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## Short Communication

# Electrochromatography in microchips packed with conventional reversed-phase silica particles

This paper shows the applicability of a disposable and inexpensive microfluidic chip for electrochromatographic separations. The chip, recently developed by us for chip-based LC, was fabricated from PDMS incorporating conventional chromatographic RP silica particles (C18) without the use of frits. Three cephalosporin antibiotics were used to demonstrate the applicability of the chip-based chromatographic packing for electrochromatographic determinations. The used sample injection method utilizes hydrodynamic pressure, thereby, reducing the propensity for sample bias during the injection.

### Keywords:

Chip / Electrochromatography / Pressure injection / Reversed-phase silica particles  
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The use of microfluidic chips has attracted great attention as they offer a number of advantages over conventional techniques including small sample volume requirements, portability, fast sampling times, ability to multiplex, and compatibility with other techniques [1]. At present, there is a high demand for miniaturized chromatographic techniques that provide very versatile applications found in LC. However, only a few chip-based chromatographic systems have been reported compared to chip-based CE devices mainly due to technical problems inherent in the former [2–4]. Recently, CEC has received significant attention because it combines the separation power of both LC and CE.

Most microfluidic devices are based on the open tubular flow designs and only a few involved packed-bed chips due to the difficulty in preparing frits [5–7]. Other devices have incorporated a physical barrier (weir) to localize stationary-phase particles [8], or derivatized beads for immunoassay [9] or heterogeneous catalysis [10]. Unfortunately, the use of frits is problematic as they are not easily fabricated within a microfluidic architecture and formation of bubbles and band broadening is frequently observed. To remedy this situation, fritless columns employing permanently tapered capillary geometries have been developed [11–13]. Hjertén and co-workers [14, 15] showed that problems in packing can be eliminated if the packed bed is replaced by a continuous bed polymerized *in situ* in the chip channels. They described both electroosmosis and pressure-driven chromatography in

quartz chips. In other work, porous polymer monoliths in chips were formed *via* photoinitiated polymerization [16, 17]. Packed bed chromatography has the benefit over continuous bed or recently reported colloidal array chromatography [18, 19] in that a large variety of stationary phases are commercially available.

In our recent work [20] we described the fabrication of a PDMS-based microfluidic chip containing RP silica beads without the use of frits or other barriers. The packing of the silica beads into the microchips is made possible by the hydrophobic nature and elasticity of PDMS. Different retaining and stabilizing effects appearing in the packed channel have been observed [20]. When pressures of approximately 2 bar are intermittently applied to compress the packing, the wall of the channel is deformed (extended). During this period, the particles fill the enlarged volume of the channel and the channel shrinks when the pressure is released thereby forming a continuous strain around the packing. The particles of the packing are pressed together by the forces of the elastic strains acting perpendicularly from the wall toward the middle of the channel (clamping effect). Finally, these forces derived from elastic strain clamp the whole packing into the microfluidic channel. The stability of the packing is also due to the strong particle–wall interactions between the C18-modified silica and the hydrophobic surface of the PDMS chip. Particles adjacent to the PDMS wall deform and partly penetrate the wall which act as anchors for the packing (anchor effect).

Herein, we extend our work in chip-based LC by demonstrating its use in electrochromatographic separations. In addition, we describe a simple pressure injection mode for the chips to inject nano- or subnanoliter volumes of sample solutions in a microfluidic channel.

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The RP chromatographic packing material consisted of porous, C18-modified, 10  $\mu\text{m}$  particles (Western Analytical Products, Wildomar, CA). Methanol was used to suspend the chromatographic beads and to prevent them from aggregating before their trapping in the chip. Stock solutions of food dyes (FD&C blue#1, and FD&C red#40, all from McCormick, MD) and three cephalosporin antibiotics (ceftazidim (Roche, Switzerland), ceftriaxon (Glaxo, UK) and cefazolin (Bristol-Myers, Italy)) were prepared in water. The buffer electrolyte for the electrochromatographic separation contained 50 mM phosphate, pH 6.8. All solutions (methanol, water) were degassed and filtered through a 0.45  $\mu\text{m}$  syringe filter.

For the sample injection a single-channel peristaltic pump was used. The samples (0.5–5  $\mu\text{L}$ ) were introduced into the peristaltic pump tubing (id 0.3 mm) which was initially filled with electrolyte. This sample was split in the junction and a small volume of the original sample was manipulated into the separation channel (approximately 0.5–5 nL). For the CEC separation, a miniaturized power supply with positive ground was used (0.5–2 kV, Cetox, Hungary). The analytes injected into the chip were detected at 265 nm by an UV-Vis fiber optic positioned directly on the chip and connected to a miniaturized spectrophotometer (Ocean Optics, USA). In case of the LC chip measurement, the above-described pressure injection and the transport of the sample through the C18 packing was carried out by a single channel peristaltic pump.

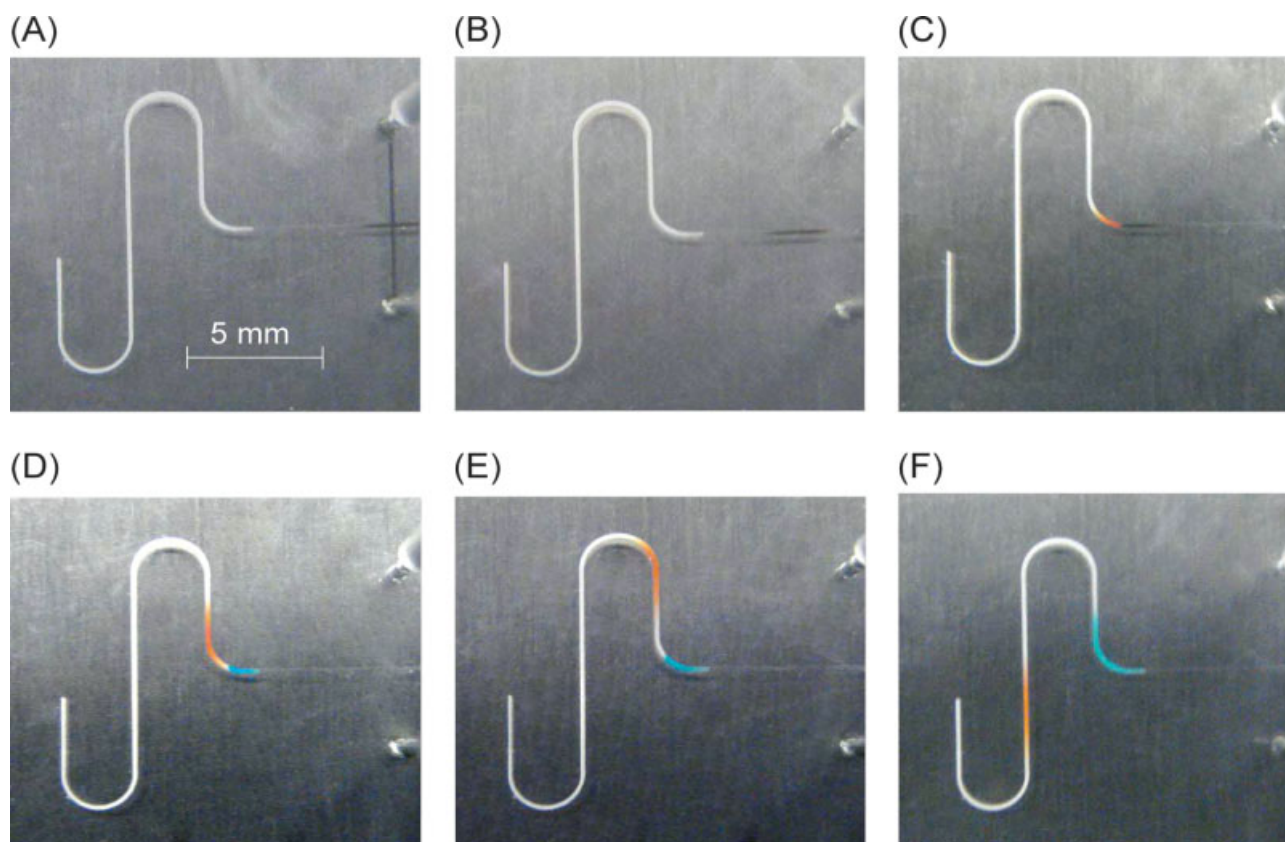
The PDMS chips were prepared by using a mold created by soft photolithography [21]. The chip with standard cross-T type channel of 100  $\mu\text{m}$  wide and 35  $\mu\text{m}$  height was fabricated using a procedure detailed in our recent work [20]. At the electrode ports buffer reservoirs made from PDMS were sealed. The chip was irreversibly sealed onto a quartz slide of 0.5 mm thickness (SPI Supplies, West Chester, PA).

The packing of the channel of a standard PDMS chip for the chromatographic studies was described earlier [20] but is discussed here briefly. The fritless packing of the chip is based on a temporary, approximate 80% taper of the channel, which traps all the particles yet allows for fluid flow through the tapered region with moderate resistance. The front end of the packing can be positioned on the chip by pressing downward on the top of the PDMS chip just above the fluid channel where the chromatographic particles are trapped. In order to temporarily taper the microfluidic channel, the top of the flexible chip is pushed downward around the point of the channel where the packing begins. About 80% taper (closure) is needed to trap the particles and to allow for flow of liquid through the tapered region. A suspension (0.05–0.5  $\mu\text{L}$ ) of freshly ultrasonicated, methanolic C18 particles was manipulated through a small-bore tubing (0.3 mm id) using a peristaltic pump, and connected to the outlet port and washed with methanol (10  $\mu\text{L}/\text{min}$ ) for 2 min. A pressure of approximately 2 bar (maximal pressure attainable by the peristaltic pump) was intermittently applied for short periods (4–5 s). After the methanol was rinsed out of the channel with water, the tapering was stopped and methanol

and water was pumped through the channel from the reverse direction (inlet port) first moderately and then with increasing pressure to obtain a smooth front edge of the packing. The packed channel was then rinsed with water and heated at 115°C overnight to maximize the stability (compactness) of the packing [13].

The injection and the quantitative determination of a small plug of sample in a microchip can be considered critical points of microfluidics. The most commonly utilized form of sample introduction in microchips is electrokinetic injection mainly due to its ease of use (no external pumps or valves are necessary to manipulate fluid in the chip). Unfortunately, difficulties in quantitation of the injected sample, caused by mobility and matrix bias, limit the use of microchips. Although it is well known that pressure (hydrodynamic or hydrostatic) injections can provide bias-free injections, only a few recent publications have detailed such procedures [22].

Using our chips packed with the chromatographic particles we found that the presence of the high-flow resistance packing in the separation channel spontaneously solved several injection problems well known in microfluidics technology. The sample injection method used in this work utilizes hydrodynamic pressure, thereby, reducing the propensity for sample bias during the injection. We used a single-channel peristaltic pump for the injection. Initially, a small volume (0.5–5  $\mu\text{L}$ ) of solution was manipulated into the peristaltic pump tubing. The sample was subsequently injected at the sample inlet port and was manipulated into the other three channels with different flow rates depending on the hydraulic resistance of each channel (Fig. 1A). Due to the high hydraulic resistance of the packing, a largely reduced flow rate was observed in the separation channel permitting the injection of a small sample plug of solution of only a few nanoliters into the separation channel (Fig. 1B). Because the hydraulic resistance in the separation channel of the used chip is estimated to be approximately 1000 times higher (that is the flow rate is 1000 times smaller) than in the other channels, when 1  $\mu\text{L}$  of sample is injected into the chip with the peristaltic pump, only about 1 nL is injected into the separation channel; the majority of the sample solution flows to the waste outlet reservoir and the buffer inlet. The sample volume injected into the separation channel is determined by the sample volume that is previously introduced into the pump tubing connected to the sample inlet port of the chip. The speed of the pumping solution has no influence on the amount of sample injected into the separation channel since the ratio of the flow rates toward the outlet ports is constant. Pumping at a higher rate only shortens the duration of the injection, but the volume of sample injected remains the same. The volume of the sample plug injected into the separation channel can be determined by monitoring the plug leaving the junction (this can be monitored microscopically using a colored sample plug). The sample volume injected into the separation channel is defined by the flow resistance of the chromatographic packing related to the flow



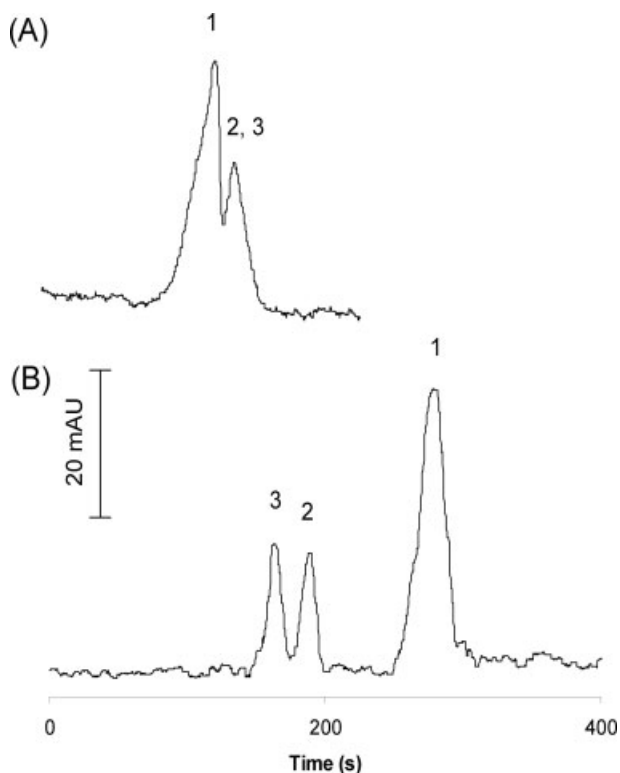
**Figure 1.** Pictures of the separation channel with the C18 packing of the PDMS chip during pressure injection (A, B) and CEC separation (C–F). Approximately 5  $\mu$ L of the sample and carrier are continuously pumped from the sample inlet port (A). When the sample plug completely entered into the separation channel, the pumping was stopped and high voltage was applied at the ends of the channel (B). The sample plug was transported to the packing by using high voltage (C), and complete separation could be achieved already in the first 4 mm of the packing (D). The blue dye was retained, while the red dye eluted from the packing (E, F) (carrier: 50 mM phosphate, pH 6.8, sample: mixture of blue and red dyes, voltage during CEC was 750 V).

resistance of the other part of the channel system. The flow resistance of the chromatographic packing depends on the geometry of the packed channel (only the length of the packed channel can be varied during the packing) and the geometry of the chromatographic particles (their size and shape). Each parameter (longer length of the packing, decreasing the size of the chromatographic particles, and improving the sphericity), by which the efficiency of the separation can be improved, increases the flow resistance of the packing that is, decreases the injected volume of the sample. It is not mandatory to know the exact amount of solution injected since the analysis is based on a relative calibration.

The precision of the injection is almost exclusively determined by the precision of introducing the sample into the pump tubing. In our experiments the required volume of sample (0.5–5  $\mu$ L) was “manually” manipulated into the tube and the precision exceeded 2% RSD. Much better repeatability (less than 1% RSD) can be expected using special commercially available microinjectors.

In our earlier work, we described a chip packed with conventional chromatographic particles that provided for

facile liquid chromatographic separations [20]. Within the packing the separation mechanisms of chromatography and electrophoresis are effectively combined when high voltage is applied across the packing. Figure 1 shows a microfluidic channel packed with C18 beads used in the separation of a mixture of two food dyes (blue and red) in phosphate buffer on application of a voltage. The dyes were injected by pressure from the sample inlet port into the separation channel through a cross-T junction and manipulated into the chromatographic packing (Figs. 1A–C). Complete separation was achieved within the first 4 mm of packing (Fig. 1D). The dispersion of the unretained red dye was relatively small as it moved through the packing. The blue dye was completely retained on the chromatographic packing even after the red dye had eluted from the packing (Figs. 1E–F). Although the blue dye may occupy a large area upon adsorption to the chromatographic packing, upon elution with a 50% methanol solution, the dye stacks on the beads and is observed as a sharp peak at the point of detection. Using a phosphate buffer containing 30% methanol, baseline separation of the dyes was achieved.



**Figure 2.** Separation of cephalosporin antibiotics in chip packed with C18-modified silica particles in (A) LC and (B) CEC mode. (sample: 1, ceftriaxon; 2, cefazolin; 3, ceftazidim,  $c = 10$  mg/mL, detection position: 2 mm after the end of the packing,  $\lambda = 265$  nm, carrier: 50 mM phosphate, pH 6.8, voltage was 750 V during CEC, flow rate (in the separation channel) was 0.4 nL/s during LC).

To further test the efficiency of the chromatographic packing in the chip three cephalosporin antibiotics (ceftriaxon, cefazolin, and ceftazidim), having relatively similar chemical structures, were injected in phosphate buffer (methanol content in the carrier fluid does not improve the separation due to the hydrophilicity of the analytes). When the sample plug was manipulated by pressure through the packing the three analytes did not completely separate (LC mode, Fig. 2A). When the same volume of sample was injected and driven by an electric field the three antibiotics separated with baseline resolution (CEC mode, Fig. 2B). The sequences of the eluted components were different in LC and CEC mode. This situation can only happen if the separation mechanism is largely different in LC and CEC. Since the contribution of the zone electrophoresis in the CEC separation of the studied components (cephalosporins) is more considerable than the contribution of the RP-LC, firstly the least (negatively) charged component will be eluted in CEC. In case of RP-LC separation the firstly eluted component is the least nonpolar one.

We have described a complete chip-based electrochromatographic analysis system in which sample injection, separation, and direct UV detection are easily per-

formed. Since RP silica particles are widely used as the stationary phase in HPLC and SPE, the described chip-based electrochromatographic system has great potential in many applications (e.g., preconcentration and purification). A complete separation of two dyes could be achieved already in the first 25% length of the packing. The CEC was also suitable to separate three cephalosporins despite of the relatively high concentration of the analytes needed for the UV detection, while the LC did not result in proper resolution. The high flow-resistance of the packing reduces common injection problems found in chip-based analysis. When 1  $\mu$ L volume of sample is injected into the chip, only approximately 1 nL of solution entered the separation channel due to the high hydraulic resistance of the packing. This type of injection should not suffer from matrix and mobility biases, which are well known in electrokinetic injections. Our recent results show that different conventional phases can be integrated in the same microchip and used for parallel analysis. Further investigation and characterization of the packing and use of other types of particles are being investigated.

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