ION LIQUID CHROMATOGRAPHY ON-A-CHIP WITH BEADS-PACKED PARYLENE COLUMN

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ABSTRACT

A parylene-MEMS ion-exchange Liquid Chromatography (LC) chip is presented here. The chip is integrated with microfluidic I/O ports, a separation column, frits/filters, and a conductivity detector. The column is packed with conventional LC stationary phase support materials, i.e. micro-beads with surface functional groups. To withstand high pressure normally encountered in High Performance Liquid Chromatography (HPLC), a self-aligned, channel-anchoring technique is developed to increase the pressure rating of the Parylene microfluidic devices from 30 to at least 800psi. On-chip injection, separation and detection of anions in water, with ~25ppm concentration, have been successfully demonstrated. To our knowledge, this is the first demonstration of microbeads-packed column ion liquid chromatography (LC) on a chip.

1. INTRODUCTION

High Performance Liquid Chromatography (HPLC) is one of the most popular, powerful, and versatile separation techniques for chemical analysis. Although LC column is normally made of capillary tubes due to fluidics limitations, the miniaturization of the column can actually improve separation performance. As shown in Fig. 1 [1] where same separation chemistry applies, separated peak width is independent of column ID, while peak heights are larger for smaller columns and/or smaller beads. In short, further down-sizing could be favorable in liquid chromatography.

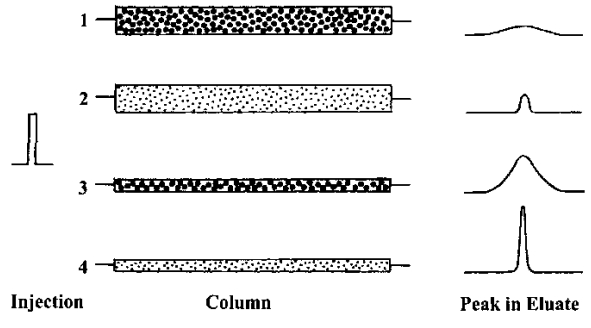

Figure 1. Separated peak shapes obtained with different column and bead size.

Compared to electrophoresis, however, little has been published about the integration of HPLC system onto a single chip. The two main reasons are the lacks of: (1) a process to integrate various components for an LC system; (2) high-pressure compatibility of the microfluidic devices needed for running HPLC. This work deals with both problems.

In terms of introduction and retaining LC stationary phase materials into the column, several approaches have been proposed, including open-tubular [2], coating micromachined posts arrays [3], coating microchannel with nanoparticles [4], monolith [5], packing [6], and packing without frit [7]. In this work, packing with conventional beads is chosen because of the following advantages. First, without introducing new surface chemistry, the extensively established separation knowledge can be utilized. Secondly, extreme flexibility is there to perform different types of liquid chromatography and/or to optimize particular separations, by choosing bead type, size, pore size, porosity, and functional group. Thirdly, packed column can achieve reproducible column performance, which is usually a problem in other methods.

2. DESIGN AND FABRICATION

The LC-on-a-chip device is made using parylene MEMS technology. The chip has four access ports, as shown in Fig. 2. LC mobile phase flows from port #1 to #2. Nanolitre volume sample injection is achieved with a cross channel injection method [8] from #3 to #4. Beads are packed externally into the on-chip column from port #1. Filters (channel with a height smaller than the bead size) trap beads in the main channel and prevent beads from entering the side injection channels. The bead-packed column inlet is near the sample output port #4, which is the injected sample plug front. The column outlet is at the filter/sensor edge (Fig. 2(b)). This integrated design minimizes dead volume, thus minimizing extra-column peak broadening. Off-chip pumps and valves are used in testing.

A conductivity sensor is used as the detector for ion sensing. Interdigital electrodes are patterned in the detector cell to monitor liquid conductivity. The conductivity of the mobile phase solution provides a baseline signal. When separated ion plugs pass by the detector, changes of solution conductivity are detected. Chromatogram is then obtained by recording conductivity of the solution flowing in the detection cell over time. To increase sensitivity, electrode width and spacing should be small, and the total electrode area should be maximized. However, the electrode design should be compromised in considerations of minimizing peak broadening and multiple-peak detection.

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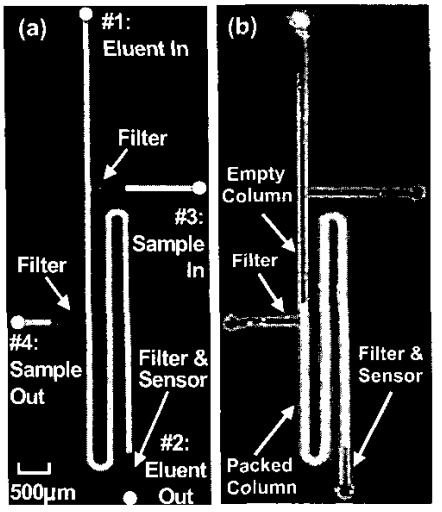

Figure 2. (a) Fluorescent overview picture of the integrated separation system. The channels are filled with Fluorescein; (b) Optical picture after packing beads.

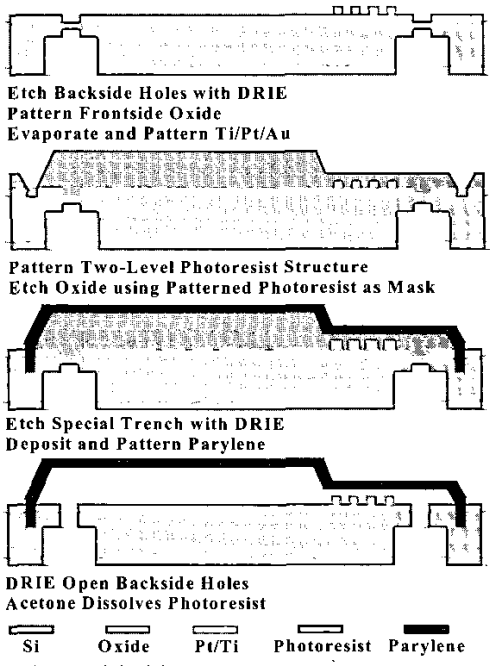

Figure 3. Simplified fabrication flow.

The fabrication process (Fig. 3) starts with growing thermal oxide on Silicon wafer. Then a two-step DRIE is performed on wafer backside to create the backside holes, leaving only a 50µm-thick diaphragm. Following frontside oxide patterning, a Ti/Pt/Au (300Å/2000Å/1000Å) layer is evaporated and patterned. Au is left only at bonding pads. Pt is used as the sensing electrode and is patterned to be interdigital 5µm-wide electrodes at 5µm intervals. 25µm-thick photoresist is spun and patterned with two masks to form two-level sacrificial photoresist structure. The 25µm-high channel part uses unexposed photoresist, while the 4µm-high filter part is partially exposed. A 15µm-wide "moat", self-aligned-to channels and filters, is fully exposed to the bottom oxide, which is subsequently etched away. Modified DRIE process [9] is then done to make 40µm-deep trench with mushroom bottom profile in the moat, or XeF_2 is used to roughen the moat area. About 10µm-thick parylene is conformally deposited filling up the trench or the roughened moat and is patterned. Backside holes are open with DRIE. Acetone dissolves photoresist and releases the device.

In the above process, a self-aligned, channel-anchoring technique is used to increase the pressure rating of the parylene chip. Figure 4 shows cross-sectional pictures of fabricated trench-anchored channel. Parylene devices without anchoring normally have a pressure limit of about 30psi, above which the top parylene layer of the channel tends to delaminate from the bottom surface. The testing results show the anchored channels do perform much better in terms of pressure compatibility.

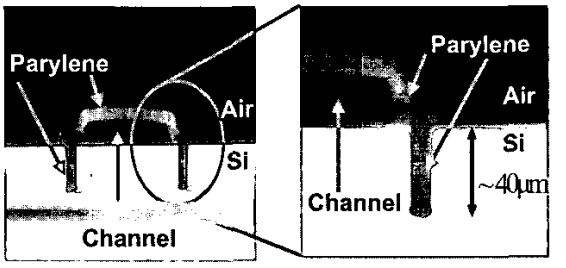

Figure 4. Trench-anchored channel cross-sectional pictures.

3. RESULTS AND DISCUSSION

A packaging jig (Fig. 5) is developed for convenient testing of the chips. A fabricated chip is clamped between a PCB and the PEEK jig. Squeezed o-rings at chip backside provide sealing. Standard fitting receiving ports are made in the jig, so fluid connections are easily obtained. Electrical contacts are made by wire bonding from the on-chip Au pads to the PCB. The jig can access 8 out of the 16 holes (4x4) simultaneously on the chip.

The pressure limits of trench-anchored and roughening-anchoring channels with an optional epoxy overcoat (Fig. 6) are obtained. Pressurized air is applied to special dead-ended testing channels. Bubble appearance in the water drop covering the channel indicates leakage. The pressure limit results are summarized in Table 1. It should be noted that the pressure limits also depend on the moat width, trench depth/shape, and parylene thickness. It is clear that trench-anchored channels perform much better than roughening-anchored ones, and epoxy overcoat can further improve the performance. Maximum pressure limit is achieved with trench-anchored, epoxy-coated channels. At the maximum testing pressure of 800psi, no leakage is found after an hour.

The failure modes for the two anchoring technique are different. Roughening-anchored channels leak out working fluid through tiny pathways in the roughened moat area at

high pressure, while the trench-anchored channels don't leak at all until the parylene breaks.

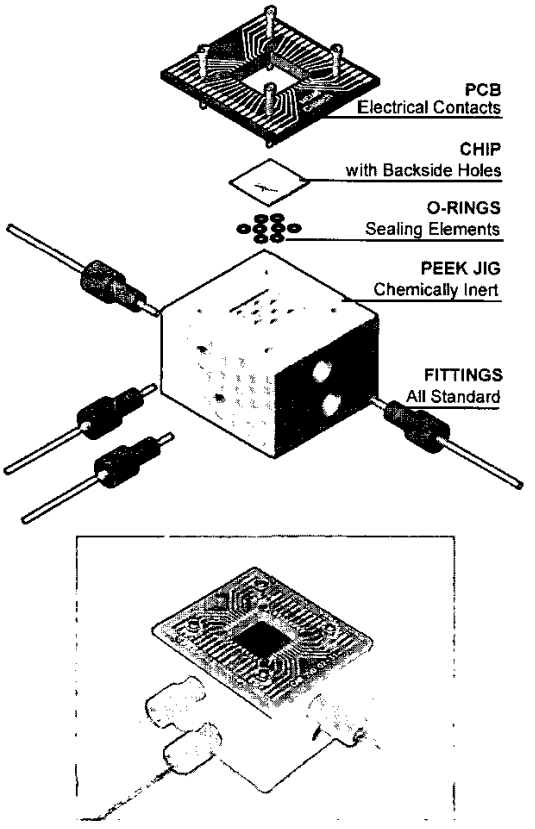

Figure 5. Parts of the testing jig and a picture of the assembled package.

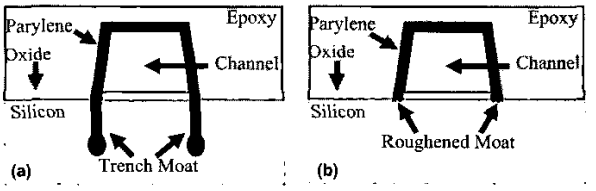

Figure. 6 Cross section of trench and roughening anchored channels with optional epoxy overcoat.

Table 1. Summary of the pressure testing result. 800psi is the maximum pressure of the testing setup. The safe pressures are for at least one hour. R: Roughening Anchoring; T: Trench Anchoring; E: Epoxy Overcoat.

	R	T	R + E	T + E
Safe Pressure	250 psi	600 psi	700 psi	800 psi
> Safe Pressure	Bubbles Appear	No Bubble until Break	Bubbles Appear	NA
Breaking Pressure	~ 350 psi	~ 700 psi	~ 800psi	> 800 psi

Packing of beads into the on-chip column is achieved with standard slurry technique (Fig. 7). In order to get good and repeatable separation, packing quality is very important. It is found that in order to get uniform and void-free column, high pressure/flowrate during packing is necessary. Beads used to produce ion-exchange columns for the following experiments are 7μm-diameter anion exchange porous PS-DVB (Polystyrene-Divinylbenzene) resin with Trimethyl-Ammonium groups from Hamilton. The resins are the same used in Hamilton's anion exchange column PRP-X110.

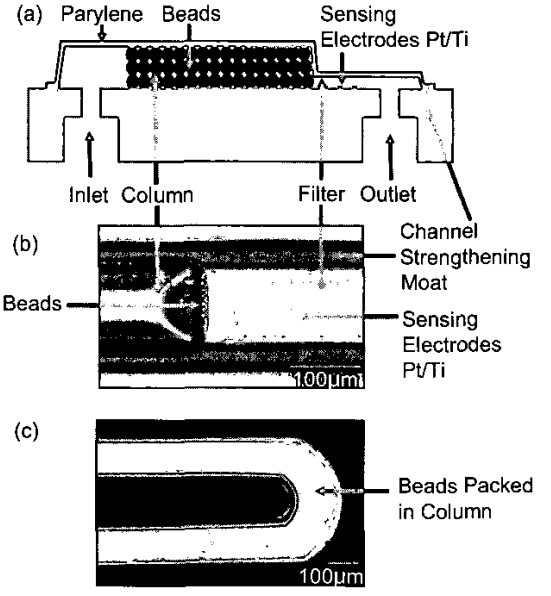

Figure 7. (a) Cross-sectional illustration of the device after packing; (b) Picture of column, packed beads, filter, detector, and channel-strengthening moat; (c) Fluorescent picture of a densely packed column.

Packed-column pressure flow-rate curve is measured for DI water (Fig. 8). The column is packed at 200psi before measurement. According to the theory [1],

$$F = \frac{\varepsilon}{\Phi}\frac{d_p^2 A_c}{\eta L_c}\Delta P$$

where

F: volumetric flow rate; ΔP: column presure drop;

ε: porosity of packed column; η: viscosity of the fluid;

d_p: beads diameter; Φ: dimensionless flow resistance;

A_c: column cross-sectional area; L_c: column length.

From the fitted linear curve, it is found that $\Phi/\epsilon = 444$. Assuming the porosity of the packed column is 0.8, which is normal for densely-packed porous-spherical-beads column, then Φ is 355, which is close to but smaller than its empirical value of 500 for slurry packed spherical porous-beads in conventional LC columns. It is believed that this is partly due to the small on-chip column to bead size ratio.

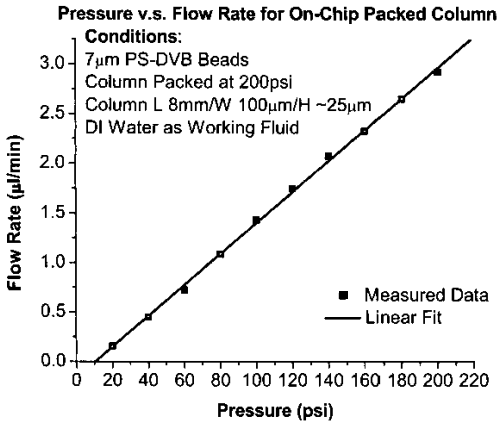

Figure 8. Pressure flow-rate curve for an on-chip packed column. Beads type, packing condition, column dimension, and working fluid are noted in the figure.

The detection cell impedance frequency response for ion-exchange chromatography mobile phase solution is measured (Fig. 9), which indicates that above about 10kHz, the impedance is almost independent of frequency. It is adequate for measuring frequency to be in this resistance dominant region to minimize electrochemical effects [10].

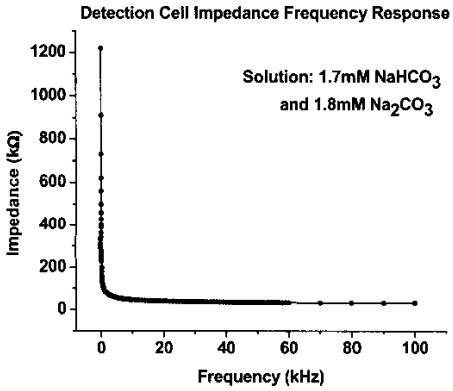

Figure 9. Impedance frequency response of the detector.

Finally, using 1.7mM $NaHCO_3$ and 1.8mM Na_2CO_3 solution as mobile phase pumped at 0.2μl/min, seven-anion mixture in water (~25ppm) have been successfully separated using ion-exchange chromatography, and the chromatogram is obtained with on-chip conductivity sensor (Fig. 10).

4. CONCLUSIONS

An IC-processed Ion Liquid Chromatography chip, integrated with on-chip column, frits, injector and detector, is demonstrated. Channel-anchoring techniques have been developed to increase the pressure compatibility of the Parylene microfluidic system to at least 800psi. When the column is packed with ion-exchange chromatography beads, multi-anion mixture (~25ppm) has been successfully separated and detected with on-chip conductivity sensor.

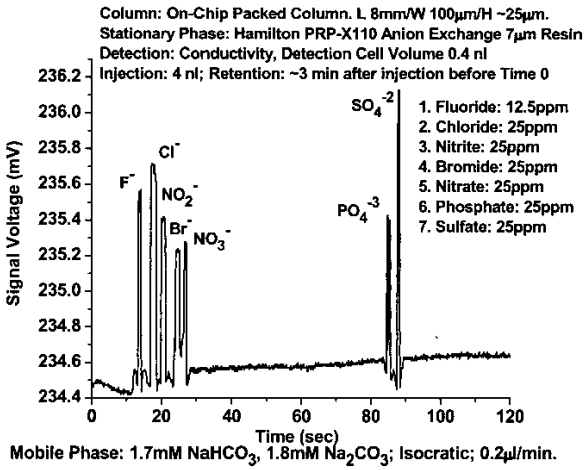

Figure 10. Ion chromatography separation of seven-anion mixture. Separation conditions are noted in the figure.

ACKNOWLEDGEMENT

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