ON-CHIP TEMPERATURE GRADIENT LIQUID CHROMATOGRAPHY

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ABSTRACT

We report here the first MEMS temperature-programmable high pressure parylene column with integrated thermal isolation and electrochemical sensor for on-chip temperature gradient liquid chromatography. Two sets of devices for different high performance liquid chromatography (HPLC) criteria were fabricated. The separation and detection of derivatized amino acids sample was successfully demonstrated. Compared with other temperature gradient HPLC systems which use "macro oven" to generate temporal temperature gradient in the column, our device's thermal performance is greatly improved by orders of magnitude without the sacrifice of chromatography quality.

1. INTRODUCTION

HPLC is one of the most powerful techniques for molecular separation. The trend of modern HPLC instrumentation has been the miniaturization of the separation column inner diameter for the reason that separation resolution can be greatly improved [1]. MEMS technology, given its strength in micro fabrication, is a perfect tool for miniaturization of HPLC system [2]. MEMS technology also provides an efficient approach for device mass production and system integration. Interestingly, while most researchers have pursued solvent gradient MEMS HPLC [3], temperature gradient HPLC [4] (defined as by changing HPLC column's temperature as a function of time to achieve analytes elution) has never been demonstrated in MEMS. In fact, MEMS temperature gradient HPLC system is extremely attractive because of the ease of achieving on-chip temperature control by MEMS as demonstrated in fields such as polymer chain reaction (PCR) [5]. Based on this motivation, we used parylene MEMS technology [6] to fabricate the temperature-controlled HPLC system-on-a-chip and demonstrated the first temperature gradient separation and detection of amino acids in MEMS.

2. DESIGN

The device to build contains components including: HPLC column which can stand column inner pressure up to 200 psi without leaking or breaking during beads-packing and separation procedures [2]; an integrated heater which can generate on-chip temperature gradient as the gradient elution mechanism; an integrated electrochemical /conductivity sensor for on-chip analyte detection; an integrated thermal isolation structure to the HPLC column for precise column temperature control and power consumption reduction.

3. FABRICATION

Two versions of devices were fabricated to satisfy different separation criteria. **Version I**: Freestanding parylene column with built-in heater and electrochemical /conductivity sensor was fabricated with the process flow shown in Figure 1. The integrated sensor is cooled by silicon substrate to maintain steady temperature during temperature gradient operation.

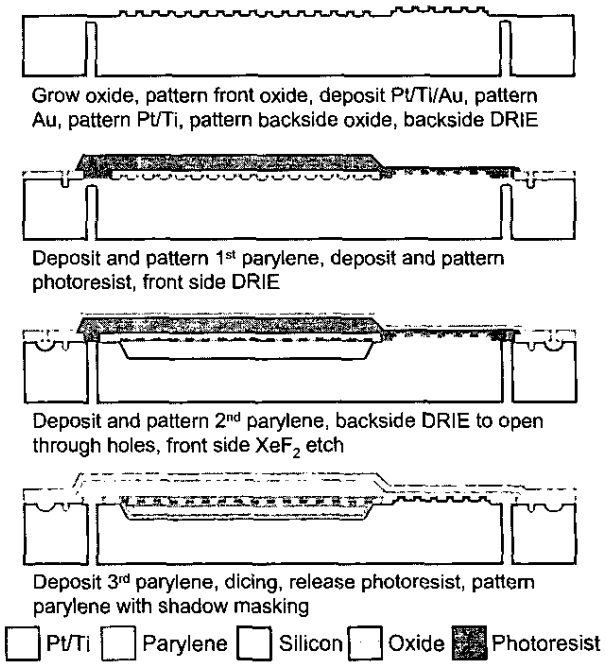

Figure 1: *Version I device process flow*

In this design, weak pressure point occurs at the parylene layers interface inside the freestanding column supporting beams (Figure 2). To achieve highest pressure capacity, several beam structures have been fabricated and tested.

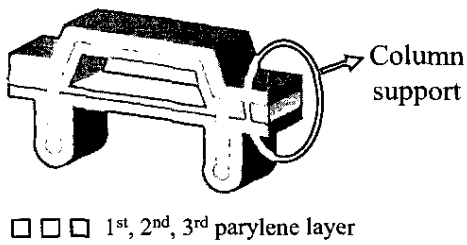

Figure 2: *Best Version I device column cross-section with pressure capacity up to 180 psi*

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The design with the highest pressure capacity is shown in Figure 2, where the 2nd parylene layer cuts through the 1st parylene layer and avoids liquid penetration through 1st / 2nd parylene interface under high pressure. The pressure capacity of this design can go up to 180 psi. Figure 3 then shows the fabricated Version I device.

Our FEMLAB simulations further showed that using Version 1 device the water-based mobile phase can be heated up to equilibrium temperature within 500 um column length with a regular linear flow velocity (~ 1 mm/sec).

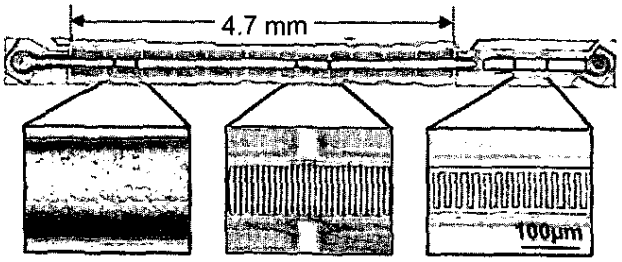

Figure 3: *Fabricated Version I device pictures: (left) column after packing with 5 μm silica beads, (middle) freestanding column with built-in heater and column supports, (right) interdigital electrochemical sensor.*

Even though 180 psi is good enough for general column beads-packing and separation tasks, it is desirable to further strengthen the column for higher pressure (> 200 psi) separation tasks. We therefore designed the Version II device. **Version II**: As shown in the process flow (Figure 4), parylene column was anchored down to the silicon substrate [7] with integrated electrochemical/conductivity sensor and distributed heater. From earlier tests, this anchored column structure can stand an column inner pressure of at least 600 psi [2].

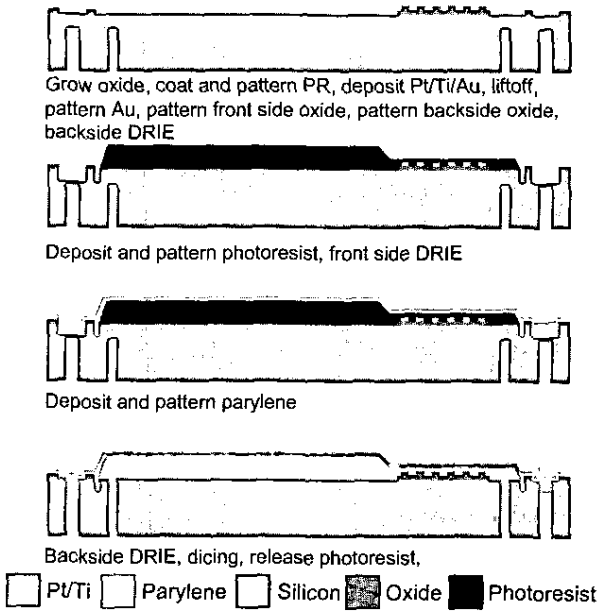

Figure 4: *Version II device process flow*

As shown in Figure 5, the silicon island is surrounded by a 150 um wide through-wafer air gap. The air gap provides thermal isolation to the silicon island so power consumption during temperature gradient operation is effectively reduced.

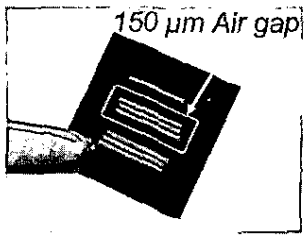

Figure 5: *Version II device picture showing 150 μm wide through-wafer air gap and silicon island (center piece)*

In order to stand the jig-clamping and wire-bonding stress during system preparation, chip integrity was enhanced by the cross-gap parylene membrane and parylene stitches structure (Figure 6). Due to the excellent heat conductivity of silicon (about 5000 times better than static air), temperature variation over the column area is greatly reduced, which is a critical quality for temperature gradient liquid chromatography.

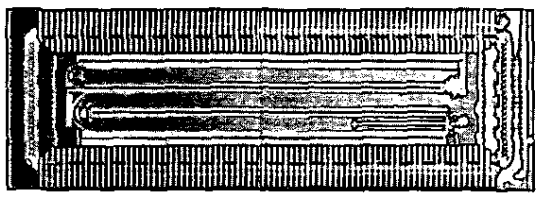

Figure 6: *Version II device close-up, dashed rectangle defines the silicon island area which is surrounded by the air gap, thin white lines are parylene stitches which cross-link silicon island to the rest of the chip, running between the HPLC column is the distributed heater.*

To make sure the 10 μm parylene column wall can stand high pressure without yielding or breaking, we studied the stress distribution in the parylene film under a 200 psi uniform pressure loading in column with FEMLAB (Figure 7). The maximum stress happens around the corner of column inner surface and is 50 MPa which is smaller than the parylene yield stress of 59 MPa [8]. This indicates that parylene column wall will not have noticeable plastic

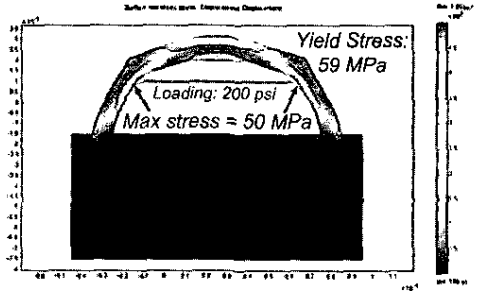

Figure 7: *Stress distribution inside 10 μm parylene column wall under 200 psi uniform pressure loading (Version II)*

deformation when operated under 200 psi.

One important factor for quality temperature gradient liquid chromatography is mobile phase solvent preheating. In other words, mobile phase solvent should be heated up to the desired temperature before it enters the HPLC column. Based on our FEMLAB simulation result (Figure 8), we found that water-based solvent is heated up to the equilibrium temperature through thermal conduction (within our flow rate range) inside the liquid access channel before it enters the HPLC column. Therefore, solvent preheating is achieved using Version II devices.

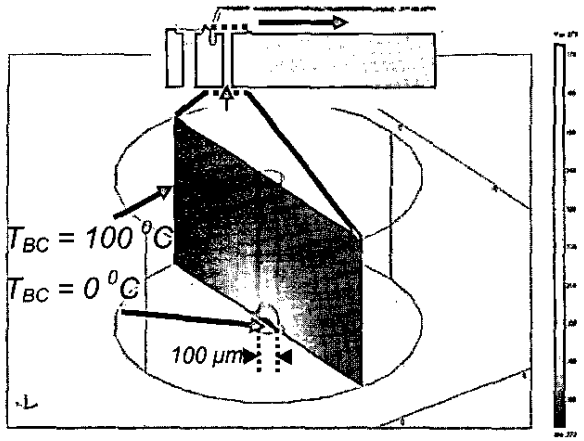

Figure 8: *Solvent preheating simulation around the liquid access channel of Version II device, linear flow velocity: 4.242 mm/sec, equilibrium temperature: 100 °C.*

4. RESULTS

Figure 9 shows the testing packaging for the temperature gradient HPLC system. The jig was made of PEEK material which has strong chemical resistance and can be machined easily. Fluid tubing and fitting were purchased from Upchurch. O-rings purchased from Apple Rubber with an outer diameter of 1.5 mm provide a leak-proof interface between the jig and HPLC chip. PCB was used to clamp down the chip against the O-rings and also it provides electrical connection to the chip via wire-bonding. Finally, Peltier cooler purchased from Melcor can be put on top of PCB when chip cooling is necessary.

In order to determine the HPLC column temperature, we used on-chip platinum heater as a temperature sensor [9]. Based on TCR measurement of the platinum heater, we can know the heater and the column temperature by monitoring the heater resistance in real time. Figure 10 then shows the power consumption comparison for both versions of devices.

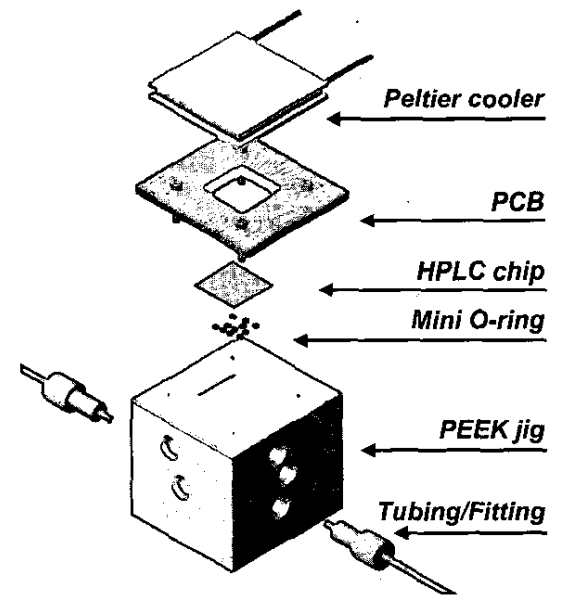

Figure 9: *Testing packaging for temperature gradient HPLC system (both Versions of devices)*

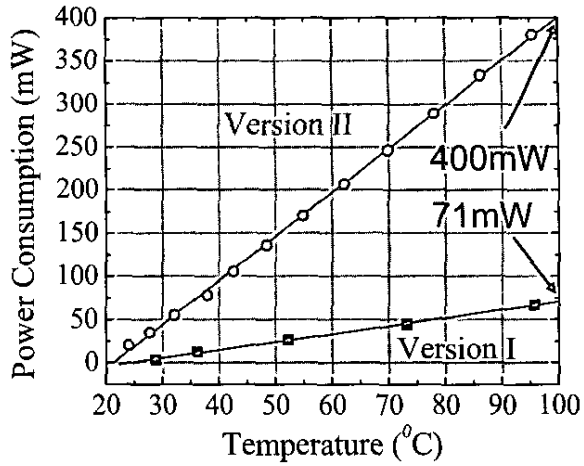

Figure 10: *Power consumption comparison, Version I device has much lower power consumption due to its smaller heating area exposed to the air.*

For basic temperature gradient liquid chromatography operation, we need to generate a constant temperature gradient which in turn requires a power input to the chip increasing linearly with time. The corresponding voltage profile is calculated and is shown in Figure 11.

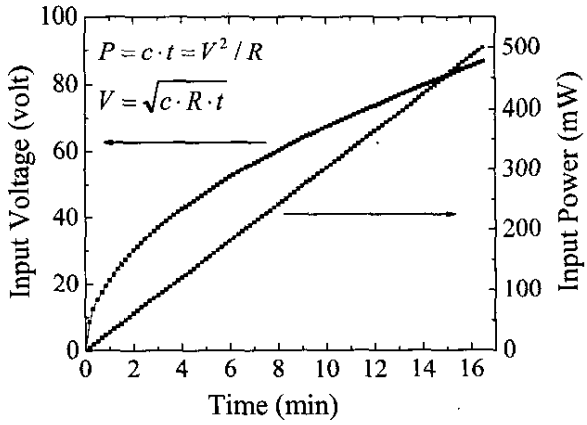

Figure 11: *Power / Voltage input profile for generation of on-chip constant temperature gradient*

In this work, we used LabView programming to control Keithley 230 function generator to output the calculated voltage profile to the on-chip platinum heater and accordingly generated linear temperature gradient.

Finally, to test the separation performance of our temperature gradient HPLC system, we have chosen a sample of mixed amino acids for separation. Amino acids separation provides information of amino acids relative composition and therefore is a very important tool for protein identification. To make electrochemical sensing of amino acid work, amino acid sample was first derivatized to become electroacitve [10]. We then carried out the cyclic voltammetry (CV) of derivatized amino acids. Figure 12 shows a typical CV of derivatized amino acid where peak current potential can be determined and used as the electrochemical sensing potential.

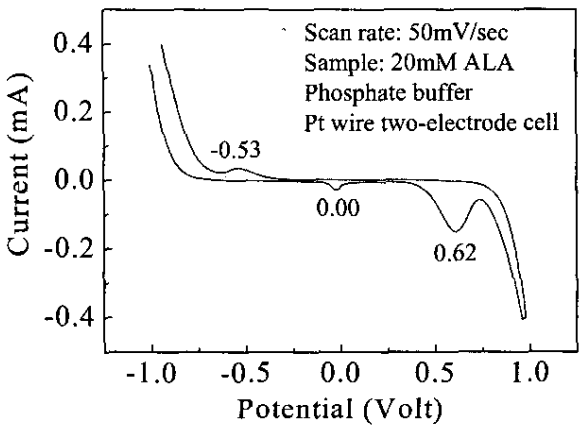

Figure 12: *Typical cyclic voltammetry of derivatized amino acid at 23 ^{0}C (20 mM ALA in this case)*

Version II devices were used for this amino acids separation test. Water-based phosphate buffer with a pH value of 6.5 and a flow rate of 2 μL/min was used as the separation mobile phase. Column temperature ramped up from 25 ^{0}C to 65 ^{0}C (while voltage source output from 0 V to 64 V) with a slope of 3.6 ^{0}C/min during the separation test. As shown in Figure 13, we successfully demonstrated the first on-chip temperature gradient separation and detection of amino acids with our system.

5. CONCLUSIONS

We have successfully fabricated two versions of temperature gradient HPLC devices which work for different chromatography criteria. Devices thermal performances were characterized. Using the fabricated devices, we successfully demonstrated the first on-chip temperature gradient separation and detection of amino acids. For future works, flexible temperature programming and feedback control capability will be added to our system so to optimize the temperature gradient profile for different separation tasks. Besides, electrochemical/conductivity sensor cooling of Version II devices for chromatogram baseline drift reduction is also under investigation.

ACKNOWLEDGMENTS

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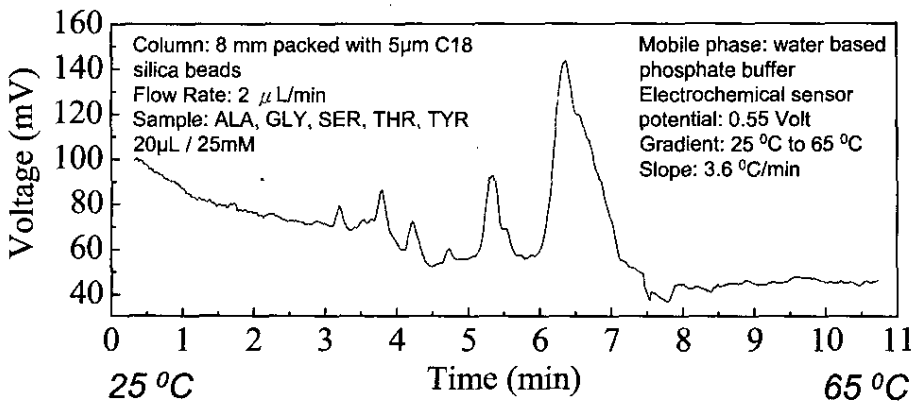

Figure 13: *Temperature gradient chromatogram of derivatized amino acids obtained using Version II HPLC system. Sample loading was done by flushing the column with 25 mM derivatized amino acids in phosphate buffer at 2 μL/min for 10 min. Column was then flushed with pure phosphate buffer for 10min before applying temperature gradient.*