Width-Modulated Microfluidic Columns for Gas Separations

Hamza Shakeel, Dong Wang, James. R. Heflin, and Masoud Agah, Senior Member, IEEE

Abstract—Microgas chromatography separation columns typically employ channels with fixed cross sections. In this paper, we demonstrate a new class of unidirectional microfabricated width-modulated columns (WMC) that afford improved chromatographic efficiency, resolution, and retention times compared with corresponding constant width (120 µm) bidirectional columns. Two new WMC architectures are introduced: 1) a linearly variable column (LVC) and 2) a step-gradient column (SGC). The width of a 1-m-long, 250-µm-deep LVC is gradually reduced from 120 to 20 µm at a 1 µm/cm gradient. While that of a 1-m-long SGC is modulated in five steps (120, 95, 70, 45, and 20 µm) each with a length of 20 cm. The effect of inlet selection (120 or 20 µm) on chromatographic performance is also evaluated. Moreover, with our improved fabrication process, multiple serially connected devices are simultaneously coated for the first time with highly stable silica nanoparticles utilizing layer-by-layer technique enabling constant film thickness.

Index Terms—Micro gas chromatography, width-modulated micro columns, silica nanoparticles.

I. INTRODUCTION

GA S chromatography (GC) is a versatile and widely accepted chemical analysis technique based on the distribution of a sample between two immiscible phases (a stationary phase and a mobile phase). A typical GC system consists of a carrier gas (mobile phase), an injector, a separation column (with a fixed cross-sectional area) coated with a thin film (stationary phase), a detector, and a data acquisition system. Figure 1 shows the schematic representation of a bench-top GC system. The sample mixture is first introduced as a concentrated plug into the instrument (GC oven) through a heated injection port and carried through the separation column by an inert carrier gas (mobile phase). Afterwards the components of a mixture are physically separated from one another by the separation column based on their interaction with the stationary phase bed. Next, the separated gas mixture along with a carrier gas enters the detector; that converts the chemical signal to an equivalent analog electrical output for measurements. Subsequently the data acquisition system plots the detector response against the time axis on a graph known as a chromatograph. For a well-separated gas mixture each peak on the chromatograph corresponds to an individual constituent of a mixture.

Due to its reliability and low detection limits, GC has established itself in a multitude of fields (petrochemical industry, pharmaceutical sciences, biological sciences, and forensic analysis) for the identification and quantification of mixture components. Despite these well-defined advantages, conventional GC systems are large, expensive, power hungry and ill-suited for field applications. The emergence of microelectromechanical systems (MEMS) along with nanotechnology has enabled the miniaturization and performance enhancement of key GC components [1]–[11].

Separation columns, being an integral part of the system, have also attracted significant attention and a number of prototype micro-GC (µGC) systems have also utilized silicon micromachined columns [12]–[14]. Typical MEMS-based separation columns have been designed with a fixed cross-sectional area, having either rectangular [15] or circular channel profiles [16], and are normally coated with polydimethylsiloxane based stationary phases [17]. Advances in silicon micromachining techniques have enabled the introduction of novel column architectures in the last decade. Partially buried columns were introduced by A. Radadia et al. [18] to enable the uniform deposition of polymer based phases. Similarly, Agah et al. [19] introduced columns with suspended microchannels using low-mass oxynitride films to achieve high-efficiency separations. Moreover, in order to enhance...
key performance parameters like sample capacity, analysis time and separation efficiency, our group also previously introduced MEMS based narrow-width multicapillary [20] and semipacked columns having embedded micropillars [21], [22]. Additionally, innovative research work on deposition of non-traditional adsorbent based stationary phase coatings for rectangular microchannels has also been performed. This includes the integration of carbon nanotubes [23], self-assembled thiol monolayers [24], silica nanoparticles [25] and MEMS compatible sputtered silica [26] thin-films inside the anisotropically etched channels.

As early as 1962, Purnell [27] suggested that gradual reduction in solvent-adsorbent interaction along the length of the column could effectively provide similar capabilities (shorter analysis times and improved sensitivity) as afforded by the programmed temperature or the programmed flow modes. This interaction could be minimized gradually by reducing either the stationary phase thickness or the column width. Although, effects of gradual reduction in stationary phase thickness have been theoretically studied and experimentally demonstrated [28], the effect of channel modulation on the separation process could not be materialized earlier due to fabrication difficulties. In this article, by employing a single lithography mask and a simple micromachining process, modulation in column width is achieved.

II. THEORETICAL DISCUSSION

The efficiency of a chromatographic column is expressed in terms of either a theoretical plate number (N) or height-equivalent-to-a-theoretical-plate (HETP = L/N). As a general rule a high efficiency column has higher theoretical plates and less band-broadening (small HETP term). HETP could be further expanded to include the effects of diffusion in the mobile phase and mass-transfer in mobile and stationary phases. For the rectangular channels HETP is given by

\[
HETP = \frac{2D_g f_1 f_2}{\bar{u}} + \left[ \frac{(1 + 9k + 25.5k^2) w^2 f_1}{105 (k + 1)^2 D_g f_2} + \frac{2k (w + h)^2 d_s^2}{3 (k + 1)^2 D_s h^2} \right] \bar{u} \tag{1}
\]

where \(D_g\) and \(D_s\) are the binary diffusion coefficients in the mobile and stationary phase, \(\bar{u}\) stands for the linear gas velocity, \(f_1\) and \(f_2\) are the Giddings-Golay and Martin-James gas compression coefficients respectively, \(k\) is the retention factor that is characteristic of a stationary phase, \(w\) stands for channel width and \(h\) for the channel height/depth [29].

To simplify the analysis, we ignore diffusion in the mobile phase (1st term) and also assume a very thin stationary phase film (small \(d_s\)-term) therefore, we can neglect the contribution of band-broadening due to diffusion in the stationary phase (3rd term) in equation (1). The HETP becomes directly proportional to the square of column width.

\[
HETP \propto w^2 \tag{2}
\]

It is clear from equation (2) that columns with smaller width will provide efficient separations and column-width is one of the critical design parameters. Moreover, if the width of a column is gradually reduced along the length, then HETP will also decrease locally and effectively the overall HETP values will reduce. Therefore, in this article the modulation in width of the column is carried out and two new microfabricated width modulated column (\(\mu\)WMC) are introduced for the first time (Fig. 2). The width of the linearly-variable-column (LVC) is modulated from 120 \(\mu\)m to 20 \(\mu\)m at 1 \(\mu\)m/cm (Fig. 2b), and the step-gradient column (SGC) is modulated in 5 steps (120 \(\mu\)m, 95 \(\mu\)m, 70 \(\mu\)m, 45 \(\mu\)m and 20 \(\mu\)m) each with 20 cm length (Fig. 2c). Moreover, for the step gradient column, the interconnections between the steps are gradually varied over the length of 700 \(\mu\)m. The separation capabilities of newly developed \(\mu\)WMCs are realized by utilizing our recently developed layer-by-layer (Lbl) technique to get a highly-stable silica nanoparticle (SNP) stationary phase. Additionally, by shifting the stationary phase deposition step to occur after anodic bonding, multiple serially connected columns are coated simultaneously providing high-throughput. The detailed fabrication process and chromatographic performance of our proposed \(\mu\)WMCs with SNPs coating is explained below.

III. COLUMN DEVELOPMENT

A. Fabrication

Fig. 3 shows the single mask MEMS fabrication process for SNP coated columns on 4 inch, 500 \(\mu\)m thick single-side polished silicon and double side-polished Borofloat wafers. 1m-long, 250 \(\mu\)m-deep \(\mu\)WMCs and regular columns are realized using photolithography, deep reactive ion etching (DRIE) and anodic bonding. The fabrication of devices starts with the standard RCA cleaning and the priming of a silicon wafer. Afterwards, an 8 \(\mu\)m thick AZ9260 photoresist is patterned using a mask aligner followed by development in AZ 400K (Fig. 3a). Next, the patterned wafer is hard baked for 4 minutes at 110 °C and then etched anisotropically using DRIE to get the desired channel dimensions. Following this, the etched silicon substrate is first cleaned with acetone and secondly by oxygen plasma. This is to ensure the removal of photoresist and the residual passivation polymer deposited during etching (Fig. 3b). Following the cleaning steps, the etched wafer is sealed with a Borofloat glass wafer using an anodic bonding station at 40 °C and 1250 V for 45 minutes.
Subsequently, the bonded wafer is diced to expose two microfluidic ports of a number of serially connected devices for the newly developed stationary phase coating technique, as shown in Fig. 4.

### B. Stationary Phase Coating

After the first dicing, the 25 cm-long deactivated fused silica capillary tubes (outer diameter 220 µm, inner diameter 100 µm) are attached to the microfluidic ports with epoxy. Next, dry nitrogen and deionized (DI) water are passed through the devices to remove any dirt gathered during dicing. The complete details of layer-by-layer (LbL) coating of SNPs can be found in an earlier report [25]. Briefly, the pH values of polyallylamine hydrochloride (PAH) solution (Sigma-Aldrich) and SNPs suspension (Nissan Chemical) are first adjusted to 7.0 (±0.1) and 9.0 (±0.1), respectively, by adding HCl and NaOH solutions respectively. The LbL coating starts by alternately passing a positively-charged long-chain inert polymeric aqueous solution (PAH) and negatively-charged SNPs suspension through a number of serially connected multiple devices for three minutes to form one bilayer. The electrostatic attraction of each solute to the oppositely-charged surface provides strong, rapid adsorption of a nanoscale layer of the solute onto the surface. Each PAH and SNP coating step is followed by a 3 minute DI water rinsing step. This removes any excess coating material deposited during the prior step that is not strongly bound by electrostatic attraction. In the present work, this process is terminated after 10 bilayers of PAH and SNPs. Similar to our earlier work for very narrow channel-width (20 µm) multicapillary columns [25], the original concentration of SNPs suspension is reduced to a third in order to achieve a uniform film thickness. At the end, the devices are thoroughly purged by passing DI water and dry nitrogen at low pressure for 15 minutes.

Next, the capillary tubes are removed and serially connected columns are diced into individual devices as shown in Fig. 4. Calcination is performed afterwards at 500 °C for 8 hours to remove the PAH layer and fuse SNPs together, resulting in a stable and homogenous film. Following the calcination step, deactivated capillary tubes are fixed to the inlet and outlet ports of individual columns using epoxy. Before performing chromatographic separations, the surface of the SNPs is deactivated by filling each column with 10 mM chlorodimethyl octadecyl silane (CDOS) diluted in toluene for 12 hours.

### IV. Device Characterization

The characterization of µWMCs with a constant SNPs stationary phase film is performed using SEM (Fig. 5). First, it is verified from the SEM images (top and cross-sectional views) that only the column width is modulated (Fig. 5 a-b) without effectively changing the channel depth. It is pertinent to note that the effect of modulation in column depth on chromatographic separation could also be studied in the future and easily achieved by changing the DRIE etch parameters. Moreover, by employing the newly developed LbL SNPs coating after anodic bonding, a complete coverage of the microfluidic channel including the glass surface is realized (Fig. 5 C). This complete coverage of the separation channel
results in improving the analyte-stationary phase interaction. The film thickness measured at different locations (top, bottom and sidewalls) inside the modulating microfluidic channel show that 10 BLs of SNPs yield roughly a constant film thickness in the range of $400 \sim 500$ nm (Fig. 5C). This is also consistent with our earlier reported LbL coating method [23] and demonstrates that the film thickness is proportional to the number of coating steps. The deviation in film thickness could be reduced further by utilizing nanoparticles with smaller average diameters for future studies.

V. RESULTS AND DISCUSSION

A. Experimental Setup

The separation performance of the LVC, SGC and regular column is evaluated using a conventional GC oven (Agilent GC-7890A) equipped with an electronic pressure controller, a flame ionization detector (FID) and an autosampler (G-4513A). Both the injector and detector temperatures are maintained at $280^\circ$C during GC testing. Ultra high purity nitrogen is used as a carrier gas for all experiments. Since nitrogen provides a higher theoretical plate number at a lower carrier gas velocity [30]. The chemicals used during the chromatographic testing are of HPLC standard and bought directly from Sigma-Aldrich. MEMS columns are attached to the inlet and detector ports using 25 cm-long deactivated (uncoated) fused silica capillary tubes.

After silane-coupling each SNPs functionalized column is first purged with dry nitrogen for 15 minutes. This is followed by column maturing in a GC oven under a constant pressure of 10 psi and an oven temperature of $140^\circ$C until a flat baseline signal is observed. This step is necessary to drive-off contaminants from the column.

B. Separation Results

Since the newly developed LVC and SGC ($\mu$WMCs) configurations have microfluidic ports with different dimensions (i.e. 20 $\mu$m and 120 $\mu$m), we can expect that the chromatographic response (retention times, flow rate and plate number) using each port as an inlet will be different. Therefore, both $\mu$WMCs cannot be considered bidirectional unlike regular columns. So the presented chromatographic parameters are first analyzed utilizing both inlets of $\mu$WMCs. As a first step, the flow rate through $\mu$WMCs and regular column is characterized against the applied inlet pressure (Fig. 6) using a gas flow meter (ADM1000, Agilent Technologies). Due to a larger cross-sectional area (along the column length), the regular column presents a higher flow rate, while the SGC shows the minimum flow rate with the smallest cross-sectional area. Following the flow rate characterization, the separation performance (plate number/meter) of the columns is evaluated according to [30]

$$N = \frac{5.54w}{t_r} \left(\frac{w}{w_{1/2}}\right)^2$$ \hspace{1cm} (3)

where N is the plate number, $t_r$ is the retention time and $w_{1/2}$ is the peak width at half height.

From Table 1, it is clear that the inlet selection for $\mu$WMCs plays an important role with a significant difference in the plate numbers and retention times. It is clearly demonstrated that the SGC provides a higher plate number (Nmax = 6850 plates/meter) compared to the LVC (Nmax = 5850 plates/meter). The higher plate numbers in the proposed $\mu$WMCs could be attributed to a gradual change in the width of the column and the stationary phase thickness. The sample molecules experience either a gradual reduction in solute-stationary phase interaction (using 120 $\mu$m as an inlet port) along the column length or get separated fast at the start of the column within the narrower column region when the injector is connected to a 20 $\mu$m column port. It has been shown (Table 1) that the later scenario provides an enhanced separation performance especially for the SGC. Furthermore, the LVC provides longer retention times than the SGC. Therefore, in order to achieve faster separations the SGC should be utilized but the LVC could provide a better separation resolution between different peaks. It can be further deduced that for both the $\mu$WMCs designs utilizing 20 $\mu$m ports as inlets provide better separation efficiencies. Therefore, all the chromatographic results presented hereafter utilize 20 $\mu$m ports as column inlets.

For $\mu$WMCs the local plate height ($\text{HETP}$) will vary with position (length) down the column due to a change in interaction between the stationary phase and the solute, as the relative linear velocities change. Since, $\text{HETP}$ cannot be measured experimentally, the apparent plate height ($\text{HETP} = \frac{5.54w}{t_r} \left(\frac{w}{w_{1/2}}\right)^2$) provided in Table 1 can be used to adjust the plate number for a specific flow rate and column length.
L/N) is calculated. From the Golay plots (Fig. 7), it is apparent that both LVC and SGC show superior performance compared to the regular columns (Nmax = 3500 plates/m) for all flow rates.

In order to optimize the GC analysis, apart from the retention time and plate number, the resolution should also be characterized. Resolution, a characteristic of the separation of two adjacent peaks, is calculated according to [30]

\[
R = 2\left(\frac{t_{rb} - t_{ra}}{w_b + w_a}\right)
\]  

(4)

where \(R\) is the resolution, \(t_{rb}\) and \(t_{ra}\) are the retention times, \(w_b\) and \(w_a\) are peak widths at half heights of corresponding compounds a and b, respectively.

Similar to HETP analysis, the LVC outperforms the fixed dimensional regular columns in terms of resolution between n-nonane and n-decane under isothermal conditions (Fig. 8). The effect of peak resolution is further demonstrated from the separation of a custom-made hydrocarbon mixture with nine compounds. It is clear from the separation results (Fig. 9) that under same flow rates the LVC is able to resolve all the nine compounds, while a regular microfabricated column is only able to resolve seven compounds in the test mixture. Moreover, it could also be concluded that \(\mu\)WMCs are more suitable for the separation of compounds with lower boiling compounds.

Additionally, we note that the SGC is only able to separate eight compounds in the sample test mixture (not shown).

VI. CONCLUSION

Two unidirectional \(\mu\)WMCs are introduced for the first time and provide better resolution, plate numbers, retention times and chromatographic separations than a regular fixed dimensional column. Multiple serially-connected columns are coated using LbL self-assembly of SNPs to enable a constant stationary phase film thickness. Moreover, the effects of modulation in column depth (controlled by micromachining parameters) and film thickness could also be studied as a future study. Similarly, the length of the \(\mu\)WMCs could also be increased to study the effect of width modulation on the separation of lighter hydrocarbons.

REFERENCES


