiment, the proteins served only as model substances and no attempt was made to optimize the separation.

Fast Electrochromatography. A C_3^- bed (no. 2) was polymerized in the separation channel (one "leg") of a chip with h-shaped configuration (Figure 2 D). A light microscope was used to verify that both ends of the bed were sharp and homogeneous and that the bed spanned exactly from the injection channel to the detection window (effective length, 18 mm). To achieve a very rapid separation, this bed was derivatized with a high concentration of SO_3^- groups, resulting in a EOF velocity of 2.1 mm/s at 500 V/cm (mobile phase, 5 mM sodium phosphate, pH 7.4, containing 15% acetonitrile (v/v)). A sample of uracil, phenol, and benzyl alcohol was injected by first filling the injection channel from reservoir X to Y (Figure 2 D) and then applying 100 V between reservoir Y and Z for 2 s. The remainder of the sample in the channel X−Y was then replaced electroosmotically by mobile phase before a run was started. An example of rapid reversed-phase electrochromatography is shown in Figure 10. All three analytes elute within a 7-s window, and the entire chromatogram in completed in less than 20 s. Provided the same mobile phase is used, the chip is immediately ready for another analysis. Separation efficiency of the slightly retained uracil was 6300 plates (650 plates/s) which is equivalent to 350 000 plates/m. Judging from the high plate numbers obtained, the bed was uniformly polymerized also in this case. This experiment demonstrates that a continuous polymer bed can be prepared in any section of the chip channel.

It should be mentioned that an even simpler method to obtain beds in a particular section of the chip may be to use radiation-initiated polymerization and simply cover the parts of the chip that should not be polymerized.

CONCLUSIONS

Continuous beds can, with advantage, be employed as stationary phases in chips for all modes of chromatography, including electrochromatography, for isocratic and gradient elution of both high- and low-molecular-weight substances. In a van Deemter plot the plate height for an unretained marker approached, at high flow velocities, a constant value (which was the same for electroosmotic elution on both a chip column and a conventional capillary column). The microchip columns thus have the same attractive feature as have standard capillary columns containing continuous beds. These findings indicate that the beds in microchips are also uniform without "wall effects" and gaps (which also has been verified by SEM pictures).

Electrochromatography of low-molecular-weight compounds as well as anion-exchange chromatography of proteins gave separation patterns similar to those obtained in straight capillaries, supporting the conclusion in the above paragraph.

With the method described for the preparation of a chromatographic bed there is no restriction as to the dimensions or geometry of the channel in the chip. For example, a bed about 30 cm long, with serpentine configuration, was synthesized in an area of less than 0.25 cm^2.

Thus, chemistry on a chip (in μ-TAS) can now be combined with high-efficiency chromatography on a polymeric stationary phase. Moreover, an array of beds derivatized with different ligands may be prepared in the same chip and used for parallel analysis.

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