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Microfabrication and Evaluation of Planar Thin-Film Microfluidic Devices

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MICROFABRICATION AND EVALUATION OF PLANAR THIN-FILM MICROFLUIDIC DEVICES

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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ABSTRACT

MICROFABRICATION AND EVALUATION OF PLANAR THIN-FILM MICROFLUIDIC SYSTEMS

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Over the past 15 years, research in the field of microfluidics has rapidly gained popularity. By seeking to miniaturize and automate separation-based analysis, microfluidic research seeks to improve current methods through decreased cost, analysis time, and sources of contamination. My work has focused on developing a novel fabrication method, based on standard microfabrication techniques, to create thin-film microfluidic devices. This microfabrication format makes it possible to generate devices that provide high efficiencies, enable mass fabrication, and provide a platform capable of integrating the microfluidic and electronic components necessary for a micro-total analysis system (μ-TAS). Device fabrication combines the processes of photolithography, thermal evaporation, plasma enhanced chemical vapor deposition (PECVD), and wet chemical etching to ultimately provide hollow-core channels. When these microcapillaries are filled with buffer and potentials are applied across them, control of the flow in the channels can be established. By designing intersecting microchannels having an offset "T" geometry, I have been

able to inject and electrophoretically separate three fluorescently labeled amino acids and obtain efficiencies of over 2500 theoretical plates. Through the addition of commercially available electroosmotic flow reducing coatings, I have been able to improve the separation of these amino acids, decreasing the run time by approximately 6 fold and increasing the efficiency by as much as 10 fold. Through the use of these coatings I have also been able to carry out electrophoretic separations of three peptides.

My most recent work has focused on the polymerization of acrylamide gels in these channels. A method for the selective placement of a gel has been developed using a prepolymer solution with a light-sensitive initiator. Further work to adjust the polymer pore size and interface with ampholyte-containing gels should allow methods such as capillary gel electrophoresis (CGE), preconcentration, and two dimensional (isolectric focusing and CGE) separations to be performed. The development of gel-based analysis methods, along with other fluidic and electrical capacities, should move thin-film microdevices toward the realization of the lab-on-a-chip concept.

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1. INTRODUCTION

1.1 Motivation

Since the introduction of chromatography at the beginning of the twentieth century, separation science has emerged as one of the largest fields in analytical chemistry. Analytical separations are used routinely in pharmaceutical development, medical diagnosis, forensics, environmental monitoring, fundamental scientific research and many other applications.[1] This wide popularity of analytical separations has inspired significant research to improve, automate and expand the capabilities of separation tools. Particularly, over the past 15 years, an exciting area of research, known as microfluidics, has emerged from the separation sciences.[2] This field seeks to develop, improve, and integrate analytical separation systems through miniaturization.

Such miniaturization is most effectively achieved through the use of micromachining technologies developed by the semiconductor industry, which allow remarkable control over micrometer-sized features. Through miniaturization, microfluidics should be able to offer many of the advantages already realized in the semiconductor industry; namely, greater capabilities in increasingly cheaper, smaller and faster devices that can be made portable. Beyond these benefits, the small dimensions of microfluidic devices naturally require smaller sample volumes than traditional techniques, providing an extra

advantage in analyses where sample volumes are typically limited, such as in forensics or cerebrospinal fluid analysis.[3] Furthermore, microfluidics has potential to decrease erroneous results due to human errors or contamination, by integrating and automating multiple sample handling steps onto a single platform.

1.2 History

Perhaps the earliest work in microfluidics was that of Terry et al. in 1979.[4] Although this initial paper remarkably incorporated a gas chromatographic column with an injector and thermal conductivity detector on a silicon chip, the work did not receive much attention. Indeed, miniaturized separations did not gain much interest until the early 1990's, when Harrison and Manz began fabricating microchip capillary electrophoresis (CE) devices.[5-15]

Following the initial introduction of the CE microchip, Harrison et al.[5] developed a microdevice that was capable of separating six amino acids in 15 s. Injection-to-detection distance was 2.2 cm, and efficiencies were as high as 75,000 plates. This same work also showed a separation of three amino acids in 4 s, using a device with a separation distance of 0.75 cm and yielding efficiencies as high as 600 plates. Shortly after this publication, Jacobson et al. performed separations in <1 s.[16] This early work established the power of microchip CE to perform very rapid separations.

The earliest microfluidic devices were fabricated from either glass or silicon, as these were materials familiar to the semiconductor industry. In a CE microchip, a channel would be etched into a glass or silicon substrate, and a cover plate with access holes would be bonded over the top. Despite the prevalence of silicon in the microelectronics industry, glass substrates became much more popular due to better electrical and optical properties. More recently, many polymers have been added to the list of substrates used for the creation of microfluidic devices due to their ease of fabrication and low cost.[17]

In 1994, Jacobson et al.[18] began studying the effects of column geometry on microchip CE. They created a serpentine channel to achieve a long separation column while maintaining small overall microdevice size. However, there were drawbacks, as differences in path lengths at the turns caused significant broadening. Though efforts to increase column length through alternate channel designs continue, the vast majority of both the early and more recent microfluidic work has used layouts based on simple cross-channel or double-T microfluidic schemes (see Figure 1.1).[5, 8, 12, 18, 19]

The cross-channel designs provide many advantages that have led to their frequent use. These include minimal sample broadening during injection and the ability to reproducibly define narrow sample plugs. Also, these designs minimize sample introduction bias by moving the head of the sample stream,

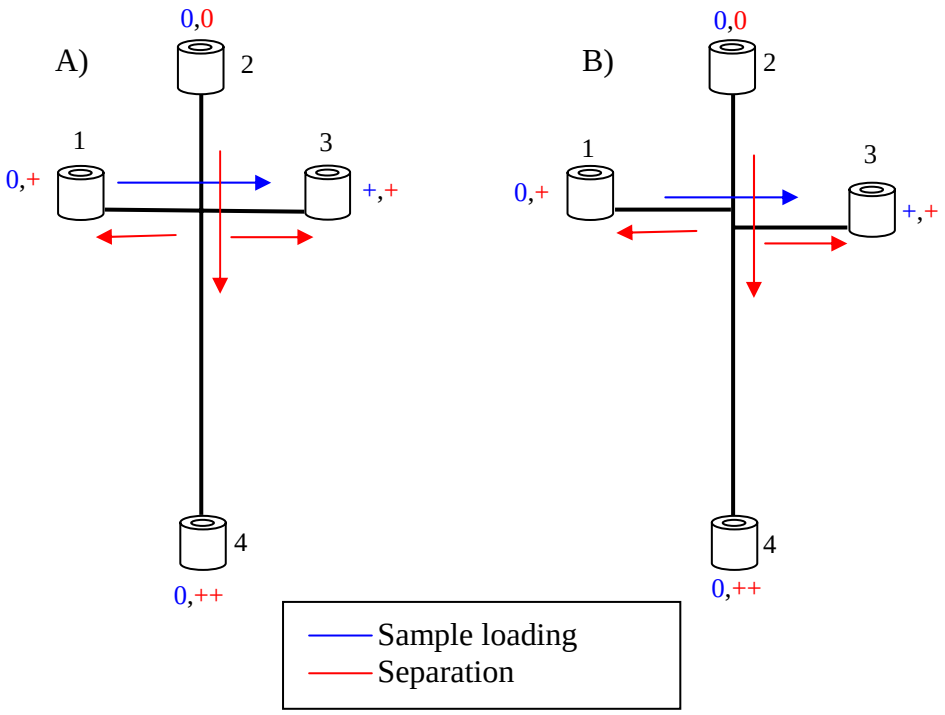

Figure 1.1. Schematics of general microdevice designs. Diagrams of (A) cross-channel and (B) double-T microfluidic device layouts. Sample loading is achieved by applying a voltage at reservoir 3 and grounding the others (1, 2 and 4). Injection and separation are achieved by rearranging the voltages such that the same voltage is applied to reservoirs 1 and 3, a larger voltage is applied at reservoir 4, and reservoir 2 is grounded.

which would resemble frontal elution chromatography, into the waste reservoir. The injection plug is then taken from a later portion of the sample stream, which has a more representative mixture of the analytes. Injection is achieved by rearranging the applied voltages, and separation occurs in a straight separation channel (Figure 1.1). The double-T layout (Figure 1.1B) varies from the cross-channel design (Figure 1.1A) in that the sample introduction arm is offset slightly from the waste reservoir arm. This offset allows a larger volume of sample to be injected, making detection of more dilute analytes possible. Although this

design loads a longer sample band, the offset is generally small enough that less than 10% of the final peak width results from the initial sample plug.

1.3 Current Directions

As rapid progress continues to be made in microfluidic work, there is increasing interest in these platforms. Improved separation efficiencies, parallel sample processing, precise fluid control, and integrated sample handling steps are all enticing capabilities that have been achieved in varying degrees. A large number of analytes, including proteins, peptides, amino acids, nucleic acids, metal ions, organic molecules and many others, have been analyzed.[20] Consequently, the use of microfluidic systems in various applications is beginning to be explored. Much of the current work being done focuses on exploring the potential of using microdevices for new applications.

One of the earliest displays of the use of microfluidic systems for rapid analyses with high throughput was shown by Woolley et al.[21] Micromachined capillary arrays were used to separate ϕX174 *Hae* III DNA fragments in 120 s, an approximate 10-fold increase in speed over conventional methods. The use of channel arrays continued with the development of a 12-lane microdevice for the parallel analysis of HFE gene variants from multiple individuals.[22] Later, the development of a 96 microchannel array DNA sequencing system showed a 5-fold increase in throughput over conventional capillary systems.[23] Finally, a

radial 384-lane microdevice was created for the analysis of the HFE gene variants of 384 individuals in 325 s.[24]

Other significant developments have occurred in the creation of DNA microfluidic systems. On-chip polymerase chain reaction (PCR) has become well established. Microchambers, commercial cyclers, on-chip resistors, IR radiation and compressed air have all been used for thermal regulation.[25] These, along with other capabilities, have led to the development of portable microdevices capable of analyzing raw genetic material.[26-28]

Other areas that have begun to be explored for microfluidic device applications include clinical analysis and diagnosis, drug discovery, food analysis, systems biology research, environmental analysis and reaction studies.[29-34] Research in these areas aims to combine the small volume control, multiple analysis steps and sensitive detection schemes of microfluidic devices with capabilities tailored for specific applications. For example, Landers and coworkers have developed microchips to detect common mutations in the BRCA1 and BRCA2 genes that have been shown to be strongly correlated with the occurrence of breast cancer.[35-39] Other clinical applications include the creation of a microchip system for analysis of total protein-bound homocysteine, a marker that is tested in patients at high risk for cardiovascular disease.[40] A PMMA microdevice with integrated PCR capabilities was developed for the

quantitative determination of hepatitis C virus.[41] Zhang's group[42] has utilized a microchip for simultaneous insulin and glucose measurements using immunologic and enzymatic assays. Other clinical applications that use microfluidics include immune disorders, bacterial infections, neurological diseases, thyroid function and hereditary diseases.[29]

Although there is still much research to be done, the creation of microfluidic devices for DNA analysis and some clinical applications is fairly well developed. As more advantages of miniaturization are realized, interest in the potential use of microfluidic devices in other areas has emerged. For example, systems biology deals with the complex and difficult task of determining the roles and interactions of biological molecules. Because these reactions are so complex and intertwining, it is difficult to develop assays that have both the fidelity and throughput necessary for effective research in this field.[32] Microfluidic array devices, however, have great potential to impact systems biology by providing high throughput for the testing of multiple, highly controlled reaction systems that can provide useful information on interaction dynamics.[32] Furthermore, work achieved in microdevices has shown the ability to precisely control and image cellular and subcellular environments.[43]

Interest in microfluidic systems for the study of non-biological reactions has emerged as well. Typically, organic reactions are performed in relatively

large batches (mL's or more), often with poor reaction control.[34] Aliquots are then analyzed, creating significant and unavoidable delay times. By moving to a microchip format, greater control and selectivity for these reactions can be achieved, as well as online monitoring performed. These capabilities may also be useful for drug discovery, where determining the most effective molecule for binding to a target site or performing a particular function, requires analysis techniques capable of high throughput and selectivity.[30]

The use of conventional CE for the analysis of food is a well-established technique. However, many improvements to current methods are needed, including decreased detection limits and increased reproducibility. By moving to integrated microchips, preconcentration and stacking techniques can be combined with the relatively sensitive detection systems most generally used for microfluidic devices – laser induced fluorescence (LIF), mass spectrometry (MS), and amperometric detection.[31] Environmental pollutant detection is another area in which microfluidic work has sparked interest. Particularly significant is the potential to provide on site monitoring without analysis delay. Microchip assays to detect phenols, aromatic amines, hydrazines, nerve agents, nitroaromatics, and chemical warfare agents have been developed.[33]

Finally, as microfluidic systems have continued to gain popularity, some commercial systems have also begun to emerge. Agilent, in combination with

Caliper Life Sciences, has developed microchip gel electrophoresis systems for protein, DNA, RNA, and cell analysis. Nanostream, Monogram Biosciences, MetriGenix, Hitachi, and CE Resources are just some of the other companies that have also begun to offer commercial microfluidic systems.

1.4 Micromachining

As mentioned previously, the micromachining techniques established in the semiconductor industry have played a significant role in the development of microfluidics. The precise control over micrometer-sized dimensions is critical in making sophisticated and reproducible devices. Because of their general importance, I will discuss a few micromachining techniques that are relevant to this work. General references for microfabrication techniques can be found in a review by Ziaie et al.[17] and in the book *Fundamentals of Microfabrication: The Science of Minaturization*.[44]

1.4.1 Photolithography

Photolithography is perhaps the most widely used microprocessing technique in the field of microfluidics. Generally, this method involves the controlled masking and exposing of light-sensitive polymers to obtain precise surface patterns. There are a variety of polymers and methods used to obtain the desired features, but I will limit this discussion to a general outline of the most relevant processes.

A positive photoresist (Figure 1.2A) is a polymer that undergoes a chemical reaction upon exposure to ultraviolet (UV) radiation. Generally, a clean silicon or glass substrate is heated to ensure that all solvents and water have been driven off. A thin layer of photoresist is then spin-coated onto the surface by spinning the substrate at several thousand revolutions per minute to disperse an even layer of the film. The substrate is then heated to drive off any excess solvent in the photoresist. This step is followed by exposing the wafer to UV

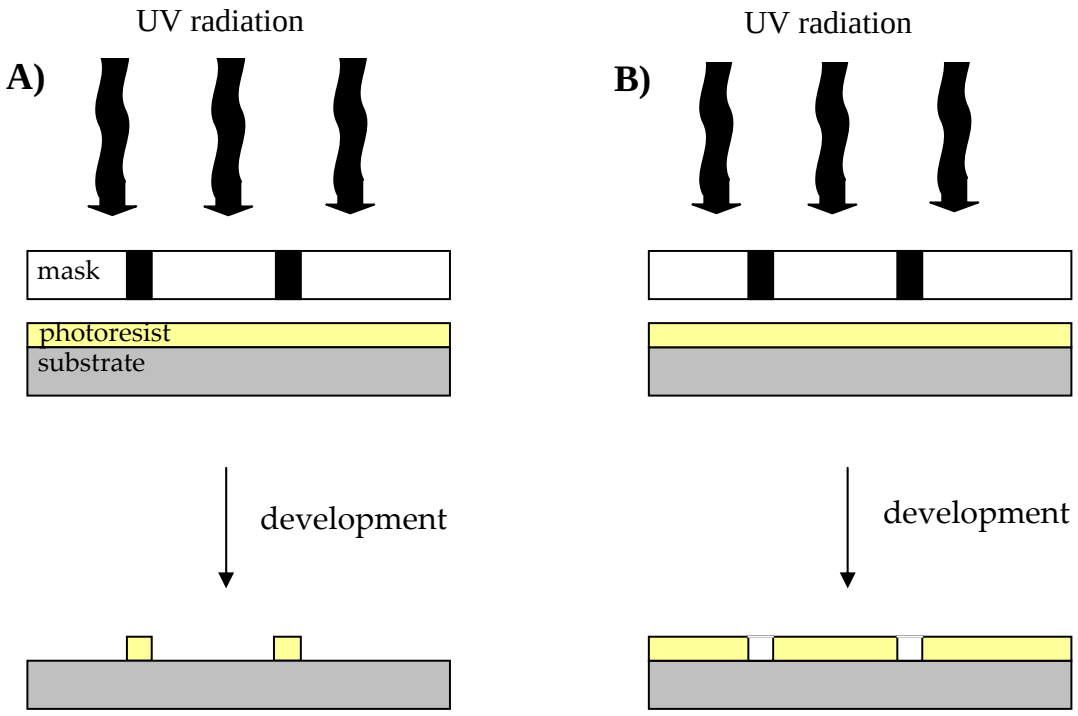

Figure 1.2. Effect of UV exposure and development on (A) positive and (B) negative photoresists.

radiation through a photomask that is placed over the substrate, protecting particular regions from UV exposure (Figure 1.2A). The substrate is then rinsed

in a developing solution, which dissolves the photoresist that has been exposed to UV radiation. Thus, a pattern of photoresist on the substrate surface that matches that of the mask is obtained (Figure 1.2A). Typically, after development the substrate is heated to a higher temperature to improve the chemical resistance of the remaining material and enhance the adhesion of the resist to the surface.

A negative photoresist (Figure 1.2B) acts, as its name suggests, in the reverse manner as a positive resist. Substrate preparation for negative resists is similar to that for positive resists. When a negative photoresist is exposed and developed, however, the pattern that remains on the wafer is the inverse or negative of the features on the mask. In a negative resist, UV light promotes cross linking, thus making the exposed regions more resistant to the solvent in the developing solution. As with positive resists, negative resists are also typically heated after developing.

1.4.2 Thin-Film Deposition

Thin-film deposition involves the placement of a thin layer of material onto a base substrate. There are many different methods used to obtain thin films. Of particular significance in microfluidics are thermal evaporation, electron beam (e-beam) evaporation, oxidation, low pressure chemical vapor deposition (LPCVD), and plasma enhanced chemical vapor deposition (PECVD).

The choice of which of these methods is used depends on a variety of factors, including what is being deposited, acceptable temperature ranges and the quality of the film required.

Thermal and e-beam evaporation methods are generally used to deposit thin layers of metals onto a substrate. Thermal evaporation heats the desired metal by passing a large current through a highly refractory metal containment structure. This resistive heating melts and vaporizes the deposition metal, which travels through the vacuum chamber and coats the substrate. The simplicity of this method makes it convenient to use; however, thermal evaporation is often subject to contamination from the metal containment structure. E-beam deposition heats and vaporizes metal using a beam of electrons emitted from a hot filament. Compared with thermal evaporation, e-beam provides higher-quality films with less contamination, due to the localized heating and evaporation of the metal. E-beam evaporation also provides the advantages of higher deposition rates and the ability to load and deposit greater sample volumes than thermal evaporation methods.

Oxidation is used to obtain an amorphous film of silicon dioxide on the surface of a silicon wafer. This process involves maintaining silicon at 600-1250 °C in a wet or dry oxygen/nitrogen atmosphere. The high temperatures aid the diffusion of oxygen (dry) or water (wet) through the SiO_2 to the silicon surface,

oxidizing it. Thus, oxidation is not technically a deposition, but rather a growth process. The resulting oxide is often under significant stress and allows the diffusion of smaller ions.

Chemical vapor deposition (CVD) techniques include PECVD, LPCVD, and other less common forms. In CVD, gas-phase constituents are absorbed onto a hot surface, undergoing reactions and forming a solid film. Typically, these methods are used to deposit inorganic materials, among the most common being silicon, silicon dioxide, and silicon nitride.

In PECVD, a radio frequency (RF)-induced plasma is used to provide the necessary activation energy for the film deposition reactions. This plasma allows lower temperatures (200-300 °C) to be used in PECVD than other CVD systems. PECVD has other important advantages such as high deposition rates, good adhesion, good step coverage, and fairly low pinhole density. The main disadvantages of PECVD films are chemical contamination, particularly from hydrogen, and nonstochiometric films.

LPCVD requires temperatures between 550-900 °C, but yields higher quality and more conformal films than PECVD. Layers deposited using LPCVD have excellent purity and fewer pinholes than PECVD films. Deposition rate, however, is about a factor of ten slower.

1.4.3 Chemical Etching

Chemical etching methods are applied almost universally in microfluidic device fabrication. Most frequently, these processes are used to etch channels into a substrate or define a template for embossing features. Generally, the photolithography step defines a pattern, and chemical etching is used to transfer the design into the substrate material. The photoresist can then be removed entirely, leaving the precisely defined pattern etched into the substrate.

Wet chemical etching is a common processing step, which uses a solution to etch the material of interest. Typically, wet chemical etching is isotropic, etching in every direction uniformly. For example, the etching of silicon dioxide in HF is isotropic. An important exception to this generality is the etching of monocrystalline substrates, such as silicon <100> wafers. Aqueous KOH etches silicon <100> more rapidly along specific crystal planes, resulting in 54.7° etch angles.

Reactive ion etching (RIE) uses an RF plasma and ion bombardment to etch materials of interest. RIE systems provide a selective, directional vertical etch, with very little etching occurring in the horizontal direction, allowing high aspect ratios to be achieved. For RIE, selectivity in etched material can be accomplished through the use of different gases. For example, an oxygen plasma

etches organic materials, a chlorine plasma etches metals and a fluorocarbon plasma will etch silicon nitride and silicon dioxide.

1.4.4 Lift-off

Lift-off is another technique used for the patterning of microstructures. Lift-off is performed by first patterning photoresist with the inverse or negative of the desired features on a substrate, and then depositing a metal film using e-beam, thermal or other evaporation techniques. Next, the photoresist is dissolved, and all the metal on the photoresist "lifts-off" the surface, leaving metal only where there was no photoresist.

1.5 Electrically Driven Separations

1.5.1 Theory

Chemical separations have been achieved using a variety of formats to provide fractionation. In microfluidics, the most common format used to drive and offer selectivity for separation is the use of electrical fields. Electrically driven separations are based on the differential migration of charged molecules when an electrical field is present. The rate of this movement is a function of the applied electric field, molecular size and charge, and the medium through which the molecule is being driven (generally either free solution or a separation matrix). Mathematically, the mobility (μ) of a molecule is proportional to the

ratio of the molecular charge (q) to the frictional force (f, dependent on size and medium) felt by the molecule.

$$\mu \propto \frac{q}{f} \tag{1.1}$$

1.5.2 Capillary Zone Electrophoresis

Capillary zone electrophoresis (CZE) establishes an electric field across free solution in a capillary to cause the separation of charged molecules. The velocity (v) of a molecule in an applied field is determined by Equation 1.2,

$$v = \frac{\mu V}{L} \tag{1.2}$$

where V is the applied voltage and L is the column length. When diffusion is the only source of band broadening, column efficiency or number of theoretical plates (N) can be shown to be

$$N = \frac{\mu V}{2D} \tag{1.3}$$

where D is the diffusion coefficient. Importantly, L does not affect N in Equation 1.3, although it is assumed that L and the length of channel over which the potential is applied (L') are equivalent. However, this assumption is often incorrect in microfluidic work (see Figure 1.1), such that the maximum theoretically obtainable efficiency must be multiplied by the ratio L/L'. Also, for two columns with equal applied voltages but different lengths, the shorter column will have a higher electric field and potentially greater Joule heating.

Consequently, if the column temperature increases, so will *D*, which could reduce *N* for the shorter channel.

Equation 1.1 indicates that electrophoretic mobility depends on molecular charge; however, uncharged molecules can also be separated by CZE, through the introduction of charged molecules that form micelles in the column. Uncharged molecules can partition into the micelles, resulting in transient, charged complexes that can be manipulated in an electric field. Since the partitioning of analyte into the micelle is a dynamic process, v is a function of the relative strengths of the analyte interactions between the micelles and free solution, which determine the amount of time spent in each. This form of CZE is known as micellar electrokinetic chromatography (MEKC).[45]

The earliest CZE separations were performed by Hjerten in 1967,[46] but the technique did not gain popularity until 1981 when Jorgenson and Lukacs[47] showed the excellent resolving power possible with CE. With glass open tubular capillaries having lengths between 80-100 cm and potentials of up to 30 kV, separation efficiencies as high as 400,000 theoretical plates were obtained. This work sparked considerable interest in CZE and led to the appearance of the first commercial CZE instrumentation 7 years later, in 1988.

There are several advantages of CZE that have contributed to its increasing popularity. The first is that efficiency, as mentioned previously, is

generally independent of column length, allowing efficient separations to be achieved in conveniently sized columns. Another benefit is that only voltages are needed to drive the separation, eliminating the need for high pressures and the associated connections and pumps. Both of these advantages make CZE readily integratable with microfluidic channels. Also, because CZE separations use relatively small volumes of solutions (<mL), which are typically aqueous buffers, hazardous waste generation is significantly lower than in other separation methods. Finally, because CZE does not rely on surface interactions (unlike liquid chromatography), the same column can be used for widely differing samples; for example inorganic, organic, and biological molecules can all be separated in similar CZE columns.[48] The initial CZE work by Hjerten separated Bi and Cu ions.[46] CZE and MEKC have frequently been used for the separation of amino acids, peptides and proteins. A wide variety of natural products, which have held historical importance in the pharmaceutical industry, have been separated using CZE or MEKC. A short list includes antibiotics, flavonoids, isoflavonoids, steroids, coumarins, alkaloids, nicotine, aflatoxins, mycotoxins and minerals.[48]

One effect that occurs in CZE, which is nonexistent in other forms of separations, is electroosmotic flow (EOF). EOF results when charges are present at the surface of a column. The oppositely charged ions in the buffer solution

form a ring of associated counter charge, called an electrical double layer, at the column-buffer interface (Figure 1.3). When an electric field is applied, the ions

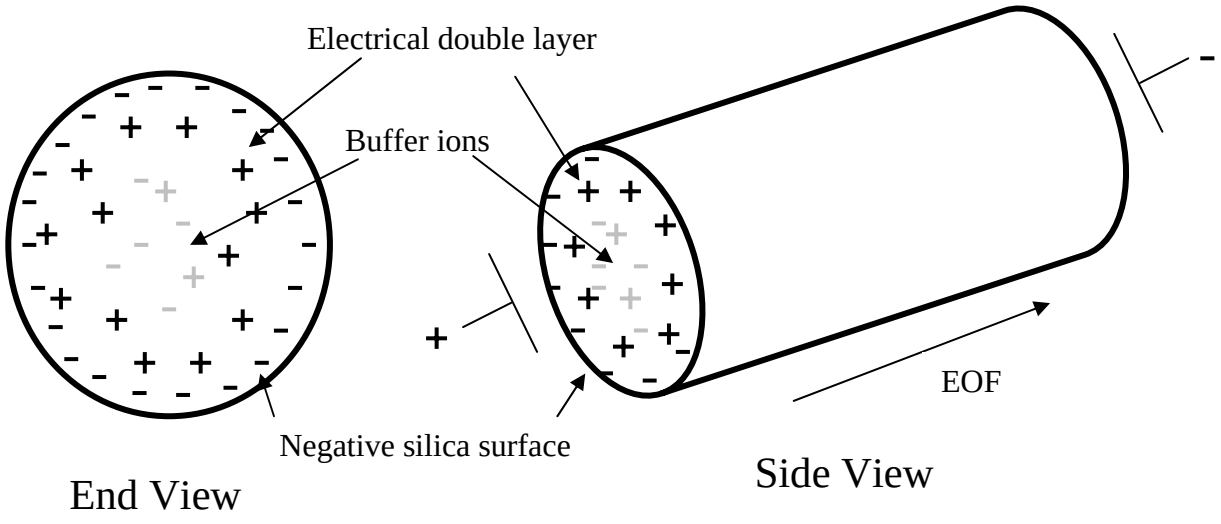

Figure 1.3. End and side view of the association between surface charge and buffer ions that gives rise to EOF in native SiO_2 columns.

that form the electrical double layer migrate and drag bulk flow in the same direction. Silicon dioxide, the most common column material for CZE, has significant numbers of negatively charged silanoate groups at the surface when in contact with solutions of pH >4. Thus, EOF is observed in typical CZE experiments (Figure 1.3).

In some instances, it is advantageous to reduce EOF, using covalent or dynamic coatings to eliminate surface charge or dramatically increase viscosity at the electrical double layer. Dynamic coatings are generally polymers that can be

added to buffers in low concentrations (<1%), forming associations with the column surface. No bonds are formed, however, as the interactions are a dynamic process. Covalent coatings permanently attach molecules, which do not ionize readily, to the surface of a column. Typically, CZE is performed in silicon dioxide capillaries, so covalent coatings are attached using silane chemistry. Recently, the emergence of polymer microdevices has inspired work focusing on the covalent passivation of polymer surfaces.[49] Beyond reducing EOF, dynamic and covalent coatings can limit interaction between analytes and the channel surface, decreasing analyte adsorption.

In other instances, EOF enhancement can be desirable. EOF can cause high flow velocities (0.4 cm/s in a 150 i.d. μm capillary[50]), particularly under circumstances with high surface-to-volume ratios, such as in microchannels. Thus the EOF velocity often exceeds the velocities of analytes in solution, such that all species move in the direction of the EOF regardless of charge. A second application of EOF is the development of electroosmotic pumps. Electroosmotic pumps take advantage of high surface-to-volume ratios to generate bulk flow through EOF. The potential for facile integration makes EOF pumps of interest for microfluidic work. Furthermore, EOF produces a flat, rather than laminar flow profile; this can reduce band broadening compared to pressure-driven flow. Despite these advantages, electroosmotic pumps have not yet been able to match

the power, reproducibility and efficiency of conventional pumping systems, thus limiting their utilization at present.

1.5.3 Capillary Gel Electrophoresis

Capillary gel electrophoresis (CGE) is similar to CZE, in that electric fields are used to drive molecules through a capillary. In CGE, however, the capillary is filled with a gel, which has important consequences: (1) EOF is eliminated, and (2) the gel can provide increased size discrimination for a specific range of molecular dimensions. This allows the fractionation of molecules that would be difficult to separate in free solution, such as macromolecules like nucleic acids. Generally, in gel electrophoresis polyacrylamide or agarose gels are used for sieving media to separate different sizes of proteins or DNA. However, in CGE, polyacrylamide is used more frequently due to its greater resistance to Joule heating.

The advantages of downscaling to capillary dimensions have caused significant growth in CGE, just as in CZE. The development of array CGE was critical in completing the sequencing of the human genome. Microchip CGE has also been used for the analysis of proteins, nucleic acids, antibodies, and other molecules.[51-54]

1.5.4 Isoelectric focusing

Isoelectric focusing (IEF) is used to separate and focus proteins, peptides, and other ampholytic species (which act as either acids or bases depending on pH) at their isoelectric points. An ampholyte's isoelectric point is the pH at which it has no net charge. In IEF, ampholytes are used to establish a pH gradient along a column. Typically, proteins or peptides are introduced into the column and migrate in an applied field. When an analyte reaches the region of pH corresponding to its isoelectric point, it is no longer charged and ceases to move under the influence of the electric field. If an analyte diffuses out of this region into an area of higher or lower pH, it again becomes charged and migrates back toward the pH of its isoelectric point. Thus, analytes are separated and concentrated according their isoelectric points. The concentration factors obtainable are limited, however, due to the reduced solubility of analytes at their isoelectric points. IEF is often combined with CGE for two-dimensional separations.

IEF has also been used in microdevices to concentrate proteins and obtain 2-D separations. Two papers published in 2003 showed the integration of IEF into microdevices.[55, 56] Work done by Herr et al.[55] successfully coupled IEF to CE separation. Voltage switching provided controlled electrokinetic mobilization and injection of focused IEF bands into an ampholyte-based CE separation.

Preconcentration factors were about 70 with these devices, and a peak capacity of 1300 for a 2-D separation was obtained.

1.6 Sacrificial Layer Technologies

1.6.1 Thin-Film, Sacrificial Layer Microstructures*

Recently, several groups have developed microfluidic device fabrication techniques that rely on sacrificial layer technology. Basically, this approach uses a sacrificial layer to define or protect microfluidic channels. Channel walls are formed by enclosing the sacrificial layer, and then the sacrificial layer is removed to provide open channels for microfluidic work.

Sacrificial approaches have the advantage of providing a means of microcapillary enclosure other than thermal bonding. In contrast, most microfluidic device fabrication involves thermal bonding, which requires stringent cleaning and high temperatures, often resulting in channel morphing and weak bonds.[57] Thermal bonding of polymer microchannels typically avoids the high temperatures and strict cleaning requirements, but these microstructures are also often bonded weakly and are subject to significant channel morphing.[57]

Sacrificial layer work has been done to create microchannels, although few separations have been demonstrated in the resulting devices.[58-64] For example,

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Lee and Lin[58] created channels by layering silicon, silicon nitride, silicon dioxide, and phophosilicate glass (PSG). Rapid etching was obtained via etch holes, which provided many points of access for the etchant to reach the silicon and PSG sacrificial layers. These openings significantly reduced the etch time, and it was noted that surface tension might prevent the channels from leaking during CE analysis; however, no such work was attempted.

Channel fabrication involving the removal of a polycarbonate sacrificial layer through its decomposition in a tube furnace (<10 h removal time) has also been shown.[60-63] This method, however, was limited to sacrificial layers from which high-temperature decomposition products diffuse through SiO_2, potentially limiting choice of channel material. Moreover, residual sacrificial layer remained on the channel walls after removal, and no separations were shown in channels created in this manner.

There has been some work to make channels for DNA fragment sizing, taking advantage of the small feature dimensions obtainable through micromachining.[59, 64] Fabrication involved depositing silicon dioxide walls over thin-film sacrificial layer (polysilicon) channels, which required 4 h etch times. The separation columns in the fabricated devices were 270 nm tall and 1 μm wide, ideal dimensions for the DNA work performed, although larger channels would be required for most other types of analysis. Although this fabrication

method could readily produce wider channels, it is limited in ability to make significantly taller channels. The length of the separation channel was not reported, but the figures suggest that it was less than 100 μm. Such short channels would permit rapid etch times, but scaling to longer channels needed for more complex devices would be a challenge. Low levels of background fluorescence were also observed in these devices, hampering the ability to detect the smallest (0.13 kb) DNA fragment in the separation.

1.6.2 Thin-Film Microfluidic Devices

Thin-film deposition is emerging as an attractive approach for the fabrication of microfluidic devices. Frequently thin-film techniques are used for the integration of electrodes, heaters, light emitting diodes and various electrical components.[65-74] There is, however, some work that uses thin films for the creation of the microfluidic channels themselves. All the sacrificial layer results discussed in Section 1.6.1 relied on the deposition of thin films to create microchannels. Microfluidic channels made using thin-film technology provide the advantage of straightforward integration with the thin-film electrical components needed for device operation. [75]

Sharma et al. used thin films for the creation of microchannels.[76] A layer of silicon dioxide was sandwiched between two silicon layers, and a thin (205 nm) channel was etched into the top silicon layer, except for at a few small bridge

points. This thin channel provided access to the SiO_2 film, which was then etched through to the second silicon layer, making a deeper and wider channel. Ti/Au contacts were deposited to provide a source and drain at the silicon bridges. To seal off the channels, a piece of PMMA was clamped over the top of the substrates. These devices were used to manipulate the flow of rhodamine B dye molecules, and current versus voltage curves for the silicon bridges as a function of the electrolyte reference potential were obtained. Surface potential changes as low as 7.9 mV could be detected.

Others have deposited thin films of silicon, silicon nitride, and parylene to create microchannels.[77] Callender et al.[78] created channel voids in SiO_2 on silicon by etching to create ridges and then using PECVD to deposit void-forming borophosphosilicate glass. Guijt et al.[79] deposited thin films of silicon nitride to form channels with 360-nm-thick walls. Spun-on films of SU-8 photoresist have also become increasingly popular for microfluidic channels.[80]

1.6.3 Polymer Substrates

Kelly et al.[81] have obtained promising results using sacrificial layers in polymeric devices. They used paraffin wax sacrificial layers to create solvent-bonded poly(methyl methacrylate) (PMMA) microfluidic devices. In this work channels were imprinted into a PMMA substrate using a photolithographically designed silicon template. A piece of polydimethylsiloxane (PDMS) with access

holes was reversibly sealed over these features. The channels were filled with heated liquid paraffin wax, which was then cooled and solidified. The PDMS piece was removed, and a second PMMA substrate was solvent bonded to the imprinted piece using acetonitrile. The paraffin wax prevented any dissolved PMMA from entering and clogging the microchannels during enclosure. Once the acetonitrile had evaporated, a bond was formed between the two substrates, and the device could be heated to liquefy and remove the paraffin wax. This fabrication process yielded devices that had significantly stronger bonds than thermally enclosed polymer devices, as well as reduced morphing during the bonding process.

1.6.4 Summary of Sacrificial Layer Approaches

Although there are some promising applications and advantages of sacrificial layer and thin film technology in the creation of microfluidic systems, little has been shown, particularly in glass microchannels, on both the creation and use of such devices. The number of fabrication papers that suggest the use of sacrificially formed channels for separations, but show no such work, suggests that features such as etch holes or residues from thermally decomposed sacrificial layers present added difficulties.

1.7 Detection Schemes

Equally as important as obtaining well-resolved and efficient separations, is the ability to detect the fractionated analytes. The small sample volumes, short path lengths, and interest in many low-abundance biological molecules place stringent requirements for the detection limits of schemes used in microfluidic work. Finding an optimum detection approach becomes more complex as the ideal of developing a μ-TAS, with detection integrated onto the chip, is considered. Consequently no detection scheme is perfect, and each detection method commonly used in microfluidic analysis has several advantages as well as disadvantages.

1.7.1 Laser-Induced Fluorescence

In laser-induced fluorescence (LIF), a laser excites a fluorophore to an exited electronic state through irradiation with a particular wavelength of light. The molecule then relaxes almost instantaneously to the lowest vibrational level within the excited electronic state and then relaxes to the ground electronic state, emitting a photon as it does. The emitted photon has a longer wavelength than the excitation photon because of vibrational relaxation, and the resulting light can be detected and converted into an electrical signal.[45]

LIF is the most widely used detection technique for microfluidic analysis, despite the relatively small number of naturally fluorescent molecules. One

reason for LIF's popularity is that it has the lowest detection limits of any system.[82] Concentration detection limits of 100 fM can be achieved with this technique and single-molecule fluorescence detection has been reported in microchips.[82, 83]

To obtain such detection limits with LIF, several important considerations must be met. First, the signal-to-noise ratio must be maximized. When laser power is increased linearly, at some point the fluorescence signal will no longer go up linearly. Instead the signal will begin to flatten out, due to either saturation or photobleaching; it is near that point that the signal-to-noise will be optimal.[82] Another key issue when using LIF is minimizing various sources of background radiation. These include scattered laser light, background fluorescence and luminescence (from the device, buffer impurities or optics), Rayleigh scattering, and Raman scattering from the solvent.[82] For most microfluidic work, the first two sources are the most significant contributors to the background signal. To eliminate background noise, optical filters are incorporated almost universally. Low-pass, high-pass, band-pass and dichroic filters are all used frequently in microfluidic work. Pinholes are also used to eliminate stray light. In addition to incorporating optical components to eliminate background signal, some groups have photobleached the buffers to lower background signals.[84, 85]

Several detectors may be used to measure fluorescence. The most sensitive work requires photon-counting detectors, although current-mode photomultiplier tubes and charge coupled devices (CCDs) may also be used.

Besides low detection limits, there are other advantages to using LIF for research focusing on the development of sample handling and separation steps. First, LIF generally does not require any special device fabrication processes. Thus, simple designs can be used, decreasing fabrication time. The second advantage LIF frequently provides is the ability to image the movement of analyte through the microsystem. This capability can be invaluable in optimizing device performance and reproducibility.

There are some weaknesses to the use of LIF in microfluidic work, however. For example, the detection setups are often large and expensive. Although research is ongoing to develop miniaturized, lower-cost systems, much remains to be done, particularly for lasers of shorter wavelengths (<400 nm). Another general weakness of LIF is that many compounds do not fluoresce. This can be overcome in part by the ability of many analytes of interest (particularly biological molecules) to be labeled with fluorescent tags, although such moieties may not be available for all analytes. Furthermore, the need for derivatization adds an additional sample preparation step that would need to be incorporated on-chip for a μ-TAS setup. Importantly, some fluorescent labeling work has

already been done on microchips using both precolumn and postcolumn approaches.[83, 86, 87]

Ocvirk et al.[88] performed on-chip cell lysis and fluorescence tagging with fluorescein-di-β-D-galactopyranoside (FDG), using a Y-junction to mix the sample and reagents. The labeled sample was separated and detected downstream two minutes after mixing, a significantly shorter time than the one-hour minimum incubation required for adequate labeling off chip. Liu et al.[89] carried out postcolumn labeling of proteins using NanoOrange. A T-junction was used to mix the sample and labeling solutions. The sample was separated by CE and passed through the mixing junction, where analytes were labeled and detected. In the mixing process, no significant band broadening was observed, and the rate of labeling was determined to be near that of diffusion. Detection limits were measured to be <0.5 pg of injected sample for model proteins.

A final disadvantage of LIF is that the signal for equal concentrations of different analytes varies significantly depending of the type and number of fluorophores on a molecule. For tagged analytes, bias is significant since the number of labeling sites, equilibrium constants, and sometimes the label used can vary between analytes in the same sample.

1.7.2 Electrochemical Detection

There are two types of electrochemical detection frequently used for microdevices, conductivity and amperometric detectors. The detectors differ in that the first monitors resistance while the second probes current. Conductivity detection measures changes in conductivity of a solution, as the conductivity of buffer often differs from that of buffer plus analyte. In amperometric detection, an applied potential drives a redox reaction of the target analytes, and the resultant current, which reflects the extent of the reaction, is monitored. Electrochemical detection holds some advantages over LIF, because it is easier and cheaper to integrate on chip than a laser and optics.

There are drawbacks, however, causing electrochemical detection to be used significantly less than LIF for microfluidics. First, the high electric fields applied for microchip CE can interfere with electrochemical detection. To minimize the interference between the detection and separation potentials, reference and counter electrodes can be placed at the end of the separation channel, where potentials are negligible. Another setback to amperometric detection is that compounds must be electroactive to be detected.[90] A final reason LIF is preferred over electrochemical detection is that electrochemical methods generally require samples of higher concentrations.

1.7.3 Other Detection Schemes

Two other detections schemes used for microfluidic analysis are mass spectrometry and chemiluminescence. The use of microfluidic systems for protein analysis has resulted in significant interest in the coupling of these devices with MS. Of particular interest are microdevices coupled with electrospray ionization and time-of-flight MS. This coupling is not trivial, however, as it generally requires the formation of a Taylor cone at the end of the separation channel.[91] Kameoka and co-workers[92] interfaced microchips with electrospray through a micro liquid junction for the separation of small polar molecules. Svedverg et al.[93] fabricated an electrospray tip directly from the end of polymeric microchips using both hand polishing and machine milling. Another approach, taken by Tachibana et al.,[94] involved affixing a spray nozzle onto the end of a microchannel. The electrospray-to-channel coupling is not the only challenge for microchip MS detection. MS systems are generally very large and can range between benchtop to room-sized instruments. Work is being done to miniaturize these systems; however, there is still much to be accomplished before MS can be integrated fully into a μ-TAS package.

Chemiluminescence is another method of detection that has been used in microdevices, although it is significantly less common than LIF, electrochemical techniques, or MS. Chemiluminescence, however, has the advantage of a simpler

setup than LIF, as a light source is not required.[91] Richter et al.[95] used chemiluminescence to detect xanthine; a bi-enzymatic mixture of xanthine oxidase and horseradish peroxidase provided analyte specificity and chemiluminescence, respectively. Enzyme solutions and coated beads were used in these studies, and both showed resulting chemiluminescence of xanthine. Other reactions that have been used include the metal-ion-catalyzed luminal-peroxide and the dansyl species conjugated peroxalate-peroxide reactions.[96]

1.8 Thesis Overview

This work discusses the development and evaluation of microfluidic structures made using thin-film and sacrificial layer technologies. Chapter 2 presents the fabrication process for the microfluidic channels and discusses some of the potential advantages of this fabrication format. I also present electrophoretic data showing the potential for rapid separations in these devices. Chapter 3 explores methods for increasing performance and capabilities of these devices though EOF-reducing coatings and localized gel photopolymerization. Finally, Chapter 4 discusses the conclusions that can be drawn concerning the devices as well as possible future research.

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2. FABRICATION AND EVALUATION OF THIN-FILM MICROCHANNELS*

2.1 Introduction

With the growing research effort to develop micro-total analysis systems (μ-TAS), there is a demand for novel fabrication techniques that increase flexibility in design, fabrication, and capability. Micromachining has long been included in the fabrication steps of many microfluidic devices.[1] Traditionally, these steps are subject to the demanding requirements of micromachining (expensive equipment and necessity for cleanroom conditions), but do not capitalize fully on the advantages of micromachining, such as parallel device construction and the ability to create intricate three-dimensional structures.[2-4] I have sought to develop a fabrication technique that takes advantage of these capabilities, and provides a format to integrate electrical and microfluidic components.

Recently, there has been an emerging interest in microelectromechanical systems (MEMS)-compatible microfluidic device fabrication.[5-11] This work takes advantage of established micromachining capabilities, using a variety of etching, bonding, deposition, and photolithographic techniques to fabricate such devices.[1,12,13] This chapter presents the use of standard micromachining

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* This chapter is adapted with permission from *Lab. Chip.*, **2005,** *5,* 501-505.

procedures and thin-film technology to create open-channel microfluidic devices on planar substrates. The devices are created using bottom-up construction techniques and sacrificial layer technologies, obviating the need to thermally or otherwise seal a cover plate to a micromachined substrate for channel enclosure, thus avoiding one of the more difficult steps in the fabrication process.[14] Importantly, this method provides unique flexibility in channel geometry, wall composition and substrate material, while taking advantage of the high precision obtainable through photolithographic patterning (feature variations of <1% are generally reported by photoresist manufacturers).

Although slower than some of the approaches summarized in Section 1.6, the method of sacrificial layer removal presented here avoids the disadvantages of the more rapid etching techniques and maintains a high degree of flexibility in channel structure. Furthermore, the etching process presented in this work is not labor intensive, and many devices can be etched in parallel. Consequently, the etch times are only a minor inconvenience in prototyping work, and are not a concern for large-scale fabrication of established device designs. Importantly, my devices have been used successfully to separate analytes. Finally, the broad substrate compatibility provided by this fabrication approach may offer a variety of readily integratable device components with multiple channel layers to develop next-generation lab-on-a-chip systems.

2.2 Experimental

2.2.1 Channel Fabrication

To create the microfluidic devices, quartz wafers (GM Associates, Oakland, CA) were used as base substrates. The basic fabrication steps are

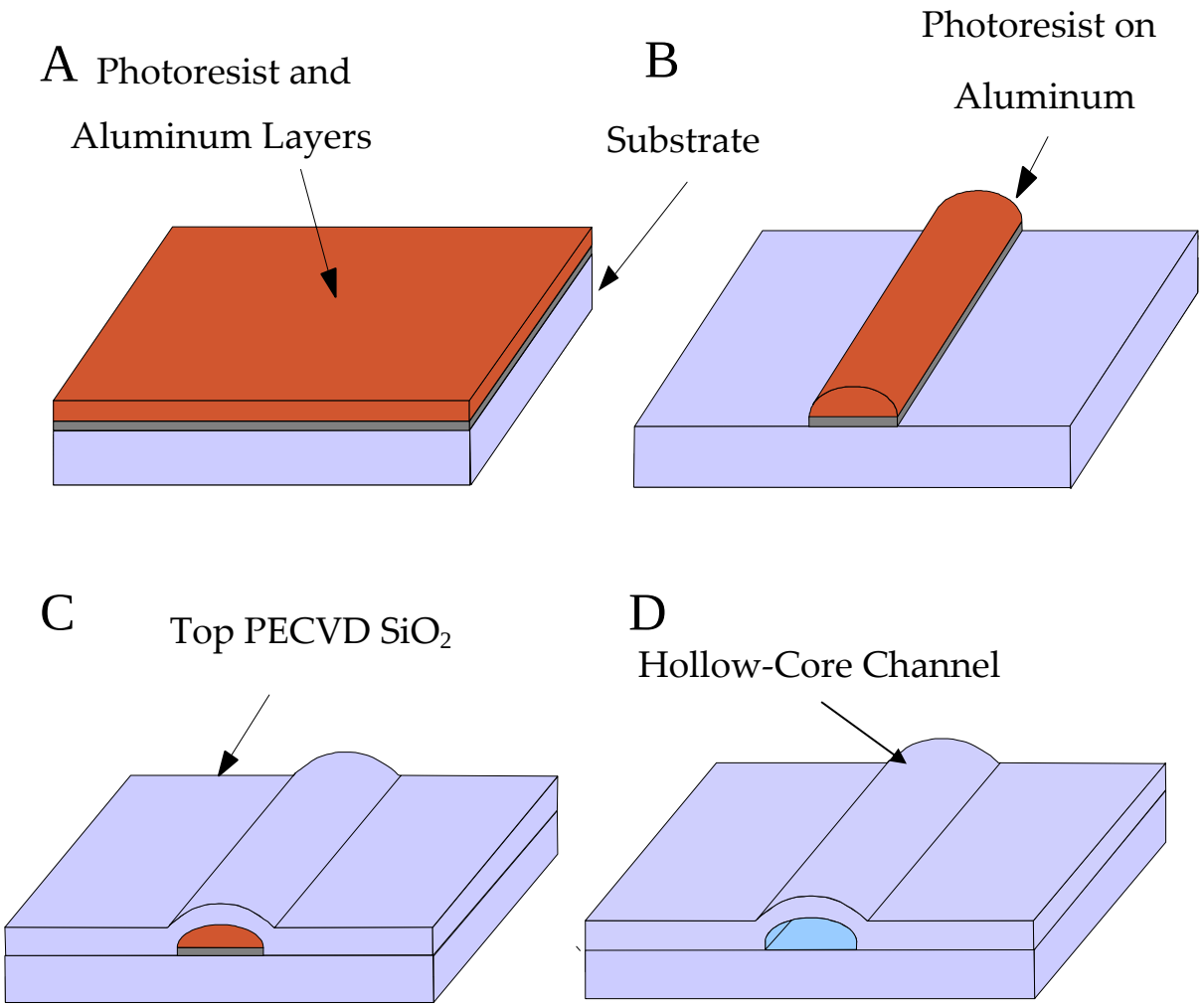

Figure 2.1. Schematic of the fabrication process for thin-film microfluidic devices. (A) Cleaned quartz substrate with 500 nm aluminum and 3 μm photoresist. (B) Reflowed photoresist with etched aluminum sub-layer. (C) Patterned quartz wafer with 3 μm oxide encapsulating the sacrificial layers. (D) Etched sacrificial layers forming a hollow tube.

shown in Figure 2.1. The wafers were cleaned in an acetone rinse, followed by rinses in isopropyl alcohol and methanol. After rinsing, the wafers were immersed in buffered hydrofluoric acid for 15 s and rinsed with deionized water. A 500 nm layer of aluminum was vapor deposited on the cleaned wafers using a CHA-600 thermal evaporator (CHA Industries, Fremont, CA). To drive off any remaining water and solvent, a dehydration bake was performed by placing the wafers in an Ultra-Clean 100 oven (Lab-Line Instruments, Melrose Park, IL) for 30 min at 120 °C. Hexamethyldisilazane (HMDS; Shin-Etsu MicroSi, Phoenix, AZ) was spun onto the wafers to promote adhesion of the photoresist. The wafers were then spin-coated with AZ-3330 photoresist (Clariant, Sulzbach am Taunus, Germany) at 2500 rpm for 60 s, yielding a 3–3.5 μm thick layer of photoresist (Figure 2.1A). The application of photoresist was followed by a 90 °C soft bake for 60 s.

The wafers were photolithographically patterned using a chrome mask that was produced in-house with a 250 CC Criss-Cross Electromask Lasometric Pattern Generator and Image Repeater (TRE, Woodland Hills, CA). The mask design contained 36 microdevices with a 100 μm offset at the double-T injectors[16] and feature widths of 10 μm. The separation channel was 0.9 cm long, while the three shorter arms were 0.3 cm in length (Figure 2.2). The wafers were exposed to UV radiation from a 250 W mercury lamp for 14 s in a MA150 CC Karl Suss

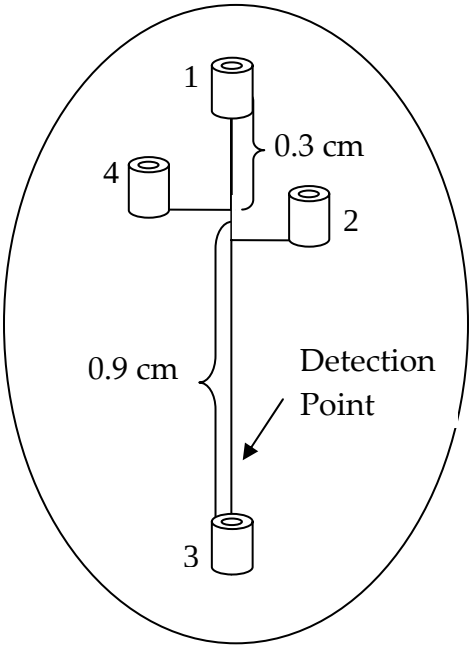

Figure 2.2. Schematic of the separation device layout. Reservoirs 1, 2, and 3 were filled with buffer solution, and reservoir 4 was filled with the amino acid sample. To inject, reservoirs 1, 3, and 4 were grounded and –600 V was applied to reservoir 2. Separation was achieved by keeping reservoir 1 grounded, applying –600 V to reservoirs 2 and 4 and applying –750 V to reservoir 3. The offset of the double-T injector is not to scale.

aligner (Karl Suss America, Waterbury Center, VT), followed by developing in AZ 300 MIF developer (Clariant) for 30 s. After rinsing, the patterned wafer was hard baked at 110 °C for 10 min, followed by removal of the unprotected aluminum in an etch bath (16:1:1:2 $H_3PO_4/HNO_3/CH_3COOH/H_2O$; Ashland Chemical, Columbus, OH) at 50 °C. Once the unprotected aluminum was removed, the photoresist was reflowed on a hot plate at 260 °C for 20 min (Figure 2.1B).

With the photolithographically defined features forming a sacrificial layer, silicon dioxide was deposited on the wafers to form the channel walls using a Sunbird System plasma enhanced chemical vapor deposition system (PECVD; SHS Equipment, Milpitas, CA). To deposit the oxide, 5% silane and nitrous oxide were flowed into the PECVD vacuum chamber at 250 °C. A high pressure

(0.900 Torr) oxide growth recipe was chosen for this work to encourage conformal deposition of the oxide. A 3 h deposition was used, resulting in a 3.5 μm layer of oxide over the entire substrate (Figure 2.1C).

After oxide deposition, removal of the photoresist/aluminum sacrificial layer was necessary to create the hollow tube channels. To gain etching access through the oxide to the sacrificial layers, two methods were used to remove the ends of the channel. The first consisted of respinning HMDS and AZ3330 photoresist onto the wafers and using standard lithography processes to pattern photoresist over the main body of the channel, while leaving the ends exposed. The exposed ends were then etched away in buffered HF. The second approach was to use a diamond scribe to break through the deposited oxide at the ends of the channels. Both methods were used successfully, although the second was simpler and faster.

Upon exposing the channels, the aluminum was etched by submerging the wafer in 2:1 HCl/HNO_3 at 80 °C for 60 h. Etch rates for aluminum in micron-sized channels can be determined by work discussed in Barber et al.[15] After etching the aluminum, the remaining photoresist was removed by soaking the wafer in Nano-Strip (Rockwood Electronic Materials, Fremont, CA) for 2–3 h at 60 °C.

Once the sacrificial materials were removed, it was necessary to provide an interface between the devices and the separation voltage sources.[16] To do this, reservoirs were created by cutting cylinders of 2.1 mm internal diameter from a 1/4" poly(methylmethacrylate) (PMMA) sheet using a laser cutter (Universal Laser Systems, Scottsdale, AZ). A variety of methods were used to attach the reservoirs to the quartz substrate. The most successful approach involved heating the quartz wafer with the reservoirs in place on a hot plate and conforming the reservoirs to the substrate. Following this step, 1 μL of acetonitrile was pipetted around the base of each reservoir to improve adhesion. Once attached to the substrate, each reservoir held 10 μL of liquid.

2.2.2 Separation and Detection

Arginine, phenylalanine, and glycine were obtained from ICN Biomedicals (Aurora, OH). The amino acids were labeled at the free amine group by combining 200 μL of 6 mM fluorescein 5-isothiocyanate (FITC; Molecular Probes, Eugene, OR) in dimethyl sulfoxide (DMSO) with 600 μL of a 3 mM solution of each amino acid for 4 days in the dark at room temperature. After labeling, the amino acids were combined and diluted to 500 nM concentrations in 100 mM carbonate buffer, pH 9.2. Pipetting 10 μL of the buffer solution into the reservoirs caused the channels to be filled by capillary action.

A schematic of the separation device is given in Figure 2.2. To perform a separation, I filled reservoirs 1, 2 and 3 with the buffer solution and filled reservoir 4 with the sample. To move the sample from the reservoir into the sample injector, reservoirs 1, 3 and 4 were grounded, and a voltage of –600 V was applied to reservoir 2. Separation of the injected sample was achieved by grounding reservoir 1, applying potentials of -600 V to reservoirs 2 and 4, and applying -750 V to reservoir 3.

The laser-induced fluorescence detection system used has been described previously.[17] Briefly, excitation was achieved by directing the 488 nm line from an Ar ion laser into an inverted microscope. Photomultiplier tube detection interfaced with a computer through LabVIEW collected and recorded the fluorescence signal at a sampling rate of 100 Hz.

2.3 Results and Discussion

The fabrication procedure described above yielded semicircular channels with heights between 3.5 and 4 μm, and widths between 8 and 9 μm. Figure 2.3 shows scanning electron microscope (SEM) images of an overhead view of the injection region and a profile view of the end of an etched channel.

The aluminum and photoresist combination was chosen for the sacrificial layer for a variety of reasons. The base aluminum layer was used because aluminum could be etched more rapidly than the photoresist and the 2:1

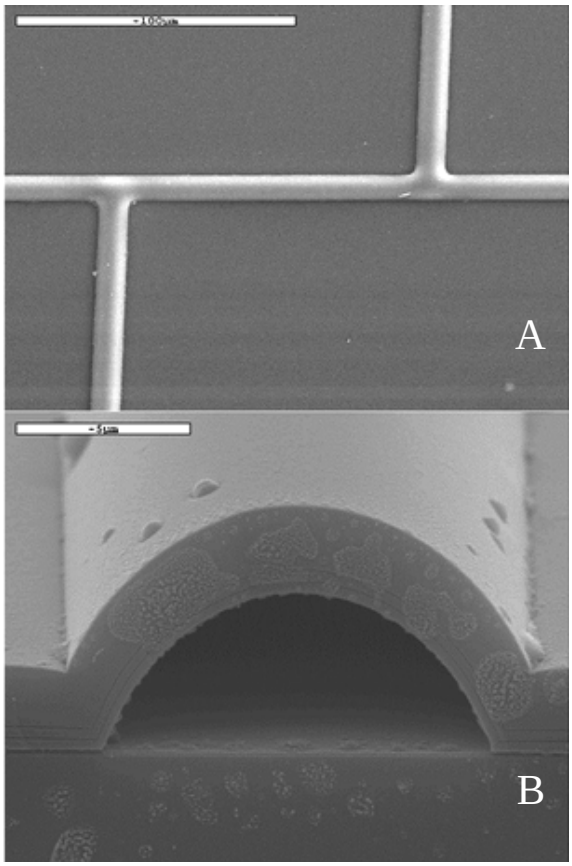

Figure 2.3. SEM images of an etched microfluidic tube made using the described procedure. (A) Top view of the offset injection region. (B) Profile view of the end of a channel.

HCl/HNO_3 mixture maintained etching potency longer than Nano-Strip. Once the aluminum had been etched, Nano-Strip could flow through the channel and rapidly etch the photoresist. The photoresist was used over the aluminum to increase the height of the channels and provide a smooth semicircular geometry, creating a stronger junction between the channel walls and the wafer substrate.

Earlier work with aluminum-only sacrificial layers reported that a width-to-thickness ratio of 35 should be maintained to provide the necessary strength to withstand the pressure while etching. The mechanical strength, defined by the critical failure pressure (P_c) of the hollow channels, is described by equation 2.1,[18]

$$P_c = 2S_t\left(\frac{t_h}{w}\right)^2 \qquad (2.1)$$

where S_t is the tensile strength of the overcoat material, t_h is the thickness of the overcoat layer, and w is the width of the hollow tube. The tensile strength of the oxide layer was calculated to be 356 MPa for aluminum sacrificial layer channels. The tensile strength for the channels created here should be similar or even greater, due to the smoother sloping geometry.

Microchip CE devices created using this sacrificial technique were used to separate an amino acid mixture (Figure 2.4). Migration times were 11.0, 19.1 and 23.5 s for arginine, phenylalanine and glycine, respectively. A small FITC peak was detected at 14.2 s. The effective separation length from the injection region

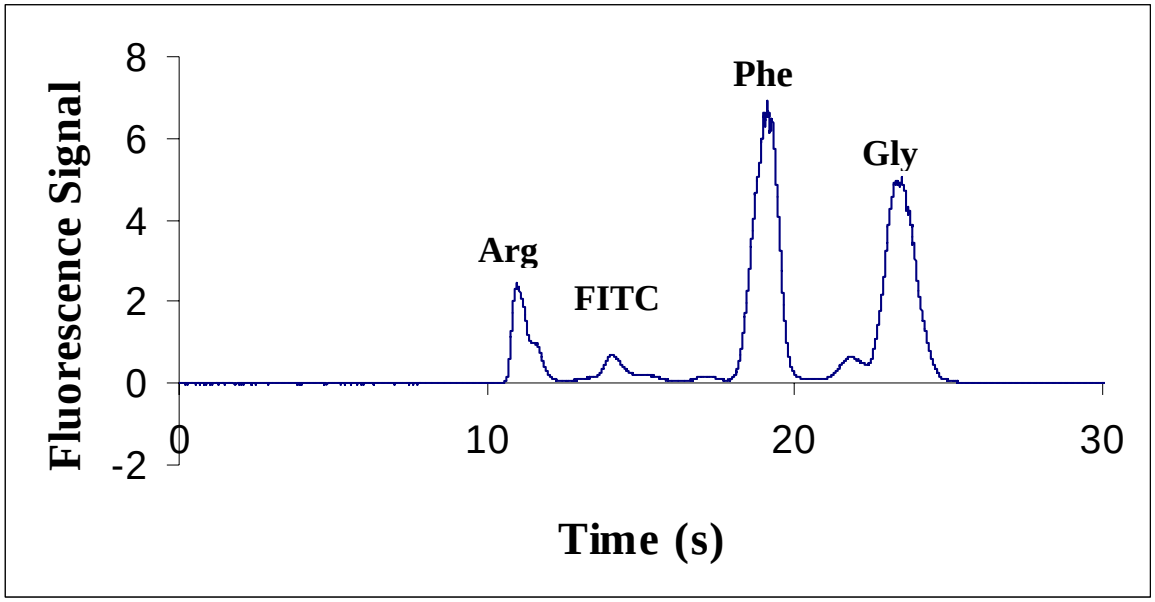

Figure 2.4. Electropherogram of FITC-tagged arginine, phenylalanine, and glycine. The injection concentrations were 500 nM of each amino acid. E_{sep}= 580 V/cm and L_{sep}= 0.65 cm.

to the detector was 0.65 cm. The separated peaks were symmetrical, and the device gave an efficiency of 2,600 theoretical plates (0.65 cm separation length) for glycine. Importantly, no background fluorescence was detected in the channels.

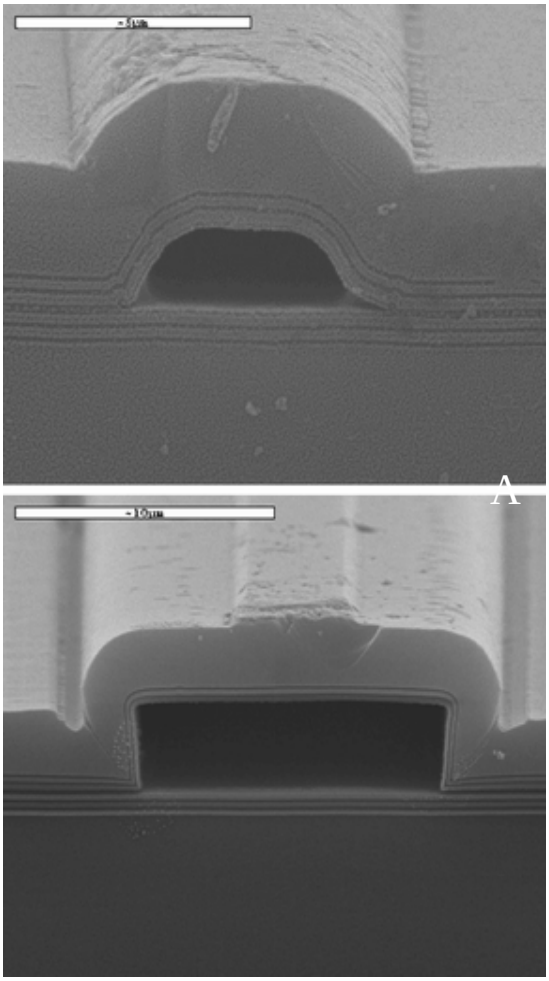

Figure 2.5. Profile SEM images of channels made using different sacrificial layers. (A) Channel made with aluminum sacrificial core, etched in 3:1 HCl/HNO_3. (B) Channel made with SU-8 sacrificial core, etched in Nano-Strip.

Beyond using the aluminum/photoresist semicircular sacrificial layers, other materials, such as SU-8 and aluminum, can be used to create channels of varying geometry and height. Figure 2.5 contains SEM images of channels made using aluminum (trapezoidal) and SU-8 (rectangular) sacrificial layers. Lines appear in the SEM images due to the layering of oxide and nitride for increased optical capabilities. Beyond the sacrificial layers detailed above, it could be expected that nearly any substance that could be defined using photolithography, would be a viable sacrificial material in this

approach. For spin-on sacrificial layers, the channel height can be controlled by varying the speed used to spin the material onto the wafer.

In addition to the variety of cross-sectional channel shapes available, a range of materials can be deposited over the sacrificial layer to form microchannels with different properties. I have deposited polysilicon and silicon nitride with success equal to that of oxide. In the BYU clean room, the ability to deposit silicon oxynitride should also be readily available. Varying the channel wall material should allow devices to be tailored for applications with unique optical, structural, and surface constraints. Further work with silicon nitride channel walls may be particularly useful due to the reported ability of silicon nitride to avoid protein surface adhesion.[10] Other materials that can be deposited or evaporated in a thermal or e-beam evaporator could also be used to create the channels. Spin-on substances, such as glass, could potentially form similar channels, without exceeding a temperature of 100 °C.

When varying the channel wall material, complications resulting from differing zeta potentials and electroosmotic flows can be avoided by depositing a thin layer of the channel material on the base wafer prior to processing. This step will ensure that the channels are composed of a uniform material and that surface properties will be consistent. This was not necessary for the work

presented here because the base substrate and deposited material were both SiO_2.

We have also observed that the substrate material channels are built on can be varied with relative ease. In addition to quartz, I have fabricated channels similar to those described previously on top of silicon and borosilicate glass. Oxide covered features have also been made on alumina. There was no significant difference between the fabrication process of the features on these substrates and the process described previously for quartz wafers.

In traditional devices, thermal bonding of glass substrates to enclose microchannels requires rigorous cleaning steps and temperatures over 500 °C. Furthermore, quartz, which has ideal optical properties for a broad range of applications,[19-23] is more difficult to bond and requires even higher temperatures, severely limiting the application to date of quartz microfluidic devices. In the work presented here, however, the high temperatures associated with bonding quartz and glass were not necessary, and the entire cleaning process for any of the substrates could be completed in under 5 min.

Other advantages of these devices are reduced radial mass transfer concerns and enhanced Joule heat dissipation abilities resulting from the small channel dimensions and thin walls. Consequently, high efficiencies should be attainable with these devices for both CE and pressure-driven, surface-

interaction-dependent separations. Due to the large volume of prior work done on silica surface modification, highly controlled surface interactions and electroosmotic flow should be obtainable in these devices.[7, 24]

It might be expected that the small microchannel size would present disadvantages, particularly due to clogging. Our work, however, found that clogging problems were rare and could typically be overcome through the application of vacuum to one of the reservoirs. Blockages that could not be removed using vacuum, as well as problems resulting from analyte adhesion to the channel walls, were successfully eliminated by soaking the devices in a mixture of concentrated sulfuric acid and 30% H_2O_2, 7:3, and/or 1 M NaOH.

To develop the full potential of these devices, further work must be done to increase the complexity of channel design and vary the surface chemistry. Well developed in the electronics industry, microfabrication readily lends itself to the creation of small, complex, intertwining networks of channels, allowing rapid parallel analysis.[1] Such three-dimensional networks, with channels crossing over each other, would provide increased capabilities for a variety of analytical applications, such as nucleic acid sequencing and protein analysis. Similar fabrication schemes are being used in making liquid anti-resonant reflecting waveguides (ARROW)[15] and electroosmotic pumps on planar substrates. In the future, the successful interfacing of these components, as well

as others, would lead to devices with integrated pumping, separation, and detection capabilities, moving closer to realizing the lab-on-a-chip concept.

2.4 Conclusions

This work presents a practical planar thin-film sacrificial layer approach, which is fully compatible with standard silicon micromachining procedures, for constructing microfluidic arrays that have been applied in biological separations. This work suggests that fabrication of much more sophisticated devices to perform more comprehensive analyses should be possible using this technique, although further research must be done to demonstrate this capability. Integration of more intricate separation systems with sample preparation and detection components should provide devices that draw nearer to the goal of μ-TAS.

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3. ENHANCEMENT OF THIN-FILM MICROFLUIDIC DEVICE PERFORMANCE AND CAPABILITIES

3.1 Introduction

With a fabrication approach established and the viability of the thin-film planar microfluidic channels for separations determined, improving the performance and capabilities of these devices became necessary to demonstrate their competitiveness with devices fabricated using other methods. To do this I have focused on two areas. The first is improving separation performance by increasing the efficiency, range of analytes that can be separated and reproducibility. The second is the incorporation of polyacrylamide gels to facilitate capillary gel electrophoresis, analyte preconcentration and isoelectric focusing processes in these devices.

3.2 Separation Performance Improvement

In view of the earlier published work (Section 1.6) that outlined similar fabrication methods, but did not actually show any separations, carrying out separations in thin-film microchips was critical in establishing my fabrication method as viable. The rapid separation times, peak symmetry, and complete resolution demonstrated in Chapter 2 were all very promising.[1] However, there were also several problems with this initial separation work, including device lifetime, reproducibility between devices, relatively low separation efficiency,

and the fact that only amino acids (among the simplest of biological molecules) had been separated.

I found that the lifetime of the initial devices was limited by the weak interface between the channels and reservoirs; very little force would cause these reservoirs to fall off. Although the devices could be cleaned, new reservoirs attached and the channels reused, the number of runs that could be done at one time was limited. Furthermore, over time the reservoirs would slowly leak buffer, providing alternate current paths outside the channels. This problem was further aggravated due to the reservoirs being in contact with each other when placed on the 3 shorter channel arms.

To overcome these setbacks, I made several improvements. First, a new mask was designed, providing greater distances between reservoirs by increasing the channel lengths (1.1 cm separation length and 0.4 cm channel arms). To attach the reservoirs, a biologically compatible double-stick tape (Adhesives Research, Glen Rock, PA) was used between the channels and reservoirs, after which five-minute epoxy was applied to strengthen the attachment and provide resistance to leakage. These improvements not only increased the lifetime of the devices, but also the more robust interfaces allowed me to apply higher voltages and increase the separation efficiency.

Perhaps the most significant problem with the initial devices was the interdevice reproducibility; in a group of 10 seemingly identical devices, reliable control of sample movement was only possible in one or two microchips. The lack of control over sample movement in the other devices resulted from strong, irregular electroosmotic flow (EOF). SiO_2 is known to adsorb molecules at its surface and have strong EOF. Importantly, surface adsorption of compounds affects EOF, potentially making it irreproducible. Moreover, the channels presented in this work are particularly sensitivity to EOF and adsorption due to their unusually high surface-to-volume ratio.

I focused on two ways to address the issue of irreproducible EOF in thin-film microfluidics. The first approach implemented better cleaning techniques to remove any surface-adsorbed analytes, and the second method reduced the EOF by using dynamic coatings.

I developed two techniques to improve device cleaning. The first approach took advantage of the new, robust reservoirs to establish pressure connections. With pressurized channels, cleaning solutions could be flowed through readily, providing a greater flush volume, as well as the ability to force solutions through channels that would not fill through capillary action alone, due to channel blockage. My second improvement in microchip cleaning

involved soaking the devices in acetic acid after cleaning in Nanostrip, which reduced problems with silica surface adsorption.

My second and more significant advance in improving microchip separation reproducibility was using an EOF-reducing coating. Two device pretreatment procedures were tested. The first flushed 1 M NaOH through the channels for 5 minutes, followed by 10 minutes of rinsing with water. The second method used devices cleaned as described earlier in this section, using Nanostrip and acetic acid. After either pretreatment, the dried channels were flushed with Ultratrol Dynamic Pre-Coat HR or LN coating solution (Target Discovery, Palo Alto, CA) for 5 – 10 min, and then buffer was flowed through the channels for at least 10 min to prepare devices for capillary electrophoresis (CE) analysis.

Implementation of the improved procedures led to significantly better CE results for the same 3 amino acids separated in Figure 2.4. Figure 3.1 shows four electropherograms of FITC-tagged amino acids separated under two different voltages in thin-film microchannels coated with either Ultratrol Dynamic Pre-Coat HR or LN. Peak elution order in these separations is reversed relative to uncoated channels (Figure 2.4), since the analyte electrophoretic velocities now exceed the EOF velocity.

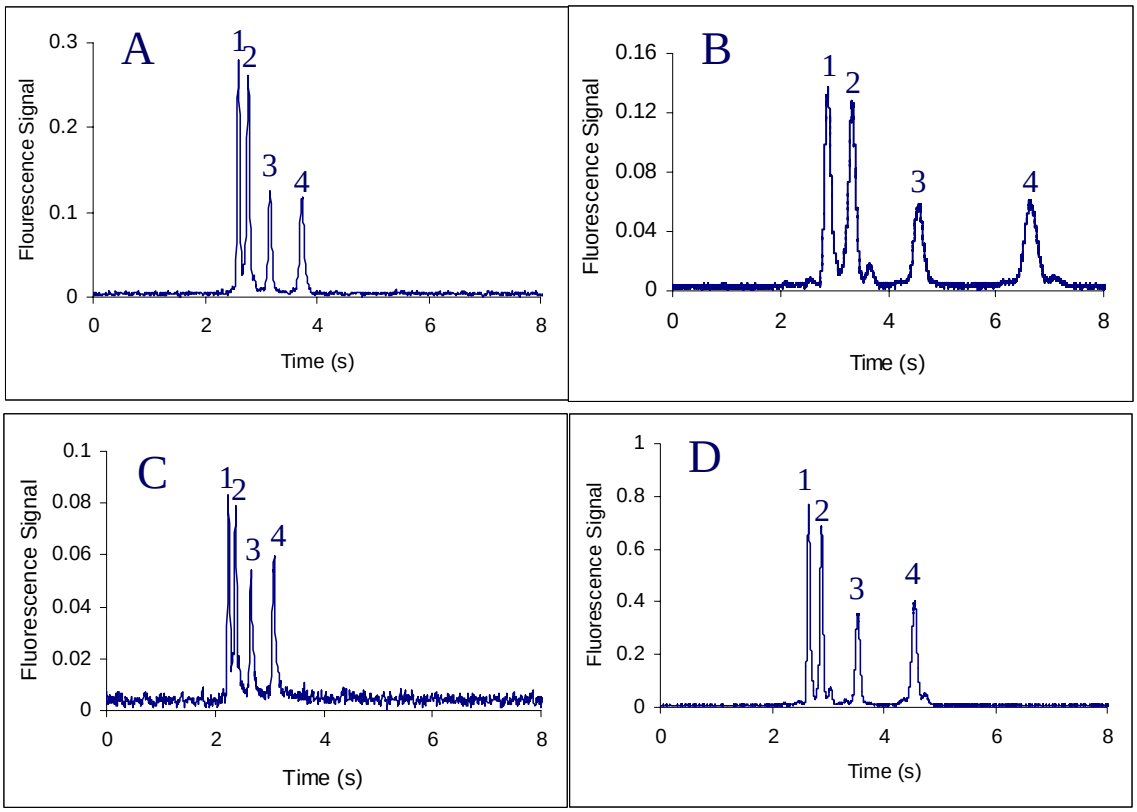

Figure 3.1. Electropherograms of the same mixture as in Figure 2.4. Coatings were Ultratrol Dynamic Pre-Coat HR in (A) and (C) and Ultratrol Dynamic Pre-Coat LN in (B) and (D). The electric fields for the separations were 660 V/cm in (A-B) and 830 V/cm in (C-D). Peaks are: (1) glycine, (2) phenylalanine, (3) FITC, and (4) arginine.

With higher applied voltages and EOF control reagents, CE peak widths well under 1 s were obtained. The separation efficiencies also increased by as much as 10 fold, yielding values comparable to other microfluidic work. A list of theoretical plate counts achieved for each peak in Figure 3.1, in comparison to Figure 2.4, is given in Table 3.1. These separations were also significantly faster than in Figure 2.4, being performed in under 8 s for the LN pre-coat and under 4 s for the HR pre-coat.

Table 3.1. Peak efficiency values (theoretical plate counts) for amino acid separations.

Peak	Ultratrol HR 660 V/cm	Ultratrol HR 830 V/cm	Ultratrol LN 660 V/cm	Ultratrol LN 830 V/cm	Native Silica 580 V/cm
Gly	5000	18000	3000	12000	3000
Phe	12000	20000	3000	14000	2000
FITC	11000	16000	3000	8000	2000
Arg	10000	15000	4000	10000	2000

By using dynamic coatings, I also decreased the surface adsorption of samples, which enabled CE experiments on FITC-tagged peptides (Figure 3.2.) The labeling procedure for the peptides is described in Kelly et al.[2] and is similar to the approach used for tagging amino acids with FITC in Section 2.2.2. Figure 3.2 also displays an increase in efficiency when higher voltages are applied.

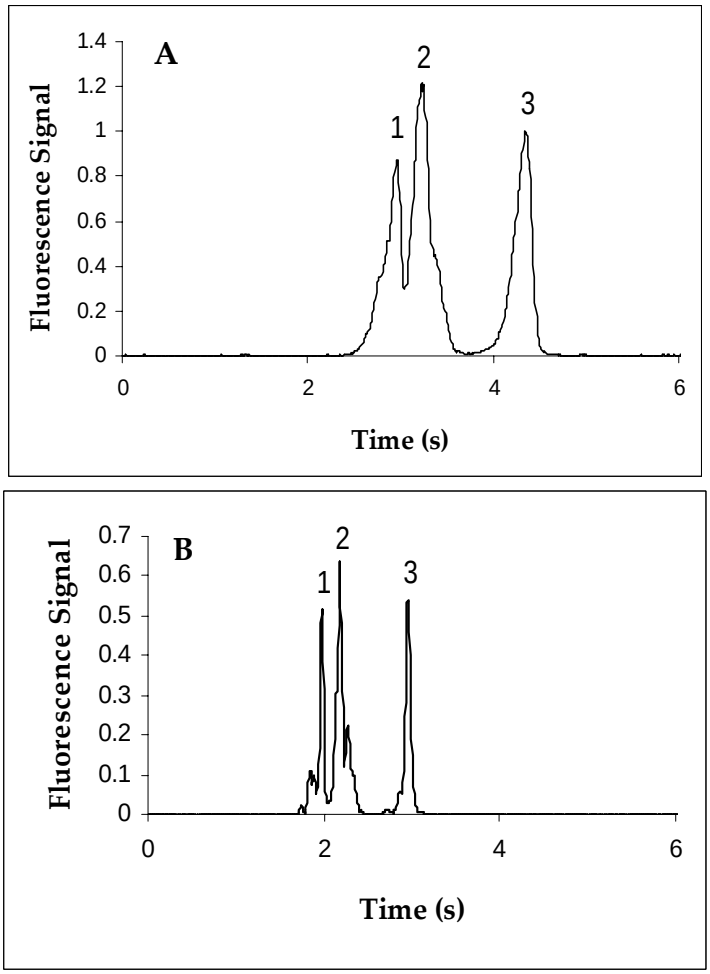

Figure 3.2. Separation of 3 FITC-labeled peptides using (A) 1000 V and (B) 1250 V in planar thin-film microfluidic channels using Ultratrol Dynamic Pre-Coat HR. Peaks are: (1) FLEEI, (2) Leu Enkephalin, and (3) GGYR.

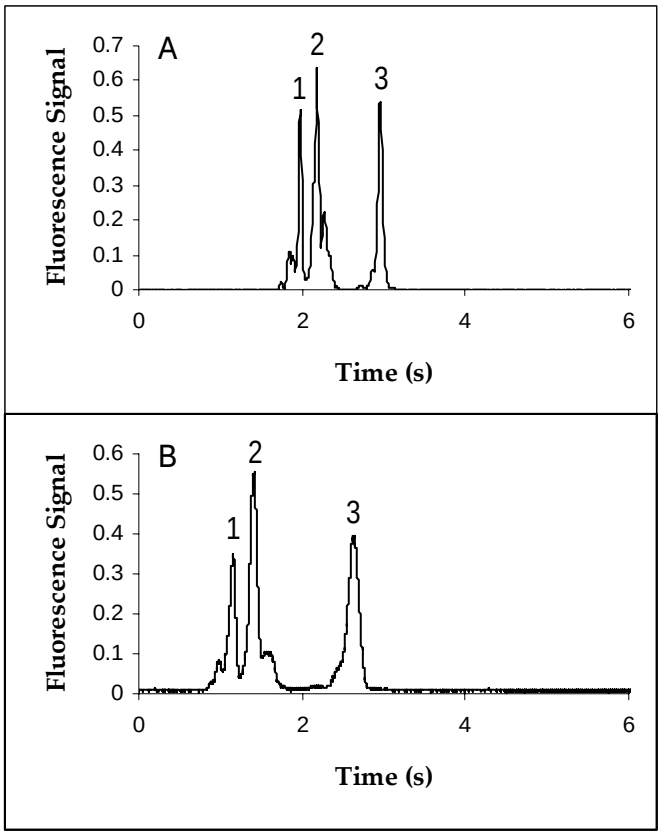

Figure 3.3. Separations of FITC-labeled (1) FLEEI, (2) Leu Enkephalin, and (3) GGYR in two different devices 6 months apart. (A) June 2005 and (B) December 2005. Separation potentials were 1250 V.

Finally, these improvements have greatly increased the separation reproducibility between devices. Figure 3.3 shows separations performed six months apart on two different microchips. The two electropherograms clearly display the same sets of peaks, with some differences in the time scale. The time variations could be due in part to differences between the actual detection points on the channels or variations in timing resulting from the manual starting of data acquisition and switching of voltages.

3.3 Polyacrylamide Gel Incorporation

Polyacrylamide slab gels are frequently used for the separation of proteins, both in one- and two-dimensional formats.[3] There are, however, several drawbacks to the use of slab gels. First, heat can damage the gel, which

limits the electric fields that can be applied, resulting in longer separation times and lower efficiencies. Second, the staining and detection methods generally used for slab gels have high detection limits, requiring relatively large sample volumes.

By moving gel electrophoresis into microchannels, some of these disadvantages can be avoided. The increased surface-to-volume ratio enhances Joule heat dissipation, and thus allows higher electric fields, which lead to greater separation efficiencies. This, combined with shorter channels, provides much faster analysis times. Finally, the potential of microdevices to integrate multiple processes should make possible the performance of preconcentration followed by separation, to enable studies of low-abundance proteins. Importantly, polyacrylamide gels can be used for preconcentrating as well as for separating by simply adjusting the cross-linker content.

I have focused on selectively defining the location of the polyacrylamide gels in thin-film microfluidic systems. This is a critical step in increasing device functionality, but it is also challenging as the small channel dimensions make it difficult to control fluid movement precisely. A reliable method for selectively polymerizing a gel within microchannels is essential for integrating multiple functionalities within a chip and realizing many of the advantages of implementing a microfluidic format.

In developing methods for selectively defining gels within microchannels, I have worked with the same double-T device layout used for enhanced CE separations (Section 3.2). Covalent attachment of the polymer gel to the channel surface is necessary because cross-linked polyacrylamide shrinks upon polymerization. This shrinking can result in gaps between the channel wall and gel, which would impair separation performance.

I have implemented a modified version of a method used by Woolley et al.[4] With γ-(methacryloxy)propyltrimethoxysilane (MAPS), I attached vinylic groups onto the silica surface. Functionalization was carried out with a 0.4% (by weight) solution of MAPS in a 50:50 (by volume) mixture of water and ethanol, adjusted to ~pH 4 using acetic acid. The channels were filled with this solution for 1 hour, then pressure was applied to flush the channels with water, and finally the water was allowed to evaporate.

The prepolymer solution consisted of 12.5% (by weight) acrylamide (Fisher Scientific; Fairlawn, NJ) with 0.5% (by weight) N,N'-methylenebisacrylamide (bis; Spectrum; Gardena, CA) in 100 mM carbonate buffer (pH 9). When polymerized, this solution formed a gel optimized for the separation of proteins (or other macromolecules) with molecular weights ranging between 12 and 80 kDa.[3] To the prepolymer solution, I added a 0.05% riboflavin solution (Eastman Chemical; Kingsport, TN), tetraethylmethylenediamine

(TEMED; Spectrum), and a 0.5% sodium fluorescein solution (Sigma-Aldrich; St. Louis, MO). Riboflavin served as a photoinitiator when illuminated with light of wavelengths under 500 nm, which excited riboflavin to a triplet state and reacted with TEMED by taking an electron. This resulted in the formation of radical TEMED cations and riboflavin anions, which in turn induced free-radical polymerization of acrylamide and bis to form a crosslinked polymer. The sodium fluorescein allowed fluorescence imaging of the location of the acrylamide solution within the microchannels.

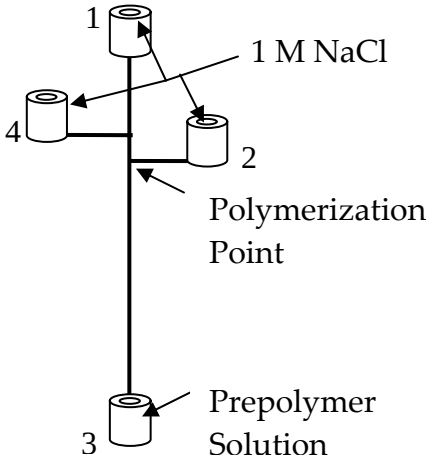

Figure 3.4. Schematic of the setup used to precisely define polyacrylamide gel interfaces in thin-film microfluidic devices.

Prior to introducing the prepolymer solution into the channels, I degassed it to eliminate dissolved oxygen, which otherwise quenches polymerization, and to remove dissolved gases to prevent the formation of bubbles during polymerization. To the degassed solution, I added the riboflavin, TEMED and fluorescein. Polymerization was most successful with 4 ppm riboflavin, 0.45% TEMED, and 10 ppm fluorescein (final solution concentrations by weight), as the reaction proceeded rapidly (~6 min), but was controlled enough to prevent premature polymerization.

The prepolymer solution was introduced into the microchannels via reservoir 3 (Figure 3.4), and the remaining reservoirs were filled with 1 M NaCl (Columbus Chemical Industries, Columbus, WI). The 488-nm argon ion laser line, focused down to ~4 μm using a 40×, 0.6 NA objective (Nikon), was positioned at the head of the separation channel (see Figure 3.4) for 6 minutes to polymerize a small plug of gel at the start of the separation channel. This well-defined interface allowed pressure or voltage to flush the shorter channels, which were designed to be gel-free, and permit polymerization of the gel in the remainder of the separation channel by whole-device UV illumination for ~1 h.

3.4 Conclusions

The successful development of improved, reproducible separations in thin-film microfluidic devices opens the door to the study of more advanced capabilities. Incorporation of monoliths, electroosmotic pumps, and surface coatings for capillary electrochromatography, are all important areas of future research.

Polymerized gels in thin-film microfluidic systems should be useful in the separation of proteins and other macromolecules. The ability to spatially define these gels should also provide utility in analyte preconcentration and in the creation of interfaces for multidimensional separations.[5, 6]

3.5 References

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4. CONCLUSIONS AND FUTURE WORK

4.1 Conclusions

I have described the development of fabrication techniques for the creation of capillary electrophoresis (CE) microfluidic systems. These devices have been shown to provide rapid separation times and good efficiencies (see Chapter 3).[1] However, unlike many other microfluidic device construction techniques, the entire fabrication process is performed using standard micromachining approaches. By relying on standard micromachining processes, this method takes advantage of the mass fabrication capabilities already established and provides a foreseeable fabrication pathway needed for the successful merging of electrical and fluidic control, a necessity for the development of a micro-total analysis system (μ-TAS).

Beyond these advantages, the high surface-to-volume ratio and thin channel walls in these microfluidic systems provide excellent Joule heat dissipation.[2] Good Joule heat dissipation is advantageous for capillary zone electrophoresis (CZE) in allowing high electric fields to be applied and diffusion to be minimized. Dispersing Joule heat well is also essential for capillary gel electrophoresis (CGE), since gels can be destroyed by significant heating. Thin-film fabrication also enables the use of materials that previously were not feasible due to fabrication constraints.

Thin-film microfluidic systems can perform rapid, efficient, and reproducible separations (Chapter 3). In an effort to establish the potential of these devices to be used in μ-TAS applications, I have evaluated the incorporation of polymerized gels, which could be utilized for CGE, preconcentration, and isoelectric focusing. Importantly, I have developed a method to precisely define interfaces of polyacrylamide gels in thin-film microchannels, a critical need for applications such as preconcentration, 2-D separations, monolith integration, or open-tubular chromatography. Since my approach uses position-specific photopolymerization, it is compatible with photomasking, and thus potentially scalable to mass fabrication.

4.2 Future Work

4.2.1 Increasing Analytical Capabilities

Thin-film microfluidics fabrication has a promising future, but additional experimentation is needed to broadly establish the potential of this approach. For example, carrying out CZE or CGE of proteins would be an important step. Another immediate application of gel-filled microchannels is as membranes for preconcentration. I have polymerized solutions with as much as 40% acrylamide (~12% bis), which should exclude small peptides, but allow the passage of buffer ions. Other polymer concentrations should provide membranes optimal for the

enrichment of proteins. Indeed, 12% polyacrylamide can concentrate larger proteins such as R-phycoerythrin (see Figure 4.1).

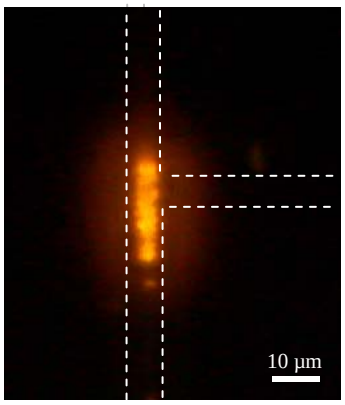

Figure 4.1. Concentration of R-phycoerythrin (240 kDa) at a 12% polyacrylamide interface.

Moreover, the addition of ampholytes into a gel would provide a means of establishing a pH gradient for isoelectric focusing (IEF). Success in this effort could lead to the interfacing of IEF with CGE in thin-film microfluidics. Slab gel 2-D electrophoresis can separate thousands of proteins, but can take days to carry out.[3] Moving 2-D separations into microchannels (see Figure 4.2) would provide much faster analysis times, and more convenient and sensitive detection platforms than in slab gels.

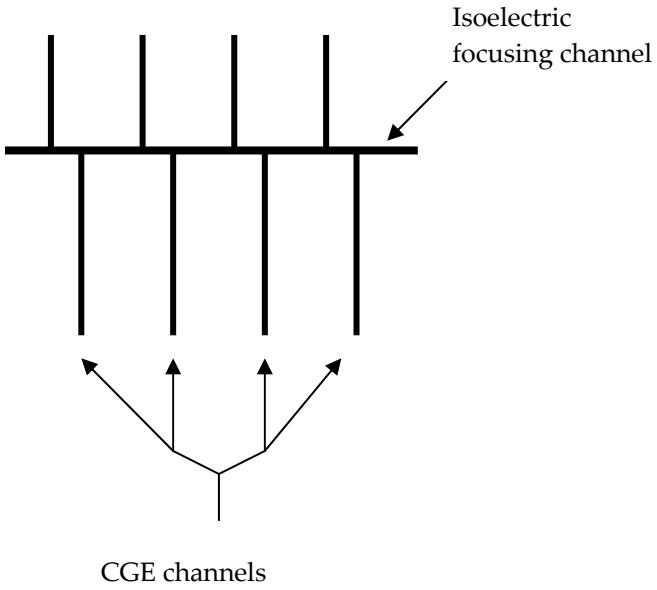

Figure 4.2. Schematic of a 2-D (IEF and CGE) microfluidic device.

The high surface-to-volume ratio in thin-film microchannels can be used to generate electroosmotic flow (EOF). A device with multiple

narrower (thinner line width) channels that converge (see Figure 4.3) should generate significant EOF when a potential is applied across the microchip. Such a device would act as an electroosmotic pump, and the resulting pressure could be used to drive chromatographic separations in the microchannels.

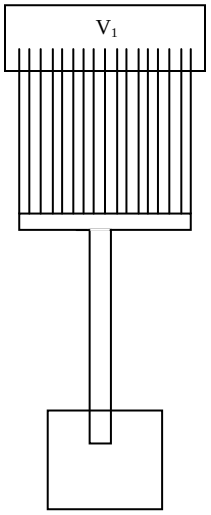

Figure 4.3. Thin-Film electroosmotic pump schematic.

The high surface-to-volume ratio of thin-film microchannels makes them well suited for open tubular chromatography or electrochromatography. A device layout that has electroosmotic pumps leading to an injector and column with stationary phase should allow electrically actuated chromatographic separation of analytes. A potential layout for such a device is shown in Figure 4.4A. The capability of such a device to inject analyte has been established (see Figure 4.4B), but since rhodamine B is uncharged and FITC-arginine has a negative charge, the separation is based on differences in electrophoretic mobilities, rather than chromatographic partitioning. To demonstrate the utility of the electroosmotic pumps in these devices for chromatographic or electrochromatographic separation, the column will need to be derivatized selectively, without surface modification of the electroosmotic pumps. Such a separation would show that

analytes can be driven by the pressure produced in the pumps and fractionated based on their interaction with a stationary phase.

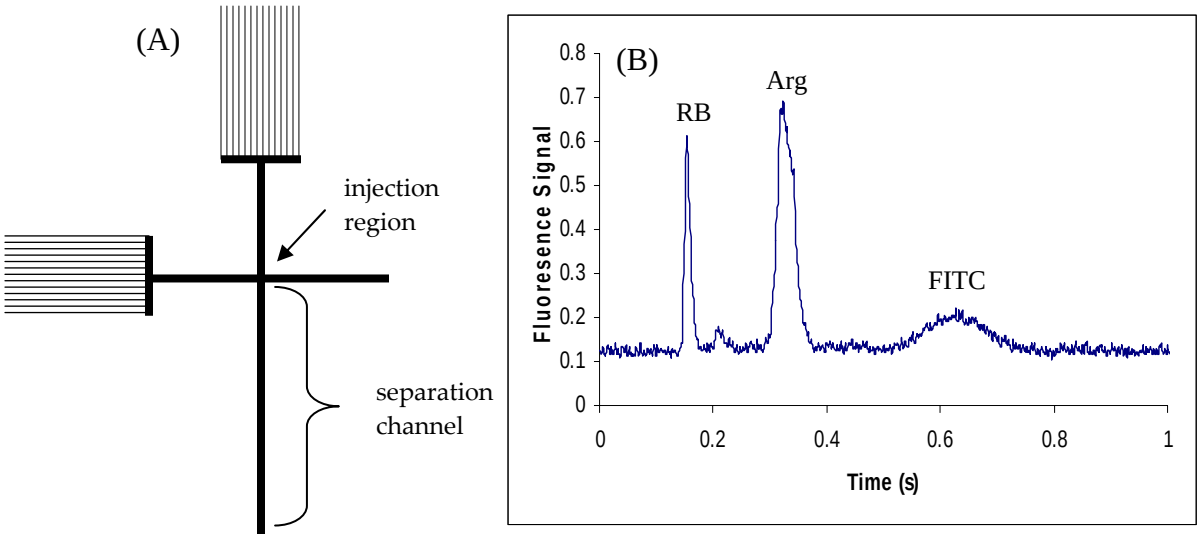

Figure 4.4. (A) Device layout for electroosmotically pumped chromatography. Chromatogram of rhodamine B (RB) and FITC-arginine (Arg) obtained at 850 V/cm in a device whose design is shown in (A).

Another area of interest is the formation of monoliths in thin-film microchannels, as considerable work has already been done on incorporating monoliths into conventional capillaries.[4-6] Monoliths are advantageous, because they provide mass transfer characteristics that are similar to those in small particle packed columns, but monoliths typically exhibit low pressure drops. Moreover, various different monoliths can be made for capturing analytes (providing filtering and preconcentration), chromatographic separation, and enzymatic digestion, among others.

Finally, the creation of microchannels with silicon nitride walls may be useful, as the inert nitride surface may provide decreased EOF and higher resistance to analyte adsorption.[7] Importantly, the thin-film microfluidics fabrication approach is uniquely well suited to making microcapillaries with silicon nitride walls.

4.2.2 Providing Electrical Interfaces and On-Chip Detection

The development of on-chip detection systems and the seamless interfacing of electrical and fluidic components, will aid in making μ-TAS instrumentation. Future work should include the fabrication of electrodes in thin-film devices to facilitate the application of voltage and to allow on-chip electrochemical detection.

Also of interest is the integration of optical waveguides for detection in these devices. Barber et al.[8] have made hollow-core waveguides or antiresonant reflective optical waveguides (ARROWs) using thin-film fabrication methods. These ARROWs should allow microchannels to act as waveguides, which could be coupled with conventional waveguides to provide an on-chip means to send light into and collect light from a microchannel. Moreover, microfabricated light emitting diodes (LEDs) could be formed on-chip to provide a fluorescence excitation source. Because thin-film waveguides have nitride interiors, the

development of nitride-walled microchannels should also provide valuable information as to the behavior of analytes in the ARROWs.

As increased sample handling and detection capabilities are developed, they will pave the way to more sophisticated devices that realize the goal of μ-TAS. Building 3-D channel networks and devices that integrate CGE separations with other modules, such as digestion monoliths, on-chip fluorescence tagging and integrated detection, can be envisioned. These lab-on-a-chip systems should prove useful in various applications, as they would realize many of the advantages of miniaturized analysis, including high throughput, portability, speed, precise fluid control and selectivity.

4.3 References

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