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Fabrication of Fritless Chromatographic Microchips Packed with Conventional Reversed-Phase Silica Particles

Attila Gaspar, Menake E. Piyasena, and Frank A. Gomez*

Department of Chemistry and Biochemistry, California State University, Los Angeles, 5151 State University Drive, Los Angeles, California 90032-8202

This paper describes the development and study of a disposable and inexpensive microfluidic chip, fabricated from poly(dimethylsiloxane) (PDMS) incorporating conventional chromatographic reversed-phase silica particles (C18) without the use of frits, permanent physical barriers, tapers, or restrictors. The packing of C18 modified silica particles into the microfluidic channels is made possible by the hydrophobic nature and excellent elasticity of PDMS. Keystone-, clamping-, and anchor-effects provide the stability and the compactness of the packing and attenuated wall-effects were observed.

Despite the high demand for miniaturized liquid chromatographic (LC) techniques, few chip-based chromatographic systems compare to chip-based capillary electrophoretic (CE) devices, mainly due to technical problems inherent in the former.[1−2] First, Manz et al. described a LC partially integrated onto a silicon chip in which 5 μm C8 particles were retained by frits in the column.[3] Since the packing process including frit preparation was enormously difficult, the very few initial chromatographic methods applied the open-tubular approach.[4] Other devices have incorporated a physical barrier[5] or tapered capillary geometries[6,7] and have been used to trap stationary-phase particles. Hjertén showed that the problems of packing can be eliminated if the packed bed is replaced by a continuous bed polymerized in situ in the chip channels.[8] Yin et al.[9] described a new approach to nanoLC−MS using a polymer microfluidic chip fabricated by laminating polyimide films. High-speed electrochromatographic separations on a silica colloidal stripe[10] and in a fully packed capillary microchip[11] have also been developed.

Herein, we describe the fabrication of a PDMS-based microfluidic chip containing reversed-phase silica beads (C18) without the use of frits or other barriers. The possible reasons for the unexpectedly easy stabilization of the chromatographic packing within the channels are described.

EXPERIMENTAL SECTION

Materials. The reversed-phase chromatographic packing material consisted of porous, C18-modified, 10 μm diameter particles (Western Analytical Products, Inc., Wildomar, CA). A suspension (20 μL) of C18 beads was prepared in methanol by sonication (10 min) and vortex mixing. Stock solutions of food dyes (FD&C blue#1 and FD&C yellow#5, both from McCormick & Co., Inc, MD) were prepared in water. All solutions (methanol, water) were degassed and filtered through a 0.45 μm syringe filter.

Microchip Fabrication. The PDMS chips were prepared by using a mold created by soft photolithography.[12] The pattern consisting of 100 μm wide channels was designed using AutoCAD software (San Rafael, CA) and printed as a high resolution (20 000 dpi) photomask (CAD/Art Services, Inc., OR). Negative type photoresist (SU-8 2025, Microchem, Newton, MA) was spin-coated onto a 3 in. 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 photomask pattern was transferred to the wafer via UV exposure for 2 min. The exposed wafer was baked at 95 °C for 5 min and developed by rinsing with SU-8 developer (Microchem, Newton, MA). 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). The PDMS mixture was degassed and baked for 30 min in an oven at 80 °C. The PDMS replicas were peeled off from the mold.

The chips contained a cross-T type channel, and it was prepared by simple cast molding. Holes (300 μm diameter) were made for the liquid connections by punching through the PDMS chip. The chip was irreversibly sealed onto a clean cover glass of 2 mm thickness (VWR microcover glass, VWR, USA) or onto a quartz slide of 0.5 mm thickness (SPI Supplies, West Chester, PA) using an Ar plasma.

* Corresponding author.

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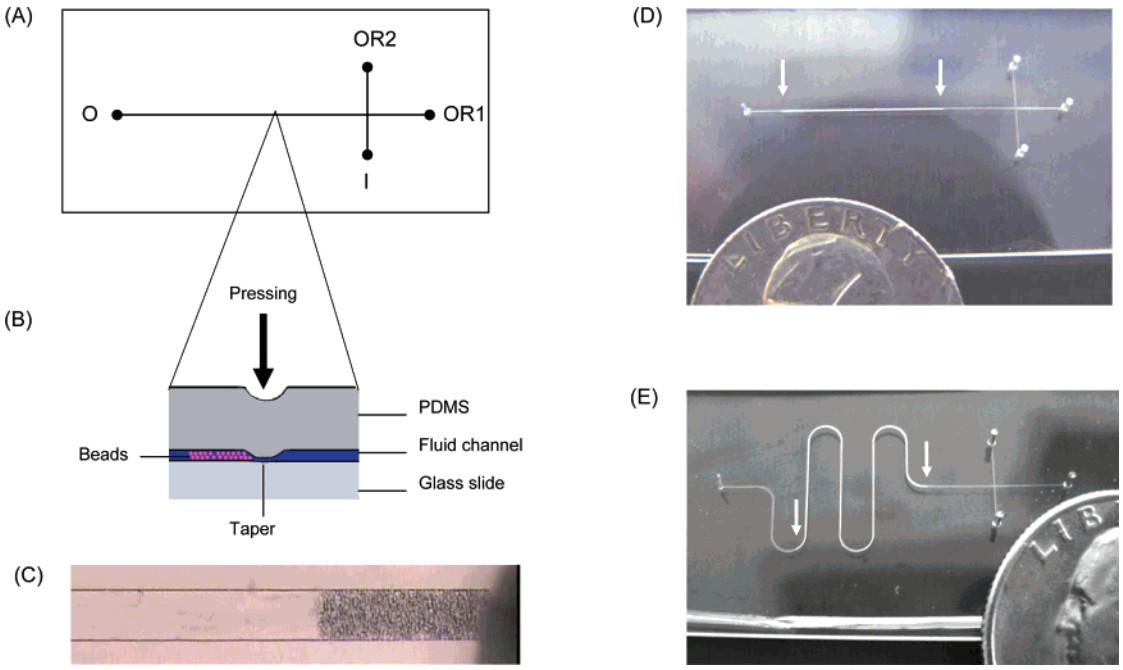

Figure 1. (A, B) Schematic illustration of the packing of a microchannel in a PDMS chip through pressing the top of the flexible PDMS chip to trap the chromatographic beads (not to scale; I, sample inlet; O, separation outlet; OR1 and OR2 are outlet reservoirs; the suspension of particles is pumped from O). (C) Optical micrographs of the fluid channel in front of the tapering after the pumping of the suspension of C18 particles. The obtained chips with straight (D) and curved (E) channels packed with C18 particles (the height and width of the packing were 30 μm and 100 μm, respectively, the length of the packing was 1 cm (D) and 3 cm (E). (The arrows indicate the beginning and end of the C18 packing.)

Packing of C18 Silica Beads. The packing of C18 particles in the channel is schematically shown in Figure 1A,B. First, the PDMS chip is rinsed with degassed methanol for 3 min to clean the PDMS wall and to remove any water from the channel. The top of the flexible chip is then pushed downward (using a metal rod or with the help of the magnetic valve[13]) around the point of the separation channel where the packing should begin. About 80% taper (closure) was needed to achieve the trapping of the particles while maintaining a continuous flow.

A suspension (0.01−0.1 μL) of freshly ultrasonicated, methanolic C18 particles was manipulated through a small-bore tubing (0.3 mm i.d.) using a peristaltic pump, connected to the O port (outlet of the separation channel of the chip), and washed with methanol (10 μL/min) for 2 min. A pressure of approximately 2 bar (maximal pressure attainable by the peristaltic pump) was intermittently applied (4−5 s). The methanol was rinsed out of the channel with water. The tapering was removed (either by lifting the metal rod or opening the magnetic valve), and methanol and water were pumped through the channel from the reverse direction (I port) first moderately and then with increasing pressure (as described above) to obtain a smooth front edge of the packing. The packed channel was then rinsed with water and heated at 115 °C for 2 h (Ceriotti et al.[7] stabilized the packing by placing the packed capillary in an oven to force interparticle bonding.). The packing was rinsed with methanol at higher pressure (2 bar) prior to use.

Injection of Sample Plug and Flow Visualization. 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 (0.1−2 μL) of solution was manipulated into the peristaltic pump tubing (i.d., 0.3 mm) which was previously filled with water. The sample was subsequently injected at the sample inlet (I) port and was manipulated into the other three channels with different flow rates depending on the hydraulic resistance of each channel. Because of the high hydraulic resistance of the packing, a reduced flow rate was observed in the separation channel, resulting in an injection of a sample plug of less than 1 nL.

To observe the movement of liquids in the microchip channels, food dyes were injected and transported via a peristaltic pump. The movement of the plugs were monitored using an inverted microscope (Nikon Eclipse TE2000-S) equipped with a color CCD camera (Panasonic GP-KR222). Movies and the images were captured by Pinnacle Studio 9 (Mountain View, CA) software.

RESULTS AND DISCUSSION

Packing of C18 Modified Particles and Reasons for Its Good Stability in the Chip. The ability to pack C18 modified particles into the microfluidic channels is due to the hydrophobic nature and excellent elasticity of PDMS. PDMS has a low Young's modulus (E = 0.5−4 MPa depending on the curing conditions) and high Poisson ratio (v = 0.5 that is essentially incompressible) making it a rubber-like material.[14] One-dimensional stress-chain analysis implies a structural deformation of about 10% under pressure of 1 bar.[15] In a rigid channel, the pressure drop varies linearly with the flow rate. On the other hand, the pressure drop

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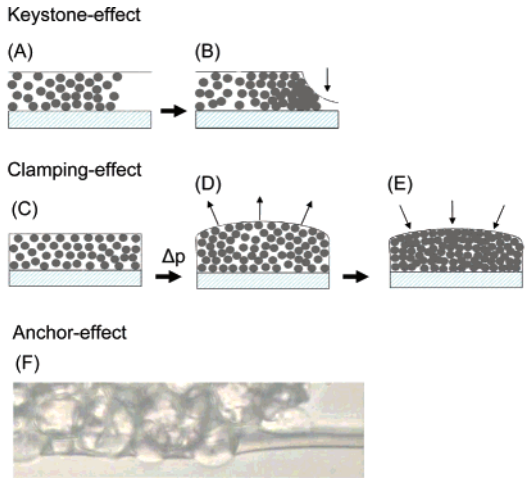

Figure 2. Schematic representation of (A, B) the keystone-effect, (C, D, E) the clamping-effect, and (F) optical micrograph of the outermost part of the packed channel showing the anchor-effect.

in an easily deformable PDMS channel is much less; hence, PDMS microchips are able to withstand higher pressures (flow rates) without leaking. Gervais et al.[15] measured 25% deformation in height in a PDMS chip (25 μm × 250 μm, E = 2.2 MPa). The authors could achieve sufficient tapering of the microfluidic channel by pushing downward on the top of the PDMS chip above the given point of the channel with the aid of laboratory tweezers.

In our experiments, an approximate 80% taper effectively traps the particles (a channel of 30 μm height was tapered to approximately 6 μm). We trapped 10 μm diameter particles when the channel height was decreased to about 6 μm yet maintained a continuous liquid flow with moderate resistance. The front end of the packing can be precisely positioned on the chip by pressing downward on the top of the PDMS chip. The length of the packed segment can be varied by controlling the amount of particles (defined by the density and the volume of suspension) introduced into the channel. The packed length of the channel is linearly proportional to the amount of time it takes to fill the channel (if the flow rate and the density of the suspension are constant). It was observed that the end of the packing is compact and relatively sharp and that the newly arrived beads adhere smoothly to the packing, increasing its length continuously. To obtain a compact packing, the intermittent application of maximal liquid pressure to the channel is essential.

The most simple type of fritless systems is the application of tapered outlet geometries.[6−7] At the taper, the density of the particles increases causing aggregation without the need of a frit or physical barrier. The first particles act as the keystones, blocking the other particles and allowing the packed section to increase in length (Figure 2A,B). Experiments demonstrated that it was only necessary to taper the capillary to approximately 10 μm (inner diameter) to achieve the *keystone effect* and to retain 3 μm sized silica beads.[6] In our system, the keystone effect is not operative after the temporary tapering. However, other retaining effects appear in our chromatographic packing technique.

When pressures of about 2 bar are intermittently applied to compress the packing, the wall of the channel is deformed

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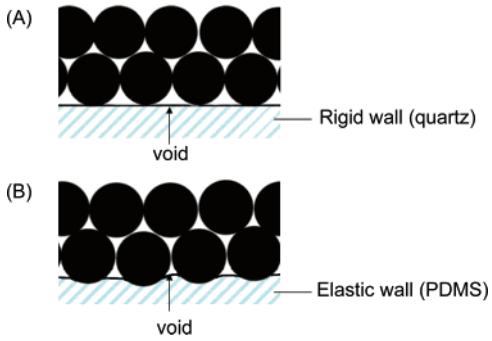

Figure 3. Schematic representation of the wall-effects in a channel with a (A) rigid wall and (B) in an elastic PDMS chip.

(extended). During this period, the particles fill the enlarged volume of the channel and then the channel shrinks when the pressure is released thereby forming a continuous strain around the packing (parts C−E of Figure 2). The particles of the packing are compressed by the forces of the elastic strains acting perpendicularly from the wall toward the middle of the channel. Finally, these forces derived from elastic strain clamp the whole packing into the microfluidic channel. This *clamping effect* is probably the main source of the stability of the packing in the PDMS channel.

The stability of the packing is also due to the strong particle−wall interactions between the C18 modified silica and the hydrophobic wall of the PDMS chip. Particles adjacent to the PDMS wall deform and partially penetrate into the wall which act as *anchors* for the packing (Figure 2F). The degree of penetration is dependent on the intensity of the intermittent pressures upon pumping and the elasticity of the PDMS.

In liquid chromatography, the *wall effect* is a consequence of the looser packing of stationary phase near the walls of the rigid column and is well-known.[1,16] The mobile phase has a tendency to flow faster near the wall due to the increased permeability. The solute molecules near the wall have greater mobility than the average molecules that make up the solute band resulting in band spreading. This effect is attenuated in our packed microchip because the outermost beads deform the PDMS wall (Figure 3). Hence, the size or density of cavities near the wall is either similar to or smaller than those found in the inner packing.

In our work, upon completion of the packing procedure, the particles remained static and degradation of the packing was not observed even upon application of an electric field or high-pressure pumping of organic solvents through the packing. At the end of the packing process, the chips were heated (115 °C) for 2 h to maximize the stability (compactness) of the packing. The packed microchip does not suffer from undesirable gravitational or hydrostatic flow due to the high hydrodynamic resistance of the packing in the separation channel (the unleveled heights of the liquids in the reservoirs generate minimal laminar flow, decreasing the precision of the successive determinations).

Chromatographic Test. Reversed-phase silica particles (e.g., C18) are widely used as the stationary phase in high peformance liquid chromatography (HPLC) and solid-phase extraction (SPE) for preconcentration and separation of analytes or to remove unwanted components from samples. In the present work, we used

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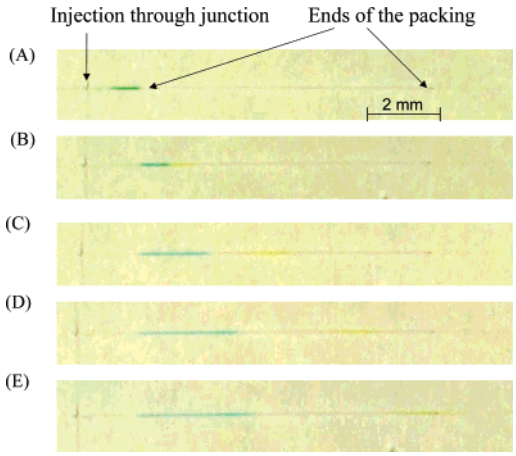

Figure 4. Pictures of the separation channel of the microchip packed with C18 modified silica particles. (A) A mixture of blue and yellow food dyes was injected by pressure applied from the bottom port (I). (B−E) While the yellow dye flows through the packing, the blue dye migrates at a slower rate and (E) has not yet reached the middle of the packing. (carrier, water; yellow dye, FD&C yellow#5; blue dye, FD&C blue#1).

mixtures of food dyes to demonstrate the applicability of the chip-based reversed-phase chromatographic separation. Figure 4 shows a packed microfluidic channel used to separate a mixture of two food dyes (blue and yellow). The dyes were injected by pressure from the sample inlet port into the separation channel through a cross-T junction and manipulated to the chromatographic packing (Figure 4A). Separation was achieved within the first 3 mm of packing. The blue dye was completely retained on the chromatographic packing even after the yellow dye had eluted from the packing (Figure 4E). Approximately 100 s after injection of the sample, methanol was pumped through the channel to elute the retained blue dye. The overall capacity was calculated to be 7.5 $\times$ 10^{-11} mol for the blue dye.

We believe the separation efficiency can be improved using smaller particles (3−5 μm). It should have great utility when coupled to other separation techniques including capillary electrochromatography (CEC). Areas needing further investigation and characterization include different types of particles and packing of the microchips.

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SUPPORTING INFORMATION AVAILABLE

Descriptions of preconcentration test, separation test, and movies and chromatograms and optical micrographs. This material is available free of charge via the Internet at http://pubs.acs.org.

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