INNOVATIVE DESIGNS FOR HIGH PERFORMANCE MICROMACHINED GAS CHROMATOGRAPHIC COLUMNS

A Thesis in
Bioengineering
by
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Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
December 2013
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ABSTRACT

Gas chromatography (GC) is a versatile technique used in diverse fields such as biological/medical, environmental protection, pharmaceutical, and food/agricultural. This is the premier technique to identify and quantify components of interest from a complex mixture. However, the current GC systems are bulky and expensive. Microfabricated gas chromatographic columns (µGCs) have been developed to miniaturize the state of the art systems, allowing for removal of the large and inefficient ovens as well as fabrication of a micro-total analytical system (µTAS) for on-site detection. The µGC devices also provide a platform for creation of novel geometries and stationary phases unique to microfabricated devices. Although there has been considerable success in development of µGC columns and µTAS devices, currently microfabricated devices have not matched the performance of commercial columns in a number of areas. Firstly, microfabricated columns have temperature limitations much lower than that of commercial columns. To date, microfabricated columns have not operated at temperatures above 250 °C due to the epoxies used in the world-to-device connections, while standard commercial columns operate at temperatures up to 350 °C. The thermal limitations of the µGC devices prevent the use of these devices with a large number of compounds typically separated using gas chromatography. Additionally, the separation efficiency of the microfabricated columns have not matched that of commercial columns and no direct comparison of µGC columns to commercial columns has been made. Finally, there is a need for a seamless world-to-device connection to eliminate detrimental dead space and improve column efficiency. Here we address the problems listed above. We have developed novel microfabricated channel designs capable of improved chromatographic performance and allowing for high throughput and lengths up to 10 meters. To operate at high temperatures (up to 400 °C) we have fabricated interfacing and packaging techniques enabling microfabricated columns and have demonstrated the first high temperature separation with a µGC column. We have optimized the world-to-device microfluidic interconnection removing dead volume at the inlet. We have benchmarked the performance of the microfabricated column against commercially available conventional columns and displayed a clear solution to improve the separation efficiency of microfabricated columns. We expect the results of this work to further the µGC field in both research and commercial aspects.
## TABLE OF CONTENTS

List of Figures ................................................................................................................................................. vi

List of Tables ...................................................................................................................................................... viii

Acknowledgements ........................................................................................................................................ iv

Chapter 1.......................................................................................................................................................... 1

1.1 Motivation and Aim ....................................................................................................................................... 2

1.2 Introduction to Gas Chromatography ........................................................................................................... 4

1.2.1 Sample Inlet: Injector ............................................................................................................................ 8

1.2.2 Gas chromatographic Column ............................................................................................................... 10

1.2.3 Flame Ionization Detector .................................................................................................................... 11

1.2.4 Gas Chromatography Applications ..................................................................................................... 13

1.3 Partition Equilibrium .................................................................................................................................... 14

1.3.1 Physical Chemistry Analysis ................................................................................................................ 14

1.3.2 Partition Equilibrium in a Theoretical System ....................................................................................... 15

1.4 Chromatography Theory and Quantification .............................................................................................. 17

1.4.1 Basic Meterics ......................................................................................................................................... 17

1.4.2 Van Deemter’s Rate Theory .................................................................................................................. 20

1.4.3 Longitudinal Diffusion: B-term ............................................................................................................. 21

1.4.4 Resistance to Mass Transfer: C-terms .................................................................................................... 21

1.4.5 Extra Column Band Broadening .......................................................................................................... 22

1.4.6 Van Deemter’s Equation: Graphical Description .................................................................................. 23

1.4.7 Isothermal and Temperature Programmed Separation ......................................................................... 24

1.5 Work in Microfabricated Gas Chromatographic Column ......................................................................... 24

1.5.1 Initial Development ............................................................................................................................... 26

1.5.2 Gas Chromatography Micro-Total Analytical Systems ....................................................................... 26

1.5.3 Column Improvement ........................................................................................................................... 27
LIST OF FIGURES

Figure 1-1. An example chromatogram with five Gaussian peaks separated in time..........6
Figure 1-2. A depiction of a gas chromatographic system........................................7
Figure 1-3. Hot Split Splitless Flash Vaporizer .......................................................9
Figure 1-4 Schematic of WCOT column. .............................................................10
Figure 1-5. Flame ionization detector schematic...................................................12
Figure 1-6. Theoretical separation...........................................................................16
Figure 1-7. Graphical description of the important variables in a chromatogram.............18
Figure 1-8. A plot of the Van Deemter equation. ....................................................24
Figure 1-9. Gas chromatography uTAS....................................................................27

Figure 2-1. Gas chromatography uTAS....................................................................32
Figure 2-2. Overview of fabrication process. ...........................................................34
Figure 2-3. The deep reactive ion etching process....................................................36
Figure 2-4. Overview of the process parameters for the flat bottom etch.......................37
Figure 2-5. Image of the etched channel...................................................................38
Figure 2-6. Schematic of the anodic bonding process.................................................39
Figure 2-7. Image of bonded silicon and glass wafer. .................................................40
Figure 2-8. Image of device with nanoport assemblies.............................................42
Figure 2-9. Display of metal manifold and compression sealing technique..................44
Figure 2-10. Initial testing on the μGC columns.....................................................46
Figure 2-11. Initial testing on the μGC columns.......................................................47

Figure 3-1. Performance comparison of the first generation μGC columns....................51
Figure 3-2. Performance comparison of the second generation μGC columns.............53
Figure 3-3. Performance comparison of the second generation μGC...........................55
Figure 3-4. Simulated distillation of ASTM 2887 in a μGC column............................57
Figure 3-5. Separation of Polycyclic Aromatic hydrocarbons in a μGC column. ...............59

Figure 4-1. Relative size of 10 meter column.................................................................63

Figure 4-2. Parallel column design.................................................................................65
LIST of Tables

Table 1-1. GC applications ...........................................................................................................13

Table 3-1. GC applications ...........................................................................................................53

Table 3-2. Comparison of the uGC column 1st and 2nd generation columns...............................54

Table 3-3. Comparison of the uGC column and commercial column .............................................56

Table 3-4. Retention times and boiling points of the ASTM mixture.............................................58

Table 3-5. Retention times and boiling points of the EPA mixture...............................................60

Table 4-1. Benchmarking our uGC device against other in the field.............................................60
CHAPTER 1

INTRODUCTION

Miniaturization of analytical systems, such as gas chromatographic systems, has gained interest as a topic of research since the development of microfabrication techniques. Analytical systems benefit from favorable scaling laws, such as increased surface area to volume ratio upon miniaturization, thus enabling the development of mobile systems for point-of-care applications. In these analytical systems, components are often fabricated individually and later assembled to form a complete system, known as a micro total analytical system (μTAS).

One of the first analytical components developed using microfabrication techniques was the gas chromatographic (μGC) column. These μGC columns provide a number of advantages over the conventional silica tubing columns. Microfabricated columns of equal length to their commercial counterparts occupy significantly less space and are less expensive to produce [1]. Furthermore, microfabricated columns have significantly less thermal mass, and therefore may be heated and cooled faster, an advantage in gas chromatography [2]. The photolithographic and etching techniques used to fabricate the columns are able to define complex geometries which increase the separation efficiency of the column, an impossible task using conventional materials [3], [4]. Miniaturization of components required for the chromatographic systems have led to the development of a μTAS chromatographic system. Similarly, microfabrication techniques such as sputtering are used to apply novel stationary phases to the μGC, which would be difficult to apply to conventional tubing [4],[5],[6]. Unlike the heavy, bulky commercial system, the portable miniaturized system is handheld and capable of providing on-site detection of environmental hazards or explosive compounds [7],[8],[9].
This chapter outlines the components required for a gas chromatographic system. Also, the common uses of these chromatographic systems are discussed. Finally, an overview of the field of μGC is reviewed.

**1.1 Motivation and Aim**

In this work we will explore:

1.) Novel microfabricated channel designs capable of improved chromatographic performance and allowing for high throughput and lengths up to 10 m.

2.) Explore interfacing and packaging strategies for microfabricated columns to allow for high temperature (up to 400 °C) operation. Currently all packaging strategies for microfabricated columns utilize epoxies. These epoxies are unable to provide a seal for temperatures above 250 °C [10]. Commercial columns operate up to a minimum of 350 °C [11]. Limited temperature operation reduces the range of components which may be separated by the column, preventing the sampling of low volatility compounds.

3.) Optimize inlet/outlet column designs and microfluidic interconnection techniques that allow for ready connections to commercial columns with the aims of (i) removing any dead volume at the inlet, (ii) successful coating of the column walls with stationary phase, and (iii) benchmarking the performance of the microfabricated column against commercially available conventional columns. World-to-device connections are non-trivial and severely reduce the quality of separations if improperly developed [12]. Classically, improving the connection of the column to the injector and detector have been topics of research due to the large impact of the extra column band broadening [13]. Coating of microfabricated columns is noted to be very difficult due to the dead volumes created by world to device connections [14]. Elimination of these dead volumes may improve the coating process. Direct
benchmarking of microfabricated columns against the state of the art commercial columns has not been performed. An indirect comparison may be made by reporting plate counts, however, plate counts are heavily dependent of the machine set-up. Additionally, important metrics such as retention index have not been report for microfabricated devices.

Microfabrication approach has been successful in improving the separation efficiency of chromatographic columns near that of commercially available conventionally drawn columns. Additionally, mobile systems for on-site detection of hazardous compounds such as explosives [15] and toxic compounds [16] have also been successfully demonstrated. These columns, however, have not been used to their full potential due to thermal limitations of the components used to fabricate the μGC columns. To date, all μGC columns have been used to perform separations under 250 °C, limiting the analysis to solely volatile components [14]. Many components require column temperatures greater than 250 °C. Such analytes include environmental pollutants, pharmaceuticals [17], fatty acids [18], and metabolites [19]. The typical conventional GC column can operate in a temperature range of 40-350 °C, high enough to move these semi-volatile compounds to the gaseous phase. The aim of this thesis is to explore innovative column designs, fabricate and characterize μGC columns which operate at high temperatures ranging from 250 – 350 °C. The microfabricated columns will be directly compared to similar commercial devices to demonstrate the efficiency of these columns. One of the reasons that the microfabricated columns have not been readily benchmarked against conventional columns is the unavailability of robust fluidic interconnection interface. Such interconnection imperfections result in separation related artifacts thereby compromising the ability to make direct comparisons. In this work we have developed a robust interconnection strategy which
minimizes such interface imperfection thereby allowing for a robust comparison with commercial columns.

1.2 Introduction to Gas Chromatography

Gas chromatography (GC) is among the most common laboratory techniques. It is used as a method to separate components of a complex mixture, which are later individually quantified and identified by a detector. Gas chromatography is performed by propelling a gaseous mixture through silica tubing, in which the components separate in time. The column is coated with a stationary phase, which is responsible for this separation of the gaseous mixture. The high resolving power, quick analysis time, and low sample consumption of GC attributes to its vast success as an analytical technique.

This technique’s popularity stems from the broad need for researchers to determine and quantify the components present in a solution of interest. Determination of components present in a solution may be accomplished using multiple techniques. Firstly, a sensor may be designed to detect a specific component. One such sensor, designated a “lock-and-key” sensor, utilizes a receptor that is synthesized to bind strongly to a specific analyte of interest [20]. While a lock-and-key approach is useful when detection of one component is needed, a researcher often will want to distinguish between multiple components in a mixture. For example, with a mixture of over 90 unknown components [21], an array of lock-and-key sensors would be an unreasonable analysis technique.

Secondly, an array of low selectivity sensors may be employed to determine compounds in a mixture. The multiple sensors output a signal from the applied solution. Signal processing of all the sensors’ outputs is used to distinguish and quantify the components of the mixture. These
sensor arrays, however, have difficulty in distinguishing components of a complex solution, especially volatile organic solutions [22]. For these reasons chromatographic columns are linked to such sensor arrays which have low specificity and high sensitivity.

Gas chromatography is the only one of these three techniques which will quickly and reliably distinguish components in diverse, complex mixtures. Through chromatography a complex mixture may be broken into a series of single components and subsequently detected. Fig 1.1 displays the output of a combined gas chromatographic column and sensor. 5 Gaussian peaks, each of which represents a single component separated from an initial mixture of these components can be seen. When the mixture is first injected all components occupy the same position in space and time and if fed to the detector would appear as a single delta function peak. However, as the mixture flows through the column the components begin to separate from one another. This separation, seen in Fig. 1.1, allows for each component to move through the detector individually, ensuring the sensor’s output is a function of only one component. The output as a function of time displays the series of components individually moving through the detector in a graph commonly known as a chromatogram. In effect, a chromatography column can be considered as a distillation column in temporal space. The chromatogram may be used for identification and quantification of components in the initial mixture.
Figure 1.1: An example chromatogram with five Gaussian peaks separated in time. Each peak depicts a single component which has been detected by a sensor.

Separation of components must occur in order to ensure proper identification and quantification of a component. When two or more components do not separate and move through the sensor simultaneously, they are said to have “co-eluted”, making quantification and identification impossible. In an ideal separation of components would have distinctly separated peaks and have peaks with a half width of a single point (delta function). While this ideal has not been achieved, a method to determine the quality of a columns separation has been developed to ensure the most optimal separation. One separation may be compared to another by choosing one of the component in both separations and comparing the time at which the peak of the component is detected and the peak’s full width at half maximum in seconds is observed. The
most efficient separation produce long retention times and/or shorter half width for the observed component.

Fig. 1.2 displays all necessary components for a gas chromatographic system. A carrier gas, typically helium or nitrogen, is constantly flowing through the column at a linear velocity set by the flow controller [23]. The sample is injected via syringe into the sample injector, where the sample is mixed with the carrier gas. The injector is constantly maintained at a high temperature such that when the sample is injected, it rapidly transitions to the gaseous phase. The sample flows through the column to a detector, in this case a flame ionization detector, which is also held at a high temperature to ensure that the gas does not condense into the liquid phase. The signal from the detector is then sent to a computer for recording.

Figure 1.2. A depiction of a gas chromatographic system. Displayed are all of the necessary components for sample separation and detection: the carrier gas, flow controller, injector, column, and detector.
1.2.1 Sample Inlet: Injector

The sample injector is the first stage of the gas chromatographic system; it vaporizes the liquid injection and mixes the sample with the carrier gas prior to the start of separation [23]. The sample inlet used in this system is referred to as a hot split/splitless flash vaporizer. A depiction of this flash vaporizer is displayed in Fig 1.3. The flash vaporizer consists of a stainless steel metal, heated frame which is used as the housing of the injector and regulates the temperature in the injector. The inlet is required to maintain the pressure of the carrier gas and therefore must be leak tight. A rubber septum is used as a means to insert the syringe into the inlet without leakage, keeping the inlet pressurized. A glass liner is inserted into the inlet to define a pathway for the carrier gas. The capillary tubing is inserted into this glass liner.

In a split/splitless injector, after sample vaporization and mixing with the carrier gas, the mixture is divided between a path to the column (carrier gas flow) and a path to the waste (split flow). This inlet uses a backpressure configuration, in which the total flow of the carrier gas is controlled by a pressure regulator and the split ratio is controlled by another backpressure regulator in the path of the split flow. Auxiliary flow is used to purge septum bleed, preventing contaminants from entering the column.

When injecting mixtures in which the components have unequal volatility, split injection will discriminate against the less volatile sample components due to: selective evaporation, incomplete sample vaporization and unequal mixing of sample vapors with the mobile phase [24]. When higher molecular weight components reach the split point, they will be composed of a semi-vaporized mixture consisting of vapor and liquid droplets. These different phases are split disproportionately, with liquid droplets likely to be moved into the split flow [25]. In a mixture with components of differing volatility, high molecular weight components are more likely to
have a high fraction in the liquid droplet form and, therefore, a lesser amount will reach the column and detector. This occurrence is known as molecular weight bias. The relation of this phenomenon to separation will be further discussed in the results section of this thesis.

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Figure 1.3. Hot split/splitless flash vaporizer. The sample is injected by the syringe and mixes with helium from the carrier gas inlet before entering the column.
1.2.2 Gas Chromatographic Column

The conventional columns used in gas chromatography are made from fused silica and coated with a stationary phase that is typically 0.05 – 0.5 μm thick [26], as seen in Fig. 1.4. These columns are referred to as “wall coated open tubular” (WCOT) columns. Due to the high diffusivity of gases, these WCOT columns have diameters of 50 μm–500 μm and are able to maintain high retention [26]. Commercial columns range from 10 meter to 30 meters in length depending on the resolution required for the separation.

Figure 1.4: Schematic of WCOT column. This depicts the layers of a typical chromatographic column consisting of: an outer polyimide coating, fused silica, and an inner stationary phase.
The stationary phase is the heart of gas chromatography, as it is responsible for the separation of components. The stationary phase’s ability to slow the movements of components is known as *retention* in chromatography. Retention in the column is caused by the *partitioning* of the gaseous components into the stationary phase and back out into the mobile phase. When the gas is in the stationary phase it is temporarily immobile and therefore will move slower through the column in comparison to a non-retained component. Most components will display different amounts of retention due to the variation in the time spent in the stationary phase. This is due to the chemical interactions between the stationary phase and the gaseous component. The chemical interactions create the varying amounts of Gibbs free energy required to move from the mobile gaseous phase to the stationary phase, as well as from the stationary phase back to the gaseous phase. Those components which move more easily into the stationary phase and move slowly back into mobile the gaseous phase will be retained longer.

### 1.2.3 Flame Ionization Detector

Flame ionization detectors (FIDs) are the most popular detector in use with gas chromatographic systems [23]. Flame ionization detectors provide low detection limits, robustness, operational simplicity, and fast signal response. The FID response results from the combustion of organic compounds which react with hydrogen radicals produced in the hydrogen-air diffusion flame. The carrier gas is mixed with hydrogen and burned at a barrow orifice chamber through which excess air is flowing. An ion collecting electrode located a few millimeters above the flame measures the ion current by a voltage established between the flame assembly tip and the collector electrode, Fig 1.5. Typically a base current of $10^{-14}$ to $10^{-13}$ A exists. As the organic vapor enters the flame combusts and produces ions, the signal is increased to $10^{-12} – 10^{-5}$ A. The
FID has a relatively large detection limit $10^{-13}$ g carbon/s and displays a linear response. The mechanism in which ions are produced in the flame is not fully understood. The non-uniform flame contains a large concentration of hydrogen, oxygen and hydroxide radicals at the hottest part of the flame. The most accepted mechanism for ion formation states that ions result from the chemical ionization of CHO produced by reaction of O' and CH' [23]. The ionization process of the organic vapor is understood to be a first order reaction explaining the linear response of the detector.

$$CH^* + O^* \rightarrow CHO \rightarrow CHO^+ + e^-$$

---

Figure 1.5: Flame ionization detector schematic. The sample and hydrogen gas mix before entering the flame assembly tip. The flame assembly tip initiates the flame. As the organic compounds enter the flame, they are broken into radicals by the hydrogen ions and form CHO$^+$ with the oxygen radicals. Oxygen, hydrogen, and hydroxide ions are formed within the flame.
1.2.4 Gas Chromatography Applications

Gas chromatography is applicable to a diverse range of applications. Gas chromatography can be used as an analytical technique or a preparative technique. The purpose of analytical chromatography is to identify and quantify components, whereas preparative chromatography is used to purify and collect one or more compounds from a mixture. Although it is possible to use GC as a preparative technique it is not common because it is difficult to scale up for commercial production [27].

The most common analytical applications are: environmental monitoring, pharmaceutical, forensic and biological applications. Biological applications include quantification of phospholipids [28], analysis of biological fluids (i.e. urine, blood, saliva) for metabolites, drugs, pesticides, and organometallics [29]. Environmental samples such as measuring the air quality, sediment and food stuffs are frequently measured for polycyclic aromatic hydrocarbons (PAHs). PAHs are toxic, carcinogenic, and mutagenic compounds which result from incomplete burning of fuels [30]. The analysis of pharmaceutical is the largest application area for gas chromatography. An application of the pharmaceutical industry is to determine the enantiomeric purity of their products [31]. A survey of the main application 1000 people using gas chromatography is displayed in Table 1.1 [32].
1.3 Partition Equilibrium

Partitioning equilibrium is defined as the ratio of the equilibrium concentration of an analyte in the stationary (liquid phase) to its equilibrium concentration in the mobile phase. Partition equilibrium is an important concept in all chromatography as it is responsible for the retention of components and variation in retention of components leading to separation of components. This section will detail the quantitative analysis of a general partition coefficient and its extension to gas-liquid chromatography.

1.3.1 Physical Chemistry Analysis

The partition coefficient, $K$, describes the distribution ratio of an analyte between two phases. Its equation is expressed in Eq. 1.1 where $C_L$ represents the concentration of analyte in the liquid phase and $C_M$ represents the concentration of analyte in the mobile phase. The variables $m_L$ and $V_L$ represent the moles of sample present in the liquid phase and volume of the liquid phase, respectively, and $m_M$ and $V_M$ describe the same quantities in the mobile phase.

$$K = \frac{C_L}{C_M} = \frac{\frac{m_L}{V_L}}{\frac{m_M}{V_M}} \quad \text{(Eq. 1.1)}$$
The partition coefficient is critical for expressing chromatographic retention. Equation 1.2 describes the retention volume, \( V_R \), which represents the volume of eluent carrier gas admitted to the column between injection of the sample and emergence of the peak maximum.

\[
V_R = V_M + K \cdot V_L \tag{Eq. 1.2}
\]

In the case of gas chromatography the partition coefficient describes the equilibrium distribution of the analyte between a liquid (stationary phase) and a gas (carrier gas). The gas compressibility effects the partitioning and therefore results in a modified partition coefficient, described in Eq. 1.3. Here \( j \) is the carrier gas compressibility correction factor.

\[
K = \frac{(V_R - V_M) \cdot j}{V_L} \tag{Eq. 1.3}
\]

### 1.3.2 Partition Equilibrium in a Theoretical System

The partitioning which occurs in gas chromatography is demonstrated in a theoretical system displayed in Fig. 1.6. The component starts in the mobile purple phase and partitions into the stationary blue phase. In gas chromatography, the distance of this theoretical zone, in millimeters, is known as the height equivalent to a theoretical plate (HETP). Upon equilibrium the mobile phase is propelled ahead to the next zone. Partitioning occurs from the mobile phase in zone 2 and from the stationary phase in zone 1 until equilibrium is achieved. The process is repeated until equilibrium is achieved in zone 4. Here \( q \) represents a percentage of the component which partitions to the stationary phase. The \( p \) value, \( 1-q \), is the percent in the mobile phase.
Figure 1.6: Theoretical separation between a purple mobile phase and blue stationary phase.
Equation 1.4 is used to determine the component in a given zone in the mobile phase, (purple, Fig. 1.6). In this equation, \( r \) represents the number of transfers, \( n \) the number of tubes, and \( f \) the factor of the initial amount in tube \( n \), after \( r \) transfers. The value \( q \) represents the percentage of components moving to the blue phase from the purple phase. The value \( p \) represents the percentage with stays in the purple phase. By extending this example to a large number of zones, one can observe the band movement and retention which will occur based on the partition coefficients.

\[
f_{n,r} = \frac{n!}{r!(n-r)!} p^r q^{n-r}
\]

(Eq.1.4)

1.4 Chromatography Theory and Quantification

The efficiency of gas chromatography requires quantification in order to determine what parameters yield the most optimal separation. Quantification revolves around the peak produced as the analyte flows through the detector. The important factors for quantification are the time retained by the stationary phase and the width of the peak. A theoretical account of the separation was developed in order to further understand the dynamics of the separation process. Van Deemter developed rate theory in order to describe the separation process. Rate theory describes how the injected band will broaden as it passes through the column. Van Deemter included a number of variables which effect band broadening, such as resistance to mass transfer, longitudinal diffusion, and Eddy dispersion. Golay further refined this theory for open tubular columns and introduced a method to display the efficiency of the column as a function of average linear velocity.

This section will introduce the metrics of separation quantification with the basic definitions required to calculate these efficiencies. Additionally, the concept of a plate and rate theory will be discussed.
1.4.1 Basic Metrics

A column’s separation efficiency is determined by a combination of the amount of retention provided by the column and the width of the sample peak. An unretained peak will require time to travel through the column, $t_m$, referred to as the holdup time. The hold-up time is solely defined by the average linear velocity and the length of the column. A retained component will take a longer time to exit the column due to the retention by the stationary phase. The retention time, $t_R$, is defined by the entire duration from the injection to the detection. The retention factor of the column is determined by the adjusted retention, $t'_r$, which is equivalent to the difference of the retention time and the holdup time. The width of the peak, $\sigma$, is critical to the determination of column efficiency. The width of the peak is calculated by the full width half maximum of each peak. These values are determined from the chromatogram and a depiction of these values is present in Fig. 1.7.
Using the terms defined above the efficiency of the column may be calculated in terms of theoretical plates. A theoretical plate is defined as the point where the solute is in equilibrium between the mobile and stationary phase. The height equivalent to a theoretical plate is defined as the distance a soluble component will travel before the component will reach equilibrium between the mobile and stationary phase. The mobile phase will continuously disrupt this state of equilibrium causing the components to diffuse back into the mobile phase. This process will occur across the length of the column, creating a Gaussian profile, as seen in Fig. 1.7.

The total number of theoretical plates, \( N \), is defined by Eq. 1.5, where the full width at half maximum is defined by \( \sigma \) and the retention time is defined by \( t_R \). It is important to note the
plate count must be carried out using an isothermal separation. The temperature of the injector must remain at a constant temperature greater than the temperature at which the least volatile component will elute from the injector.

\[ N = 5.54 \left( \frac{t_R}{\sigma} \right)^2 \]  
(Eq. 1.5)

The height equivalent of a theoretical plate, Eq. 1.6, is calculated by the product of the length of the coated column, \( L \), and the inverse of the number of theoretical plates produced by the column.

\[ HETP = \left( \frac{L}{N} \right) \]  
(Eq. 1.6)

The most common method to quantify the efficiency of the column is to report the HETP for alkane mixtures. For specialty columns the HETP will be reported for the component of interest. However, other variables are critical when comparing columns as well. The retention factor, \( k \), of the column for each analyte should be reported to express the extent to which the columns’ coatings are retaining the gaseous compound. The retention index is defined in Eq. 1.7, where \( t_M \) is the time in which it would take a non-retained component to reach the detector.

\[ k = \frac{t'_R}{t_M} \]  
(Eq. 1.7)

To observe the true separation power of the column, a combination of the retention and the column efficiency, the resolution equation is applied. The resolution, \( R \), is dependent on the distance between two peaks and the width of both peaks, as expressed in Eq. 1.8. The resolution equation is the best quantitative value to express the extent to which the column can separate two components. However, because the resolution equation is dependent on both the coating and the column efficiency it is not commonly employed, rather the HETP is used to compare to columns. The equation relates the resolution between two peaks A and B, in which B is retained longer.
Here $\sigma_A$ and $\sigma_B$ describe the full width at half maximum of the two components, A and B. The variables $(t_R)_A$ and $(t_R)_B$ describe the retention time of components A and B, respectively. Likewise, the variables $k'_B$ and $k'_A$ are the retention values for components A and B.

\[
R = \frac{2[(t_R)_B-(t_R)_A]}{\sigma_A+\sigma_B} = \frac{\sqrt{N}}{4} \left( \frac{k'_B}{k'_A} \right) \left( 1 + \frac{k'_B}{k'_A} \right) \quad \text{(Eq. 1.8)}
\]

1.4.2 Van Deemter’s Rate Theory

In 1959, Van Deemter developed an equation to express how band broadening occurred based on phenomena occurring in the column, such as longitudinal diffusion and resistance to mass transfer. Van Deemter’s original equation related the HETP to a number of constant values A, B, C and D which represent different phenomena that cause band broadening. These terms are scaled differently by the average linear velocity of the carrier gas. The A term was used to describe packed columns rather than the current open tubular columns used today. Therefore the A term is omitted. Equation 1.9 describes plate height of a separation by the contributions to the band broadening process where B describes longitudinal diffusion, $C_s$ and $C_m$ resistance to mass transfer, D extra column band broadening and $u$ the linear velocity. These contributions to plate height will be discussed in detail in the following sections.

\[
\text{HETP} = \frac{B}{u} + (C_s + C_m) \cdot u + D \cdot u^2 \quad \text{(Eq. 1.9)}
\]

1.4.3 Longitudinal Diffusion: B-Term

The B-term is representative of the longitudinal diffusion which naturally will occur in the column. This term is inversely proportional to velocity, therefore as the velocity increases the
band will spend less time in the column and have less time to diffuse. The calculation of the B term is described in Eq. 1.10, where \( D_G \) is the diffusion constant of the analyte in the mobile phase.

\[
B = 2D_G
\]  
(Eq. 1.10)

### 1.4.4 Resistance to Mass Transfer: C-Terms

The C-term defines the contribution of the resistance to mass transfer to and from the stationary phase. This component is broken into two parts, \( C_M \) representing the mass transfer in the mobile phase and \( C_S \) representing mass transfer in the stationary phase. The \( C_S \) term represents band broadening which occurs due to partitioning of the molecules into the stationary phase. As mentioned earlier, a HETP is the distance required for the components to reach equilibrium between the mobile phase and the stationary phase. When equilibrium is reached, the mobile phase will propel the solutes in the gas phase along the column. This allows the molecules in the stationary phase to diffuse back into the mobile phase as the concentration in the mobile phase is now depleted. However, this process will take time and the majority of the band is past the point where the gas will diffuse back into the mobile phase. Therefore, band broadening has occurred.

Equation 1.11 quantifies the band broadening due to the resistance to mass transfer in the stationary phase. The \( k \) value represents the retention factor which has previously been defined. The \( D_S \) term represents the diffusion of the solute in the stationary phase, \( d_f \) represents the film thickness and \( k \) the retention coefficient. The Van Deemter’s equation shows that this term increases with velocity. As the velocity increases, the diffusion of solutes in the stationary phase must diffuse quickly back into the mobile phase to maintain equilibrium. When the velocity is too high the diffusion from the stationary phase will be delayed and the band will broaden, decreasing the efficiency of the separation.
Resistance to mass transfer in the mobile phase is a result of the parabolic flow profile in the column. The particles are experiencing different velocities and therefore band broadening occurs. The band broadening caused by the resistance to mass transfer in the mobile phase is quantified in Eq. 1.12. The $r_c$ term represents the radius of the capillary and $D_M$ represents the diffusion in the mobile phase.

\[
C_M = \frac{(1+6k+11k^2)r_c^2}{24(1+k)^2D_M}
\]  
(Eq.1.12)

1.4.5 Extra Column Band Broadening: D-term

The extra column band broadening term was not originally a part of Van Deemter’s equation. Extra column band broadening refers to band broadening caused by dead volumes in the inlet, column and outlet. The extra column band broadening effects due to these dead volumes are intensified in short columns [33]. Equation 1.13 quantifies the extra column band broadening. The $\Delta t$ term represents the dead time and $L$ represents the length of the column.

\[
D = \frac{\Delta t^2}{L(k+1)^2}
\]  
(Eq. 1.13)

Additionally, gas decompression of the mobile phase contributes to band broadening. “Because of the easy compressibility of gas the velocity, $v$, and with it, $H$, vary along the column” [34]. Due to the short length of our column this phenomena provides a greater effect on the band broadening process. Equation 1.14 quantifies this effect in terms of $\sigma$ the full width at half maximum.

\[
\sigma^2 = \int_0^L H \frac{p_z}{p_o} dz
\]  
(Eq. 1.14)
H refers to the local plate height, \( p_i \) is the initial pressure, \( p_f \) is the final pressure, and \( L \) is the length of the column. This variation in linear velocity will change the efficiency of the column, specifically due to the resistance of mass transfer. To eliminate this effect in our studies, we attached five meters of uncoated “guard column” to the end of the wafer and commercial column. This reduced the unwinding effects of the carrier gas in the area where the column was coated and allowed for examination of the columns with minor contribution from extra column band broadening.

### 1.4.6 Van Deemter’s Equation: Graphical Depiction

A graphical description of the Van Deemter equation is displayed in Fig. 1.8. The longitudinal effects of band broadening are reduced with increasing linear velocity, while resistance to mass transfer is increased with increasing velocity. The contribution from both of these components are displayed Fig 1.8 as the green line and blue line, respectively. The combination of these components is displayed in a black dashed line, a result of the Van Deemter equation. The black dashed line has a minimum point where the separation is optimized. This point is reported as the column’s efficiency.
Fig. 1.8: A plot of the Van Deemter equation. The Van Deemter equation produces the combined Golay plot, in black. The contribution from longitudinal diffusion is shown in blue and contribution from the resistance to mass transfer in green.

The Van Deemter equation is derived to obtain an optimal velocity \( u_{\text{opt}} \), which is the linear velocity at which the plate height is minimized, described in Eq. 1.14. To determine the optimum velocity, test mixtures consisting of alkanes are separated in the column at a range of velocities and a Golay plot, displayed in Fig 1.8, is generated.

\[
 u_{\text{opt}} = \sqrt{\frac{B}{C_S + C_M}} \tag{Eq. 1.14}
\]

1.4.7 Isothermal and Temperature Programmed Separation
There are two modes in which separation may occur. The first is an isothermal mode in which the temperature of the oven remains the same throughout the separation. Typically isothermal separations are only used to characterize the column. The second mode is termed temperature programmed mode, in which the oven starts at an initial temperature before the temperature is increased linearly to a final temperature. The temperature programmed mode is the standard operating mode while performing a separation. The metrics used to quantify the efficiency of a column stated throughout section 1.4 are only valid during isothermal separations, as temperature effects the diffusion properties in the stationary and mobile phases complicating the quantification of plate height. Therefore, testing of the column’s efficiency must be determined using an isothermal separation. Both isothermal and temperature programmed separations were performed to characterize the column and for proper separations respectively.

1.5 Work in Microfabricated gas chromatographic columns

Development of μ-GC columns has been motivated by a number of aspects, most notably the miniaturization of the bulky oven and column used in the commercial devices [7], presented in section 1.2. With the miniaturization of additional components required for GC, a complete gas analysis system may be developed which significantly reduces the complex heating and bulky system currently used. Additionally, the small, portable devices may be utilized for continuous monitoring of hazardous and explosive compounds in the environment [16]. Novel microfabricated column designs may be introduced to reduce inefficiencies in the device such as the B and C terms mentioned in the Van Deemter equation in section 1.4. This development in μ-GC columns has also led researchers to explore novel geometries to improve the resolving power of the columns. Others have used silicon processing techniques to deposit novel stationary phases on the μ-GC for separations with specific requirements [5],[6],[36].
1.5.1 Initial Development

Micro-machined gas chromatographic columns were first developed in 1979 at Stanford [37]. The group used photolithography and wet, isotropic, etching to define a 1.5 m long by 30 µm deep column. This μGC column was integrated with a miniature thermal conductivity detector, which was separately fabricated. The motivation for the development of this column stemmed from the difficulty to connect microfabricated detectors to commercial GC columns. The μGC column displayed a column efficiency of 1,533 plates per meter, a value much lower than that of commercial columns which operate around 10,000 plates per meter. The lower resolving power of the μGC column resulted from the large band widths. These large band widths can be attributed to poor connections between the carrier gas and the μGC column, as well as the connection between the μGC column and the microfabricated sensor. Although, the column was not comparable to the resolving power of a commercial column, the first μGC column was developed and successfully integrated with a microfabricated detector and injection valve.

1.5.2 Gas chromatography Micro-Total Analytical System

Research on microfabricated chromatographic columns remained relatively dormant until 2004 when researchers at the University of Michigan started a collaborative effort to develop μTAS systems for hazardous compounds. Figure 1.9 displays the development of the μTAS device including all necessary components for a GC system.
Fig. 1.9: University of Michigan uTAS. The system uses a two-dimensional design for increased separation. Each dimension has separate heating capabilities and a thermal modulator is placed between the columns. *Adapted from Kim et. al* [38]

The pumps, pre-concentrator, injector, column, and detector are all included on chip [38]. This device was successfully tested on-site to detect the explosive marker, 2,4 DNT [39], and hazardous volatile organic compounds (VOCs) commonly found in the workplace [16]. Although this impressive device was successful in the field detection of these hazardous compounds, it is limited for many applications due to restrictions on the maximum operating temperature of the device. These restrictions are imposed by the epoxy used at the world-to-device connection, which degrades at around 250 °C. While these devices have been unable to operate with temperatures above 250 °C, gas chromatographic systems require temperatures
ranging from 250-450°C in order to separate semi-volatile pollutants such as poly-aromatic hydrocarbons, and crude oil residues. Furthermore, environmental pollutants will be unable to move to the vapor phase at lower temperatures. These temperature limitations have severely impacted the utility of the device.

1.5.3 Column Improvement

While extensive research has been conducted on the on-chip integration of the μTAS gas chromatographic system, less research has been conducted to improve the gas chromatographic column’s efficiency. One example is the semi-packed column [3], which employs photolithography to pattern pillars in the path of the gaseous mobile phase. This design was adapted from common designs in liquid chromatography. This is one of the few cases where the power of the microfabrication technique was advantageously utilized. Figure 1.10 displays the semi-packed design in detail. This column packing strategy doubled the efficiency of the column in comparison to an equivalent open tubular column. This increase in efficiency is a result of the decreased mass transfer distances between the open tube and the stationary phase, as well as low variation in the velocity profile. Although the column efficiency was increased, only volatile compounds may be separated because an epoxy was used to create the seal between the world-to-device connector. The maximum temperature of operation was limited to 250°C, once again reducing the usefulness of the device.
Fig. 1.10: Display of the semipacked column. The pillars between the larger walls reduce the diffusion distance and alter the flow characteristics to enhance separation. *Adapted from Ali et al.*

Other research explored the improvement of the column by applying novel stationary phases to the μGC columns. In 2011, Vial et al. sputtered silica on the μGC column before sealing the column with a glass wafer. The authors sputtered the silica on a typical open tubular column as well as a semi-packed column†[5], seen in Fig. 1.11. The authors reported the maximum efficiency 5,000 plates/meter in the semi-packed column, half the value of the semi-packed column with the typical stationary phase, reported in Fig. 1.10. However, they did report that the
retention doubled after moving from an open tubular design to a semi-packed design. This phenomenon did not occur when using a semi-packed column with a typical stationary phase.

Fig. 1.11: Images of open tubular (top) and semipacked (bottom) columns. The silica stationary phase has been sputtered on both. Adapted from Vial et. al

Furthermore, Stadermann et al. have used carbon nanotubes as the stationary phase, as the higher surface area may improve retention [6]. The authors included an on-chip heater on their short column (50 cm in length) coated with the carbon nanotubes. The authors designed this column for fast separation of components, and achieved successful separation of alkanes in under a minute. The plate height of the microfabricated column, however, was not reported.
1.5.4 Summary

The microfabricated chromatographic columns provide various advantages over commercial counterparts. Firstly microfabricated columns are smaller in comparison to their commercial counterparts. This will enable the miniaturization of the bulky oven. The low thermal mass of the silicon devices allows for fast temperature cycling, which in turn leads to quicker and more efficient separations. These devices are able to utilize complicated geometries such as the semi-packed design, which increase the efficiency of the column. Miniaturization of the components and developments of a μTAS devices enable on-site detection of hazardous compounds. These μTAS devices are inexpensive in comparison to the bench-top commercial devices.

1.6 Project Outline

Prior to this research, only low temperature (<250 °C) separations have been conducted on chip-scale chromatography columns. This is a result of the use of materials in the μGC column, which are incompatible with high temperature operation. However, the operation of μGC columns for the separation of semi-volatile chemicals of interest require operation at temperatures >250 °C. Additionally, the μGC columns have yet to provide a significant improvement over the commercial columns. This is in part due to the extra column band broadening effects of the short μGC columns and inefficiencies in the world-to-device connection. In this work the μGC columns are benchmarked against commercial column with and without experimental conditions which eliminate the extra column band broadening due to short columns.
CHAPTER 2

Fabrication and Experimental Methods

This chapter describes the design and fabrication of the microfabricated gas chromatographic columns. In section 2.1 the design of the photomasks used to pattern the column and through-holes are discussed. Section 2.2 details the fabrication process flow of the μGC column, including an explanation of the deep reactive ion etch, anodic bonding process, backside alignment of the through-holes, and coating of the column with a stationary phase. The experimental set-up and experimental parameters are explained in section 2.3. This section includes the GC system used to carry out the testing of the devices. The characterization of the efficiency of the microfabricated and commercial column, as well as the quality of the coating is described. Finally the methodology for the temperature-programmed separation is outlined.

2.1 Mask Layout

All photomasks were designed with L-edit software. Figure 2.1 displays the two masks required for the fabrication of this device. The first photomask, displayed in Fig. 2.1a, is used to pattern the double Archimedean spirals channels of the μGC column. Cut lines surround each device on the mask for ease of dicing and to accurately define the size of each chip. Alignment marks, the plus symbol and squares, are present on the spiral design for the backside alignment process. For creation of a seamless world-to-device connection, through-holes were etched into the back of the device. Figure 2.1 b displays the mask required for the backside alignment and etching of the through-holes for the μGC column; these through-holes are 360 μm in diameter. This mask also includes alignment marks which match with the alignment marks in the spiral mask.
Fig. 2.1: Two photomask designs required for device fabrication. (a) The six double Archimedean spiral devices are shown with respective cut lines. The alignment marks and inlet/outlet are displayed in the magnified inset. (b) The through-holes used for backside alignment match with the inlets/outlets of the Archimedean spirals. A magnified inset shows the alignment marks and the through-holes.
2.2 Fabrication Process Overview

Fabrication of the μGC columns requires three processes originally developed for the semiconductor industry: photolithography, deep reactive ion etching (DRIE) and anodic bonding. Figure 2.2 shows the fabrication steps of the μGC column. First, the photoresist (SPR955.2.1) was spin coated on a silicon wafer, Fig. 2.2A. The column’s double Archimedean spiral pattern was transferred to the wafer, Fig. 2.2B,C using photolithography and was developed. Formation of the microfluidic channels was accomplished with a deep reactive ion etch, in which 100 μm of silicon was removed from the wafer, Fig. 2.2D. After the etching process, the microfluidic channels were closed by anodically bonding a borofloat glass wafer to the face of silicon wafer containing the channels, Fig. 2.2E. Creation of the seamless design was accomplished by fabricating through-holes on the backside of the wafer. Photolithography was used to define the through-holes. Photoresist was spin coated onto the bonded silicon wafer, as depicted in Fig. 2.2F. The through-holes were then defined by photolithography, as shown in Fig. 2.2G,H. A deep reactive ion etch was used to remove silicon material until the etched channels on the opposite side appeared under optical microscopy 2.2I. Finally, the column was coated with a stationary phase, Fig. 2.2J. A detailed description of the fabrication process is provided below.
Fig. 2.2: Overview of fabrication process. Steps (A), (B), and (C) describe the photolithographic process. Step (D) shows the deep reactive ion etching (DRIE) and (E) displays the anodic bond. Steps (F), (G), and (H) show the backside photolithography. Step (I) shows the backside DRIE and (J) displays the addition of the tubing and the stationary phase.

### 2.2.1 Photolithographic Processes

Photolithography is a standard technique to define patterns in the semi-conductor industry. In the photolithographic process, a photoactive compound (PAC) is set atop the material which will be processed. The molecular structure of the PAC is altered by the application of ultra-violet (UV) light. In the case of positive photoresists, the application of UV
light breaks a bond of the PAC’s structure and produces a compound which is acidic. This acidic compound is dissolved in a basic solution, referred to as a developing solution, to remove the acidic compounds. By selectively allowing the light to contact parts of the photoresist, a pattern is formed. The photolithographic process used in this work is described in detail below.

A four inch, 525 μm thick (100) silicon wafer is used for the base of the μGC column. Firstly, hexamethyldisilazane (HDMS) was applied to the wafer to promote adhesion of the photoresist to the wafer. The HDMS was spin coated on the wafer at 3000 rmp for 35 seconds followed by a bake step at 110 °C for 30 seconds. The SPR-955 positive photoresist was spin coated at 3000 rpm for 35 seconds to obtain a film 2.1 μm thick. The photoresist was soft baked at 105 °C for 1 minute. The wafer was immediately taken to the EVG-620 mask alignment system for ultra-violet exposure. The mask in Fig. 2.1a was loaded and the wafer was placed into hard contact. The wafer was exposed at 8 mW/cm$^2$ for 9.5 seconds. The photoresist was then placed in a CD-26 developing solution for 2 minutes, after which the wafer was cleaned with DI water. The wafer was placed on a hotplate at 120 °C for 5 minutes to further remove the solvent in the photoresist.

The backside etching process requires a thicker film of SPR-955. To obtain this thick film, the SPR-955 was spin coated at 1200 rpm for 35 seconds and then baked for 2 minutes at 105 °C before being cooled to room temperature, producing a film of 3.35 μm. This process was repeated to obtain a thick film of 7.1 μm, as this thick film is required for a longer backside etch.

2.2.2 Deep Reactive Ion Etching

Deep reactive ion etching (DRIE) is an anisotropic process used to fabricate smooth sidewalls with high aspect ratios. The deep reactive ion etching process combines physical etching via ion bombardment with chemical etching [40]. An SF$_6$ reagent was used as the etchant, C$_4$F$_8$ was the
polymer used to passivate the sidewalls and prevent isotropic etching, and O₂ was used to balance the chemical and physical etching rates promoting the formation of flat bottom channel with no tapering. Oxygen radicals compete with the fluorine radicals to passivate the surface, reducing the rate of chemical etching [41]. Fig. 2.3 gives a cartoon description of the process used to fabricate the trenches. This process was used to form 100 μm deep trenches in a double Archimedean spiral design.

Fig. 2.3: The deep reactive ion etching process. Firstly, the passivation polymer is applied to the wafer. The passivation layer is partially removed by the oxygen plasma and oxygen passivates the silicon. SFₓ ions chemically and physically remove bulk material.
In this work an Alcatel Speeder 100 Deep Silicon Reactive Ion Etcher was used to perform a radiofrequency (RF) etch process. The RF recipe used for fabrication of the trenches required: 300 standard cubic centimeters per minute (sccm) on SF$_6$ for 3 seconds, 300 sccm on C$_4$F$_8$ for 1.5 seconds and 100 sccm of O$_2$ for 1 second, with the full list of parameters presented in Fig. 2.4. This recipe was developed to create a flat bottom rather than a curved bottom, which would occur from the standard etching technique (Bosch process). Additionally, this process eliminates the scalloping of the sidewalls that results from the Bosch process. The etch rate was ~5 \( \mu \)m per minute and a typical etch was run for 19.2 minutes to define the 100 \( \mu \)m deep trench. The result of the high RF etching process is displayed in Fig. 2.5. The double Archimedean design is displayed along with a small section of the etched area.

![Fig. 2.4: Overview of the process parameters for the flat bottom etch. The gas flow and duration are shown on the left side of the graph in Standard cubic centimeters (sccm) and seconds (s), respectively.](image)
Fig. 2.5: Image of the etched channel. A cartoon of the channel (top) and an SEM image of the inlet/outlet of the etch channels (inset) are displayed.

2.2.3 Anodic Bonding

Anodic bonding is a process used to bond a silicon wafer with a borofloat glass wafer from schott glass. The borofloat glass wafer’s coefficient of thermal expansion is matched to that of silicon minimizing the stress between wafers. This is critical to the bonding process, a mismatch of the coefficient of thermal expansion will result in broken wafers or improper bonding [42]. The bonding of these wafers occurs in a chamber held at high temperatures (200-400°C) and under vacuum [43]. After stabilization of the pressure and temperature, a voltage
(100-1500V) is applied between the two electrodes in contact with the silicon and glass wafers. This electric field facilitates the bonding process. Figure 2.6 depicts the basic setup of the anodic bonder. The silicon and glass ‘stack’ are fixed between a bond electrode (top) and larger chuck (bottom). The bond electrode acts as the anode and bottom chuck as the cathode. Although the chemical mechanism in which bonding occurs is not entirely understood, it is accepted that the applied field assists in bonding by moving the positively charged ions in glass (e.g. sodium ions), away from the bonding interface. Furthermore, the electric field forces O$_2^-$ ions in the glass to be pushed toward the interface, promoting the formation of SiO$_2$ bonds between the silicon and glass interface. The application of higher temperatures is known to decrease the resistance of ion movement in the glass substrate.

---

Fig. 2.6: Schematic of the anodic bonding process. An electric field in applied across the silicon and glass wafer stack. The negative oxygen atoms move toward the interface of the glass-silicon while the positive sodium ions move away.

---

To bond the 500 μm thick borofloat glass to the 525 μm thick silicon wafer, a process similar to that described above was used with an EVG-501 bonder. Firstly, the remaining photoresist on the wafer was removed by allowing the silicon wafer to soak in Remover PG for at least 1 hour,
followed by a 10 minute rinse in Nanostrip. Both the silicon wafer and glass wafer were cleaned with common cleaning procedures, RCA 1 and RCA 2, for 10 minutes each. The silicon wafer then was placed in a 6:1 BOE for 10 minutes to remove the oxide layer developed by the RCA cleaning processes. The silicon and glass wafer stack were assembled and placed into the chamber. The chamber was then set to a temperature of 400 °C, along with a vacuum of ~10⁻⁴ Torr. Following stabilization of temperature and pressure, a voltage of 500 V was applied until the current reached .7mA. This process was repeated for applied voltages of 750 and 1000 V. After this stage, the chamber was vented and cooled. The wafer was not removed until the chamber reached room temperature to prevent thermal shock. The bonded silicon and glass wafer is displayed in Fig. 2.7.

Fig. 2.7: Image of bonded silicon and glass wafer. The silicon wafer with etched channels (bottom), is anodically bonded to the glass wafer (top).
2.2.4 Backside Lithography, Alignment and Etching

For a seamless design, ports the size of the outer diameter of the capillary (360 μm) were etched 400 μm into the silicon backside of the bonded wafers. A thick layer of photoresist, 7.1 μm, was spin coated on the silicon side of the bonded wafer. To accurately etch the through-holes such that they align properly to the inlet and outlets of the Archimedean channel, the alignment marks on the photomasks were used, as shown in Fig 2.1.

Firstly, the mask was loaded into the EVG-620 bonder and digital marks were placed on top of the alignment marks on the mask. The mask was removed and the bonded wafer was then loaded into the EVG. The micromanipulators were used to move the wafer until the etched alignment marks on the silicon wafer were covered by the digital alignment marks. After alignment, UV light was applied to the photoresist for 20 seconds and the wafer was developed for 5 minutes in CD-26. After development, the wafer was etched for 1 hour and 3 minutes using DRIE and same process that was listed above. After etching, the wafer was inspected using an optical microscope to ensure that the channels were fully visible. If the channels were not visible or partially obstructed, the DRIE process was run again for 30 seconds and reevaluated optically.

2.2.5 Packaging

The devices were diced using an ADT 7100 dicing saw, cutting the bonded wafer into 6 devices. Nanoport assemblies were then aligned using an optical microscope and attached to the silicon wafers to create a leak-tight seal between the capillary and the microfabricated column. The nanoport assemblies use an epoxy which is cured by heating. The wafers with epoxy and nanoports in contact were placed into an oven at 160 °C for 1 hour.
Fig 2.8: Image of device with nanoport assemblies. The top-left panel displays the 1st generation column with nanoports on the glass side. The 2nd generation column is displayed in the bottom image where the nanoport assemblies are creating a world-to-device connection using the silica tubing. The top-right side displays the relatively small size of the 2 meter device.

2.2.6 Coating Stationary Phase

Two methods exist to coat a chromatographic column: static coating and dynamic coating. In static coating the column is filled with the stationary phase and one end of the column is plugged. Then vacuum is applied to the other end intending to remove the solvent and leave a
film of the stationary phase within the column. This method is particularly difficult to accomplish with microfabricated devices, however, it allows the user to quantify the film thickness [14]. The dynamic coating method involves infusion of a solution of stationary phase and solvent through the entire column. Then a gas is used to push the solution out of the column. This also leaves a film of stationary phase and is easier to perform dynamic coating, although quantification of the film thickness is difficult.

The column was coated with a film of non-polar polydimethylsiloxane (PDMS). A dynamic coating procedure was used to coat the columns after difficulty in attempting the static coating. A solution of 0.737 g/ml PDMS in a pentane solution was used for the dynamic coating procedure. The column was initially filled with a solution of pentane to remove any impurities in the column. The pentane was then eluted by helium gas at high pressure. Next, the PDMS coating solution was infused into the column at a rate of 2 μl/min. Once the column was entirely filled, it was connected to a helium tank at one end and the PDMS solution was pushed out of the column, leaving a PDMS film. The side which was attached to helium was marked; this side was also placed into the injector. After coating, the coated leads were removed and uncoated leads were placed on either side of the column. For use with high temperature testing, the columns were heated to 350°C and allowed to sit under 20 psi for one hour.

2.3 High Temperature Manifold

To enable high temperature applications, a face sealing technique was developed, which uses commercially available column nuts and graphite ferrules. A stainless steel manifold, displayed in Fig. 2.9, was fabricated at the Pennsylvania State University’s machine shop. A divot with the dimensions of the column was milled into the bottom manifold. The top manifold was precisely milled such that when it was fixed to the bottom manifold, insertion of the column
nuts would align the capillary to the inlets of the column in the bottom manifold. Once the chip was in the bottom column, the top column was screwed onto the bottom. The capillary was then threaded through the column nut and the graphite ferrule was compressed on the column one quarter turn past hand tight. The graphite ferrule and column nut are rate for temperature operations up to 1000 °C. This sealing technique removes the thermal limitations present in previous sealing techniques.

Fig. 2.9: Display of metal manifold and compression sealing technique. A cartoon of the metal manifold details the column nut and graphite ferrule required for the compression seal. As well as how the manifold fits together. A picture displays the manifold assembled and ready for use.
2.4 Experimental Set-up and Testing Parameters

This section will describe the experimental parameters of the gas chromatographic system as well as the parameters for determining column efficiency. Initially the first and second generation columns were benchmarked against commercial columns. After this, the μGC and commercial columns were compared with a five meter uncoated silica tubing to remove extra column band broadening. Finally, complex mixtures requiring high temperatures were separated to outline the ability of our μGC columns to properly operate under standard conditions. The specifics of these experiments are described in detail below.

2.4.1 Determination of Column Efficiency

All columns were tested using an Agilent 7820A Gas Chromatograph with FID detector and split/splitless inlet. A solution of heptane or mixture of hexane, heptane, and octane was placed into a vial with a crimped lid. The solution was allowed to sit so that the alkanes partitioned into the vapor phase from the solution, creating a large concentration in the head space of the vial. Two microliters of vapor was collected by the automated syringe and injected into the inlet through a split/splitless double goose neck SPME liner. The split ratio was set to 1:200. The two meter microfabricated columns, commercial columns, and silica tubing’s coated in-house were tested at various linear velocities ranging from 3 cm/s to 150 cm/s. The data was processed into a Golay plot from which the optimal HETP was determined. Uncoated 40 centimeter long silica tubing was used to connect the μGC column to the inlet and outlet, as seen in Fig. 2.10.
2.4.2 Determination of Column Efficiency with Restrictor Column

To eliminate the extra column band broadening presented in chapter 2, a five meter uncoated silica tubing was connected to the outlet of the μGC and commercial columns. Again, 40 cm tubing was connected to the inlet and outlet of the μGC column. A standard connector was used to connect the 5 m tubing to the 40 cm tubing on the outlet. Although the 5 m tubing could be connected directly to the outlet of the μGC column, the standard connector was used in order to make a direct comparison to the commercial column, as the connector may induce band broadening.

Fig. 2.10: Initial testing on the μGC columns. The nanoports are attached to create a world-to-device connection between the inlet/outlet and the 40 cm long capillary tubing.
The commercial tubing was connected to the inlet and a 5 m uncoated silica tubing was attached to the outlet of the commercial column, as shown in Fig. 2.12. Again, a standard connector was used to attach the commercial column to the uncoated silica tubing. Similar to the initial testing, a mixture of heptane, hexane, and octane was used to determine the plate height in each column. The column was tested at a range of velocities in order to generate a Golay plot and determine the maximum column efficiency. However, the increased resistance due to the 5 m uncoated tubing restricted the range in which the GC can operate to 75 cm/s, down from 135 cm/s.

Fig. 2.12: Modified testing of the μGC columns. A 40 cm long tubing is connected to the inlet and 5 meters of uncoated tubing is connected to the outlet of the μGC columns.
2.4.3 Experimental Set-up and testing methodology

The EPA method 8310 was used to display the separation of polycyclic aromatic hydrocarbons (PAHs). The PAHs are dissolved in a solution of acetonitrile, and each has a concentration of 500 μg/ml. A liquid injection of 1 μl was deposited into a split/splitless double goose neck SPME liner. The split ratio was set to 1:100 and the injector and detector were set to 250 °C. The oven temperature was initially set to 45 °C, after which the oven was ramped by 15 °C until it reached 325 °C. Helium was used as the carrier gas and operated at a linear velocity of 35 cm/s.

The simulated distillation was performed by separating an 18 component mixture of alkanes using method ASTM D2887. Each alkane is in a 1% w/w solution with carbon disulfide. The mixture was then diluted 1:20 in carbon disulfide to prevent fronting of the peaks. One microliter of the solution was injected on the column with a split ratio of 1:100. The injector and detector were set to 250 °C and the oven temperature was initially set to 45 °C, after which the oven was ramped by 15 °C until the oven reached 350 °C. Helium was used as the carrier gas and operated at a linear velocity of 35 cm/s.
CHAPTER 3

Results and Discussion

This chapter presents the separation efficiency results of our μGC columns by measuring the height equivalent of a theoretical plate (HETP) of alkanes. Furthermore, separation of complex mixtures of environmental pollutants requiring high operating temperatures is displayed. In these studies, an Agilent 7890B GC with an FID detector was used for injection of the gaseous mixtures and detection of the elution of the μGC column. Section 3.1 displays the separation efficiency of μGC column against commercial equivalents by quantifying the separating a simple mixture of alkanes consisting of: hexane, heptane and octane. Development of an improved second generation column is described and its’ separation power is displayed in section 3.2. In section 3.3 a five meter restrictor column is placed on the end of the μGC and commercial columns to eliminate the extra column band broadening and compared the columns as if they were of a standard length. The first μGC with the ability to operate in the standard GC temperature range (45-350 °C) is displayed in section 3.4. To demonstrate this complex mixture of alkanes (C5-C44) was separated using our μGC with high temperature manifold. Section 3.5 demonstrates the utility of our μGC device by separation of common semi-volatile environmental pollutants deemed important by the environmental protection agency because they are toxic, mutagenic, and carcinogenic [44]. This separation demonstrates both the separation ability and the high temperature operation for this μGC column with a real world application.
3.1 Initial Determination of Column Efficiency

Initial testing of our first generation μGC column’s efficiency was benchmarked directly against 2 meter commercially-coated columns with inner diameters of 100 μm. The columns were compared by determination of the maximum plate height in the Golay plot produced by each column, Fig. 3.1. The column efficiency was determined by the column’s separation of a gaseous mixture of heptane. Figure 3.1 displays the commercial column with film thickness of 0.4 μm provided the lowest HETP and therefore most efficient separation. The commercial column with a film thickness of 0.1 μm produced a plate height closer to that of the μGC column. The microfabricated column’s optimal point of separation equivalent to the silica tubing coated in house. The microfabricated column, however, displays a dramatic increase in HETP once the velocity is set past the point of optimal velocity. This suggests two possible issues: either the film thickness is very large contributing to the C terms or extra column band broadening is very large contributing to the D-term. This was attributed to the poor alignment of the inlet and outlets contributing to the D-term rather than a thick film. A second generation column was developed with the alignment issue in mind. Elimination of the thick film contribution was due to the low retention value of the microfabricated column discussed later.
Fig. 3.1: Performance comparison of the first generation μGC columns with standard silica tubing. The μGC column HETP compared against commercially coated columns (top). The HETP of silica tubing which were coated in house.

Figure 3.1 also illustrates the deviation of each point from the theoretical Van Deemter curve. The theoretical Van Deemter was generated by fitting the data to the points to solve for the co-efficient of Van Deemter’s equation. The collected data’s deviation from the theoretical
The curve is at least in part due to the pressure controller, which is developed for columns ranging from 10-30 meters in length. These columns provide significantly large resistance than the commercial and μGC columns used here. The inconsistencies of the pressure maintained by pressure controller are amplified when using a short column, and therefore may propel the mobile phase at a velocity different from that of the set point. Additionally, band broadening from extra column effects, D-term, in the Van Deemter equation will occur due to the short column length. To reduce the error from the inconsistencies of the pressure regulators and eliminate the D-term, a 5 meter restriction column was applied to the back end of the columns, detailed in section 3.3.

Table 3.1 presents the retention coefficients and minimum HETP for the commercial GC columns and the μGC column. As mentioned in chapter 1, the retention coefficient displays the ability of a column to impede movement of an analyte. The commercial column with 0.1 µm film thickness theoretically should display a lesser retention coefficient than the 0.4 µm film thickness column. The retention of the 0.4 µm column is 3.26 while the 0.1 µm column was 2.26, these values will act as standards to determine the quality of the dynamic coating procedure used to coat the μGC column. The retention coefficient of the μGC column was 1.64, significantly lower than that of both commercial columns. This value indicates that the quality of the coating of the μGC column is poor compared to the commercial columns. Multiple factors may be responsible for the decreased retention value. One factor is that the dynamic coating method used to coat the column has produced a thin film ( <.1 µm) column and therefore its retention is less than that of its’ commercial counterparts.
With the performance of the first generation column was poorer than expected a second generation column was developed. The first generation design had poor alignment of the inlets and outlets, which was suspected to contribute significantly to band broadening. To address this issue, we developed a process that uses the precise alignment ability of the EVG-620 mask aligner as describe previously in chapter 2. Column efficiency of the 2nd generation column was determined in an identical manner to that of the 1st generation column. Figure 3.2 displays the large contribution the misaligned holes played in reducing the efficiency of the column. The HEPT at the optimal velocity is now comparable to that of the commercial columns. Additionally, the plot displays the large reduction of the C and D contributions to the HETP.
Fig. 3.2: Performance comparison of the second generation μGC columns and standard silica tubing with commercial columns.

Table 3.2 display the exact values of the minimum HEPT for each column. The second generation column improves upon the first generation column as noted by the reduced optimal HETP. To ensure the improved HETP is due to the new design and not caused by a change in film quality the retention values are compared. The retention value varies slightly and the variation is not within standard error.

<table>
<thead>
<tr>
<th>Column Type</th>
<th>Retention Co-efficient</th>
<th>Optimal HETP (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commerical Column (.4 μm Stationary Phase)</td>
<td>3.26 +/- 1.40</td>
<td>0.81 +/- .262</td>
</tr>
<tr>
<td>Microfabricated Column (1st gen.)</td>
<td>1.64 +/- 0.33</td>
<td>1.46 +/- .266</td>
</tr>
<tr>
<td>Microfabricated Column (2st gen.)</td>
<td>1.67 +/- 0.39</td>
<td>0.82 +/- .282</td>
</tr>
</tbody>
</table>

3.3 Determination of Column Efficiency with Restrictor Column

The two meter microfabricated and commercial columns separation efficiencies do not compare well to a standard commercial column of length 10-30 meters. To determine if the
microfabricated columns could replace typical commercial columns the extra column band broadening of both the commercial and microfabricated columns were removed. The second generation μGC columns were tested with a five meter uncoated silica tubing attached to the end of each column. We determined theoretically that 5 meters of guard column is optimal to maximize plate height. These uncoated columns are referred to as restrictor columns and the uncoated tubing was attached to the end of the column to simulate longer tubing. As discussed previously, short columns (<5 meters) will suffer from extra column band broadening. Attachment of a restrictor column eliminates the extra column band broadening effects. Additionally, the pressure gauge used to control the carrier gas velocity was designed for columns greater than 10 meters. The error in the pressure gauge will create larger error in the carrier velocity in shorter column and therefore attaching the restrictor column will decrease this error. However, addition of the guard column will increase the band broadening due to longitudinal diffusion.

These columns with the restrictor were tested with a mixture of hexane, heptane and octane. Figure 3.3 displays the Golay plot of the μGC column in comparison to the 0.4 μm film commercial column. The 2nd generation μGC column displayed a superior maximum HEPT than that of the 0.4 commercial columns. The μGC column’s minimum plate height was 0.004 cm for heptane and 0.006 cm for octane. The commercial column had a minimum plate height of 0.007 μm for heptane and 0.007 cm for octane. It is clear the 5 meter restrictor significantly increased the column efficiency of both the commercial and μGC columns.
Fig. 3.3: Performance comparison of the second generation μGC columns with 5 meter restriction column and standard silica tubing with commercial coating.

Table 3.3 displays the retention time, band width, and height equivalent to a theoretical plate of the μGC column and the commercial column at their respective minimum HETP for pentane, heptane, and octane.

<table>
<thead>
<tr>
<th></th>
<th>μGC</th>
<th></th>
<th>Commerical</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t_r (s)</td>
<td>σ(s)</td>
<td>HEPT(cm)</td>
<td>t_r (s)</td>
</tr>
<tr>
<td>1. Pentane (C6)</td>
<td>0.331</td>
<td>0.003</td>
<td>0.00296556</td>
<td>0.485</td>
</tr>
<tr>
<td>2. Heptane (C7)</td>
<td>0.389</td>
<td>0.004</td>
<td>0.00381717</td>
<td>0.753</td>
</tr>
<tr>
<td>3. Octane (C8)</td>
<td>0.657</td>
<td>0.009</td>
<td>0.00677446</td>
<td>1.404</td>
</tr>
</tbody>
</table>
3.3 Simulated Distillation with American Society for Testing and Materials (ASTM) 2887

A simulated distillation is a typical gas chromatography technique in which a mixture of alkanes is eluted in order of boiling point. The test method used here was ASTM 2887, which covers materials which have boiling point from 55 °C – 538 °C, with components of ASTM 2887 listed in Table 3.3. A common use for simulated distillation is to determine the boiling points of crude oils. However, we are using the simulated distillation to display our column’s ability to operate at temperatures up to 350 °C, the maximum oven temperature of the GC. Figure 3.4 displays all eighteen components of the ASTM 2887 mixtures have eluted. The oven temperature started at 45 °C and was ramped to 350 °C at a rate of 15 °C/min. The GC will fault if a seal is broken prior to the completion of this separation and therefore will indicate the columns ability to operate in the standard temperature range. This separation was performed over twenty times with the same column, outlining the longevity of this set-up.
Fig. 3.4: Simulated distillation of ASTM 2887 in a μGC column with 5 meter guard column. The top chromatograph displays the full separation. The bottom chromatogram displays a zoom-in of the first 4 peaks. The simulated distillation was started at 45 °C and was ramped to 350 °C at a rate of 15 °C/min.
The first high temperature separation in a μGC column is displayed in the chromatogram in Fig. 3.4. All 18 components of the ASTM mixture were resolved in the column and detected by the FID. As mentioned in chapter 1, while using a split-splitless flash injector components with higher molecular weight tend to move towards the waste outlet of the injector rather than being injected into the column. The effects of the injector’s molecular weight discrimination can be observed in components 15-18, and is typical while using a split/splitless flash injector.

### 3.4 High Temperature Separation of Polycyclic Hydrocarbons

The simulated distillation displays the column’s ability to operate at temperatures up to 350 °C. However, simulated distillation includes components that are separated due to distinct differences in boiling point, rather than retention from the column. To display the column’s separation power, a mixture of polycyclic aromatic hydrocarbons was separated. A solution of EPA method 8310 was injected into the column, the components of which are displayed in Table 3.5. The EPA method 8310 solution requires separation in the column as well as temperature ramping due to the similar structures and therefore boiling points of its’ components. Without separation, multiple components would co-elute as a single peak. Polycyclic aromatic hydrocarbons (PAHs), environmental pollutants that are formed when oil, coal, and trash
undergo incomplete burning, have been determined to be toxic, mutagenic, and carcinogenic. Gas chromatography is commonly used to determine the concentration of the carcinogens in ground water and air.

The chromatogram produced by separation of EPA8310 is displayed in Fig 3.6 and shows that the column was able to resolve most components. However, the column had some difficulty resolving components 7 and 8, which have a similar boiling point and retention time. In general, PAHs are separated in columns which have lengths of 15-30 meters. The column length of the µGC was only 2 meters. For a column of this size, the µGC performed an excellent separation of this complex mixture.

![Fig. 3.6: Separation of Polycyclic Aromatic hydrocarbons in a µGC column with 5 meter guard column. The separation was started at 45 °C and was ramped to 325 °C at a rate of 15 °C /min. The temperature ramping was stopped at 325 °C because the least volatile component only required a temperature less than 325 °C, unlike the simulated distillation.](image-url)
Table 3: Retention times and boiling points of the 18 component EPA 8310 separated in the μGC column in order of elution

<table>
<thead>
<tr>
<th></th>
<th>b.p. (°C)</th>
<th>t_r (m)</th>
<th>b.p. (°C)</th>
<th>t_r (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. 1-methylnapthalene</td>
<td>240</td>
<td>4.704</td>
<td>11. Benzo(a)anthracene</td>
<td>437</td>
</tr>
<tr>
<td>4. Acenapthylene</td>
<td>270</td>
<td>6.313</td>
<td>13. Benzo(b)fluoranthene</td>
<td>481</td>
</tr>
<tr>
<td>6. Fluorene</td>
<td>295</td>
<td>7.642</td>
<td>15. Benzo(a)pyrene</td>
<td>495</td>
</tr>
<tr>
<td>8. Anthracene</td>
<td>340</td>
<td>9.301</td>
<td>17. Benzo(g,h,i)perylene</td>
<td>542</td>
</tr>
<tr>
<td>9. Fluoranthene</td>
<td>384</td>
<td>11.118</td>
<td>18. Indeno(1,2,3-c,d)pyrene</td>
<td>534</td>
</tr>
</tbody>
</table>
CHAPTER 4
Conclusion and Future Work

4.1 Conclusion

A microfabricated gas chromatographic column (μGC) was developed and fabricated. This device is the first microfabricated column which able to operate up to temperatures of 350 °C, enabling microfabricated columns to operate in the typical range of a commercial column. These columns displayed high separation efficiency up to ~26,000 plates/meter superior to that of the commercial columns which displayed plate counts of ~13,000 plates/meter.

These devices were used to initially separate mixtures of hexane, heptane, and octane in a commercial gas chromatograph with a flame ionization detector. Table 4.1 displays the developments and separation efficiencies produced by other groups. To date our column with the 5 meter restriction attached produced the device with the highest efficiency. The large impact the extra column band broadening contributes to the separation efficiency. These results suggest possible advancement in the μGC device. The most basic solution would be the fabrication of a longer column. However, clever additions to the μGC may reduce the extra column band broadening without requiring a longer column.
This device was also used to separate complex mixtures of alkanes (C5-C44) and polycyclic aromatic hydrocarbons. In these applications a metal manifold was used to seal silica tubing to the chip forming a world-to-device connection which is compatible to 350 °C. Alkanes over C15 were separated using a μGC device, which to date has only been accomplished by our group. Additionally, all components in the EPA 8310 mixture were separated using our μGC.

### 4.2 Future Work

The findings of this work make it clear that longer μGC columns are extremely desirable for optimal performance. We are in the process of exploring μGC columns, 10 m in length. Figure 4.1 displays the size of a fabricated 10 meter column in comparison to a 2 meter column and an American quarter.
Thus far, fabrication of 10 meter columns has proven difficult. The anodic bonding process is more complex for the 10 meter design than the 2 meter design due to the increased space, in which no silicon glass bond exists. Additionally, the increased resistance to flow proves problematic after bonding. After the anodic bonding process, optical inspection of the column indicates that bonding was successful. However, with application of pressure to the column, it is apparent that part of the glass-silicon bond has separated. Due to the strength of anodic bonding, we have concluded that the column would break prior to the glass-silicon de-bonding. Therefore, the bonding procedure must be improved. The cleaning procedure of the wafers will be reviewed to ensure all organics or metal particles are removed from the wafer. If the problem persists, the design may be modified to increase the wall thickness of the microfluidic channels.
Secondly, our benchmarking of microfabricated columns concluded that the retention index of microfabricated columns is lower than that of commercial products. Static coating of the columns might improve the overall performance of the device further. However, this requires process development. Each company closely guards their coating techniques. Currently, our collaborating professor is discussing collaboration with a commercial GC company, Restek, to coat our columns using their proprietary technique.

The separation efficiency of gas chromatographic columns is dependent on the diameter of the columns. A small diameter column will theoretically provide a better separation due to the decreased distance of diffusion from the channel center to the wall [3]. Parallel columns that include narrower diameters but maintain high throughput will be able to provide further improvements in $\mu$GC technology. Our innovative double Archimedean spiral design is inherently capable of incorporating parallel channels. To minimize band broadening the lengths of the columns must be equal. Our Archimedean spirals provide a geometry, which allows multiple columns to connect to one inlet and one outlet while maintaining the same path length. The parallel column design is displayed in Fig. 4.2. Thus far band broadening induced by small variations in etching of each column has reduced the separation efficiency in each column. Improvement of the etching process will remove the band broadening due to the variations of channel width and depth.
Fig. 4.2: Parallel column design. Our Archimedes spiral design consists of 10 channels originating at one inlet and connecting to one outlet. All channels are equal in length.
4.3 References


