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Research Article

Microfluidic ‘‘thin chips’’ for chemical separations

This paper describes the design, development and application of microfluidic ‘‘thin chips’’ fabricated from PDMS. Thin chips consist of multiple layers of PDMS chemically bonded onto each other. Unlike thicker PDMS chips that suffer from lack of sensitivity due to PDMS absorption in the VIS and UV range, the thinness of these chips allows for the detection of chromophoric species within the microchannel *via* an external fiber optics detection system. C18-modified reversed-phase silica particles are packed into the microchannel using a temporary taper created by a magnetic valve and separations using both pressure- and electrochromatographic-driven methods are detailed.

Keywords:
Electrochromatography / Microfluidic thin chips / PDMS / Reversed-phase silica particles

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1 Introduction

The development of microfluidic chips and devices offer a number of advantages over conventional analytical techniques including small sample volume requirements, portability, fast sampling times, ability to multiplex and compatibility with other techniques [1]. Microfluidics has been the motivation for various biochemical application advancements in point-of-care diagnostics, bioterrorism detection and drug discovery. Potential applications include biotechnology, pharmaceuticals, life sciences, defense, public health and agriculture.

PDMS is a favorite material to construct microfluidic chips due to its cost, ease of fabrication and robustness [2–7]. One difficulty with PDMS is that it absorbs in the VIS and UV range, thereby making detection problematic unless alternative detection systems are imbedded within the chip. There are a number of studies that detail novel detection schemes for PDMS-based microchips, but they either require the compounds of interest to be labeled (in the case of fluorescent detection) or integration of detection components (for example, conductivity and amperometric) embedded into the chips. Although labeling of molecules is now well defined, the use of label-free detection modes is growing and will surely increase in usage in lab-on-a-chip devices. Similarly, incorporation of sensor components into chips is quite developed [8–16]. On the other hand, it would be beneficial if neither labeling nor chip modification was needed to detect small molecules in the ultraviolet and VIS range using a PDMS-based microchip.

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There is currently a high demand for miniaturized separation techniques that are easy to use, versatile and inexpensive to fabricate. Recent studies have documented the development of both LC and CEC-based platforms on-a-chip for separating proteins, peptides, DNA and viral and bacterial cells [1]. One area of interest is using the microchips for separations chemistry. Packed bed chromatography is one area of separations that is amenable to microfluidics-based techniques. Reversed-phase silica particles (*e.g.* C18) are widely used as the stationary phase in HPLC and SPE for preconcentration and separation of analytes or to remove unwanted components from samples. In our recent work [17], we described the fabrication of a PDMS-based microfluidic chip containing reversed-phase 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 (clamping and anchor-effects) appearing in the packed channel were observed.

Herein, we extend our work in chip-based LC and CEC by demonstrating the use of ‘‘thin chips’’ in separations. The chips consist of multiple layers of PDMS and allow for facile detection of analytes *via* a fiber optic assembly configured perpendicular to the chip. The thinness of the chips minimizes PDMS absorption in the VIS and UV range. Pressure- and electrochromatographic-driven separation methods are detailed.

2 Materials and methods

2.1 Chemicals and reagents

The reversed-phase chromatographic packing material consisted of porous, C18-modified, 10-ìm particles (Western

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Analytical Products, Wildomar, CA, USA). Neodymium sphere magnets (1/8″ diameter), block magnets (1/8″ × 1/8″ × 1/16″ thick) and cylinder magnets (1/16″ diameter × 1/16″ thick) were purchased from K&J Magnetics (Jamison, PA, USA). 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 blue1, FD&C yellow5 and FD&C red40, all from McCormick, MD, USA) and three cephalosporin antibiotics (ceftazidim from Roche, Switzerland, ceftriaxon from Glaxo, UK and cefazolin from Bristol-Myers, Italy) were prepared in water. The buffer electrolyte for the electrophoretic and the electrochromatographic separation contained 50 mM phosphate, pH: 6.8. All solutions (methanol, water) were degassed and filtered through a 0.45 μm syringe filter.

2.2 Instrumentation

For the sample injection, a single-channel peristaltic pump was used. The samples (0.5–5 μL) 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 by a UV–VIS fiber optic positioned directly on the chip and connected to a miniaturized spectrophotometer (Ocean Optics, USA). The fibers were arranged perpendicular to the microfluidic channel (above and below the chip) using an adjustable stand (x–y translational stage). Since the detection was performed externally, the fiber optics could be positioned at any point along the chip. The PDMS chip with quartz slide could be used for the detection at 265 nm. 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.

2.3 CEC chip fabrication

2.3.1 Chip fabrication

The PDMS chips were prepared by using a mold created by soft photolithography. The pattern consisting of standard cross-T-type channel of 100 μm wide was designed using AutoCAD software (San Rafael, CA, USA) and printed as a high resolution (20 000 dpi) photomask (CAD/Art Services, Bandon, OR, USA). Negative type photoresist (SU-8 2025, Microchem, Newton, MA, USA) was spin-coated onto a 3″ silicon wafer at 3000 rpm for 30 s to a thickness of 30 μm. The photoresist-coated wafer was baked for 15 min at 95°C. The pattern on the mask was transferred to the wafer through UV exposure for 2 min. The exposed wafer was baked at 95°C for 5 min and unexposed areas were removed by rinsing with SU-8 developer (Microchem). The PDMS chip was fabricated by cast molding of a 10:1 mixture of PDMS oligomer and cross-linking agent (Sylgard 184, Dow Corning, Midland, MI, USA). The PDMS mixture was degassed and baked at 80°C for 30 min. The PDMS replicas were peeled off from the mold. Holes (300 μm diameter) for the liquid connections were punched through the PDMS chip. 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, USA) (Fig. 1). The fabrication process to incorporate magnetic valves (Fig. 2) was detailed earlier [18].

2.3.2 Fritless packing of chip with chromatographic particles

The packing of the channel of a standard PDMS chip for chromatographic studies was described earlier [17] but is discussed in brief below.

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. 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 μL) 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 μL/min) for 2 min. A pressure of approximately 2 bar 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

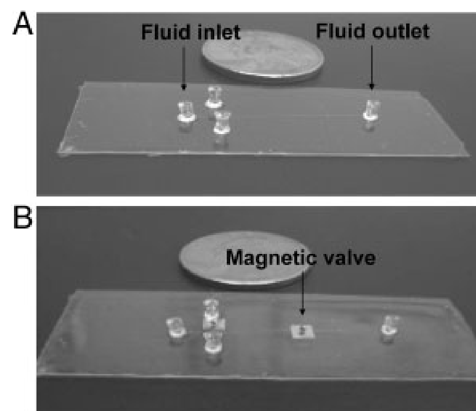

Figure 1. Microfluidic thin chip (A) with and (B) without magnetic valves.

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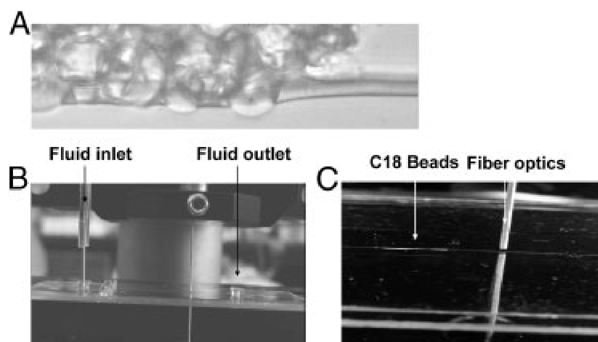

Figure 2. (A) C18 beads packed into microfluidic channel. (B) Picture of the thin chip set-up with single liquid input and fiber optics. (C) Optical micrograph of a representative section of the channel in the thin chip packed with C18 beads with fiber optics detection set-up. (Width of the channel: 100 μm).

increasing pressure to obtain a smooth front edge of the packing. Figure 2A shows the beads packed in the microfluidic channel. The packed channel was then rinsed with water and heated at 115°C overnight to maximize the stability of the packing. The packing was washed with methanol at pressure of about 2 bar before use. Figure 2B shows the fiber optic set-up using a microfluidic thin chip. Figure 2C is a close-up showing the C18 beads packed in the microchannel prior to the fiber optic configuration.

3 Results and discussion

3.1 Sample injection with hydrodynamic pressure into the chip

The injection and the respective volume measurement 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) [19]. 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 [20–22].

Using the 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 μL) 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

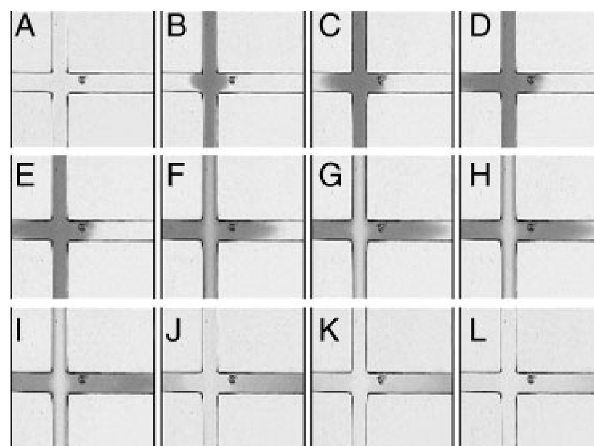

Figure 3. Representative series of pictures capturing the flow of a plug of green dye in a microchannel of a thin microfluidic PDMS chip packed with C18 beads in the outlet channel. Frame a shows the cross-section of a standard T-channel filled with deionized water without sample. Frame b shows the initial introduction of green food dye through the side inlet channel at the top. Frames c-l show that the majority of sample is pushed through the two outlet ports (left and bottom of the frame) forcing a small amount of fluid through the separation channel. The time at which each frame was taken is displayed in seconds in the lower portion of each picture.

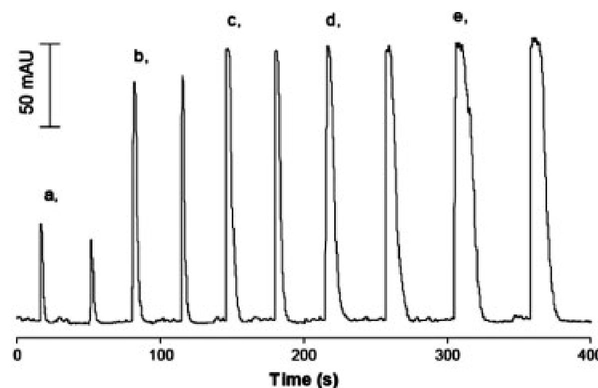

Figure 4. Absorption signals of manually injected plugs of green food coloring in a thin microfluidic chip. Plugs were detected directly on the chip before the chromatographic packing. The volumes of the samples pumping into the sample inlet port were (a) 0.5 μL, (b) 2.5 μL, (c) 5 μL, (d) 10 μL, and (e) 20 μL (sample: FD&C blue1, $\lambda = 620$ nm)

on the hydraulic resistance of each channel (Fig. 3A). Because of 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. 3B). Because the hydraulic resistance in the separation channel of the used chip is estimated to be approximately one thousand times higher (that is, the flow rate is one thousand times smaller) than in the other channels, when 1 μL of sample is injected into the chip with the peristaltic pump, only about 1 nL is injected into the separation

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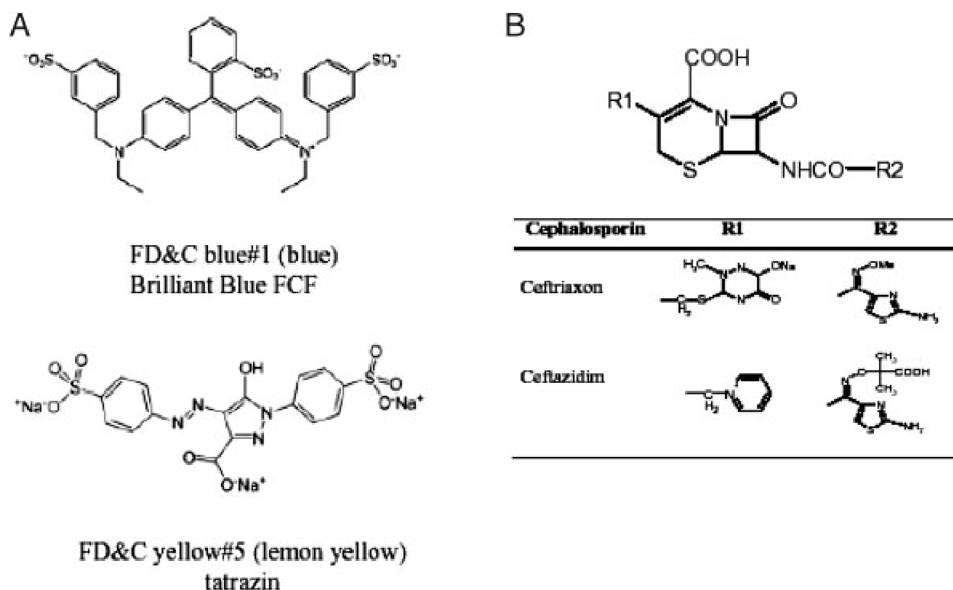

Figure 5. Structures of (A) dyes and (B) cephalosporin antibiotics.

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). It is not mandatory to know the exact amount of solution injected since the analysis is based on a relative calibration. Electrokinetic injections are biased making it difficult to determine the exact amount of sample volume injected.

The absorption signals of different volumes of dye solution introduced into the inlet port are shown in Fig. 4. The sample plugs are detected before the chromatographic packing. When small plugs (length to width ratio of the plug is smaller than 10; the volume of the plug is smaller than 3 nL) are injected into the separation channel, the dispersion of the solution resulted in reduced signal heights. Larger and constant absorbance values were obtained for sample volumes greater than 3 nL and the areas of the peak increased with increased sample volume.

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 μL) 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.

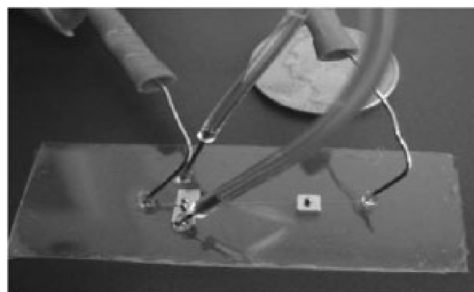

Figure 6. Picture of the chip configured with magnetic valves and electrodes. Width and height of the channels are 100 and 35 μm, resectively. Distance between the electrodes is 3 cm.

3.2 Electrochromatographic tests

In our earlier work, we described a chip packed with conventional chromatographic particles that provided facile liquid chromatographic separations [20]. Hence, we suspected that electrochromatographic-based separations on microfluidic chip would result in greater separation efficiencies because of the flat flow profile induced by EOF since convective band broadening would be diminished. Within the packing the separation mechanisms of chromatography and electrophoresis are effectively combined.

To further test the efficiency of the thin chip, three cephalosporin antibiotics (Fig. 5) (ceftriaxon, cefazolin and ceftazidim), having relatively similar chemical structures, were examined for separation on the thin chip using C18 as packing particles. Figure 6 shows the microfluidic thin chip set-up with connected cathode and anode electrodes. This chip consists of three magnetic valves although not all were required for this experiment. The sample was injected in phosphate buffer from the sample inlet port into the separation channel through a cross-T junction and

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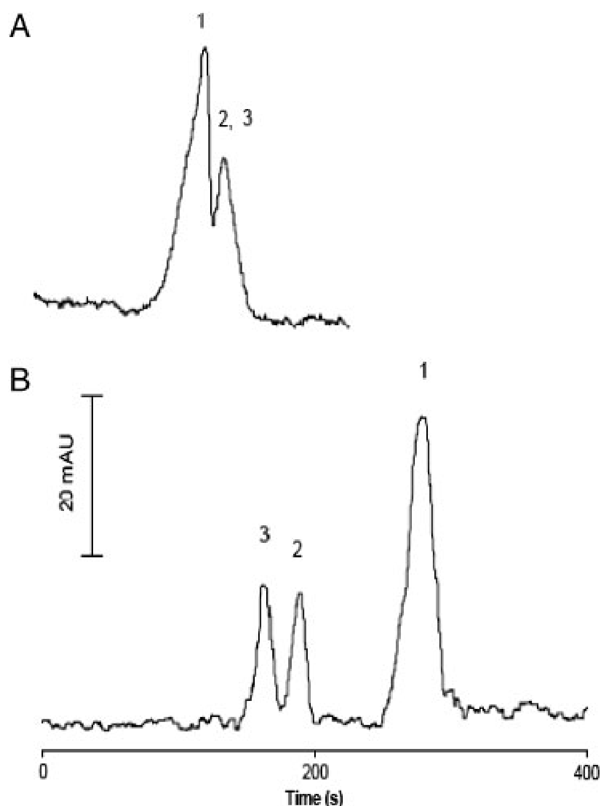

Figure 7. Separation of cephalosporin antibiotics in thin chip packed with C18 modified silica particles in (A) LC and (B) CEC mode. (sample: 1, ceftriaxon; 2, cefazolin; 3, ceftazidim; c = 10 mg/mL; detection position: 2 mm after the end of the packing; λ = 265 nm; carrier; 50 mM phosphate, pH 6.8, voltage was 750 V during CEC, flow rate in the separation channel was 0.4 nL/s during LC).

manipulated into the center microfluidic channel (methanol content in the carrier fluid does not improve the separation due to the hydrophility of the analytes). When the sample plug was manipulated by pressure (LC mode) through the packing, the three analytes did not completely separate (Fig. 7A). When the same volume of the sample was injected and driven by an electric field (CEC mode), the three antibotics separated within 5 min and with baseline resolution (Fig. 7B).

4 Concluding remarks

We have described the design, development and application of microfluidic "thin chips" by detailing pressure- and electrochromatographic-driven separation methods. Sample injection, separation and direct UV detection *via* a fiber optic assembly configured perpendicular to the chip are easily performed using the chips. The thinness of the chips minimizes PDMS absorption in the VIS and UV range. Thin chips allow for the integration of facile fiber optic-based detection systems without the need of other complex detection systems that reduce the benefits accrued from a microfluidic platform. First, proof-of-concept is demonstrated, whereby detection on-chip is shown using a manually controlled magnetic valve. Second, using a similar chip packed with C18 particles, the efficiency of the chromatographic packing in the chip was tested by examining the separation of three cephalosporin antibiotics (ceftriaxon, cefazolin and ceftazidim). Complete separation and resolution was achieved under the CEC mode and not the LC mode. Future work will focus on developing the thin chip methodology for other applications including protein separations.

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The authors have declared no conflict of interest.

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