Development of Miniaturized Devices Using X-Ray Microlithography in Poly(methyl Methacrylate) for Chemical Monitoring and Microfluidic Applications.

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DEVELOPMENT OF MINIATURIZED DEVICES USING X-RAY MICROLITHOGRAPHY IN POLY(METHYL METHACRYLATE) FOR CHEMICAL MONITORING AND MICROFLUIDIC APPLICATIONS

A Dissertation

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in

The Department of Chemistry

by

Christopher Scott McWhorter
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Table of Contents

ACKNOWLEDGEMENTS ................................................................................................................ ii

LIST OF TABLES .......................................................................................................................... vii

LIST OF SCHEMES .................................................................................................................... viii

LIST OF FIGURES ....................................................................................................................... ix

ABSTRACT ................................................................................................................................... xii

CHAPTER 1. MICROCHIP CAPILLARY ELECTROPHORESIS DEVICES ............................... 1
  1.1 Introduction .......................................................................................................................... 1
  1.2 Current Technologies for Developing μ-CE Devices ......................................................... 4
    1.2.1 Film Deposition ............................................................................................................. 4
    1.2.2 Photoresist Processing (Photolithography) ................................................................... 7
      1.2.2.1 Photoresists ........................................................................................................... 8
    1.2.3 Etching Processes ........................................................................................................ 9
      1.2.3.1 Wet Chemical Etching .......................................................................................... 12
      1.2.3.2 Dry Etching ......................................................................................................... 14
      1.2.3.2.1 Reactive Ion Etching ....................................................................................... 14
      1.2.3.2.2 Laser Photoablation ....................................................................................... 16
      1.2.3.2.3 X-ray Synchrotron ......................................................................................... 16
    1.2.4 Device Molding ....................................................................................................... 21
      1.2.4.1 Hot Embossing ................................................................................................. 21
      1.2.4.2 Thermoplastic Injection Molding ....................................................................... 22
      1.2.4.3 Casting .............................................................................................................. 24
      1.2.4.4 Imprinting ......................................................................................................... 26
    1.2.5 Device Assembly ....................................................................................................... 27
  1.3 Topographical Characterization Tools ............................................................................... 28
  1.4 Fluid Pumping in μ-CE Devices ...................................................................................... 30
  1.5 References ......................................................................................................................... 32

CHAPTER 2. DETECTION STRATEGIES IN MICRO-CAPILLARY ELECTROPHORESIS ........... 38
  2.1 Introduction ......................................................................................................................... 38
  2.2 Optical Detection Strategies .............................................................................................. 39
    2.2.1 UV Absorbance Detection ......................................................................................... 39
    2.2.2 Laser-induced Fluorescence Detection ...................................................................... 41
    2.2.3 Raman Detection ..................................................................................................... 47
    2.2.4 Refractive Index Detection ........................................................................................ 51
  2.3 Electrochemical Detection .................................................................................................. 54
  2.4 References .......................................................................................................................... 57
List of Tables

Table 2.1 Detection Limits of Various Detection Strategies ................................... 40

Table 3.1 Chromatographic Efficiencies, LOD's and Amplification Numbers ......................................................... 84

Table 5.1 Results from Terminal transferase reaction and GA-functionalized PMMA surface labeling ...................... 134

Table 6.1 Limiting equivalent ionic conductances for several common anions and cations ........................................ 155
List of Schemes

Scheme 5.1 Reaction scheme for (1) the formation of N-lithio ethylene diamine and (2) poly(methyl methacrylate-co-N-(2-amino ethyl)-methacrylamide) ......................... 121

Scheme 5.2 Reaction scheme for the attachment of the Hae III enzyme to 6 mm amine-terminated PMMA beads ............... 125

Scheme 5.3 Radiolabeling of a 34-mer ssDNA using terminal transferase enzyme. The labeling was accomplished with an α-32P ddATP to ensure the addition of only one radiolabel per ssDNA .................................. 126
List of Figures

Figure 1.1 Flow chart demonstrating the micromachining processes involved in developing μ-CE devices ................................................................. 5

Figure 1.2 Post processing effects on resists as a function of Tg. The aspect ratio (ratio of height of structure to the width of the structure) is shown to decrease because of the fluidic nature of polymers when heated above their Tg ......................................................... 10

Figure 1.3 Representation of interaction of a chain scission type polymer with a photon of light ................................................................. 11

Figure 1.4 Microfabrication process for glass μ-CE devices ................................................................. 13

Figure 1.5 Schematic of an apparatus to perform reactive ion etching ................................................................. 15

Figure 1.6 Processing steps used in UV laser ablation of polymeric devices ................................................................. 17

Figure 1.7 Schematic representation of LIGA micromachining process ......................................................... 20

Figure 1.8 Diagram of a hot embossing machine used to Micromachine polymeric devices ................................................................. 23

Figure 1.9 Schematic representation of a machine for injection molding of plastics ................................................................. 25

Figure 2.1 Optical micrograph showing launch of 488 nm beam from lower fiber with the beam path illuminated by fluorescein at 520 nm (503-533 filter) ................................................................. 42

Figure 2.2 A simplified Jablonski diagram which represents the fluorescence and nonfluorescence processes ................................................................. 45

Figure 2.3 Schematic of the CE chip and the laser-excited, confocal fluorescence detection system ................................................................. 48

Figure 2.4 Schematic representation of energy level diagrams depicting Normal Raman and resonance Raman transitions ................................................................. 50

Figure 2.5 Diagram of Raman spectroscopy/microchip isotachophoresis system ................................................................. 52
Figure 3.1 An illustration of the polymerase chain reaction (PCR) process ........................................... 62

Figure 3.2 Diagram of the conductivity detector cell and the solution ion migration when a field is applied between the electrodes ........................................................................... 66

Figure 3.3 Schematic of the conductivity cell .................................................................................. 71

Figure 3.4 Schematic of the μ-RP-HPLC system ........................................................................... 74

Figure 3.5 Slab gel electropherogram of (A) 25 cycle and (B) 30 cycle PCR mixes run in parallel with a low DNA mass sizing ladder ........................................................................ 76

Figure 3.6 Calibration plots for KCl (•) and DNA (Δ).................................................................. 77

Figure 3.7 Chromatograms of (A) a standard low mass DNA ladder, (B) 25 cycles and (C) 30 cycles, 500 bp PCR product separated by μ-RP-HPLC .......................................................... 81

Figure 4.1 Volume flow rate (solid lines, closed symbols) and pressure drop (dashed lines, open symbols) as a function of linear flow velocity .......................................................... 89

Figure 4.2 Schematic representation of the piezo-driven micro-syringe pump .................................. 92

Figure 4.3 (A) Operational modes of diffuser/nozzle system. The arrows represent the direction of fluid flow and the magnitude of fluid flow through the device during operation. (B) Scanning electron micrograph of the diffuser/nozzle system micromachined in PMMA ............................................................................. 98

Figure 4.4 Linear displacement of piezo-head, non-amplified (squares) and amplified (circles), versus applied voltage ..................... 102

Figure 4.5 Volume flow rate versus applied voltage to the amplified PA head ................................ 104
Figure 4.6 (A) Volume flow rate as a function of ramp speed (Vs⁻¹) for 20 μm id tubes of various lengths. The lengths of the capillary tubes were changed in 0.3 m increments so as to alter the load pressure on the PA head. (B) Volume flow rate versus load pressure at five different ramp speeds (Vs⁻¹). ........................................ 107

Figure 4.7 Pump stability at three different volume flow rates ......................... 110

Figure 5.1 Electroosmotic profiles for PMMA and fused silica taken as a function of the buffer pH .............................................................................. 117

Figure 5.2 Contact angle measurements of unmodified and amine terminated PMMA ........................................................................ 130

Figure 5.3 Laser-induced fluorescence confocal microscope images of (A) unmodified PMMA and (B) amine-terminated PMMA labeled with FITC ........................................................................ 131

Figure 5.4 Capillary electrophoresis (CE) separations of (A) a free solution and (B) a PMMA immobilized enzyme digest of φX174-RF DNA (0.025 μg/μl) ........................................................................ 132

Figure 5.5 Fluorescence scan of DNA hybridization ........................................ 136

Figure 6.1 Topographical layout of the μ-CE chip .............................................. 147

Figure 6.2 Optical micrograph of the conductivity cell ..................................... 148

Figure 6.3 Scanning electron micrographs of the Ni mold insert ....................... 151

Figure 6.4 Scanning electron micrographs of the hot embossed PMMA ............ 153

Figure 6.5 Calibration plot for KCl. The plots was constructed by pressure pumping KCl through the conductivity cell averaging the signal over three runs ........................................ 156

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Abstract

The research presented in this document is focused on the development and characterization of novel microfluidic devices using X-ray photolithography in poly(methyl methacrylate) (PMMA). A conductivity detector cell was developed and characterized as a sensitive detector for DNA and KCl using flow injection, then was integrated into a high-performance liquid chromatography system for the purification of PCR amplicons. A planar conductivity cell was then developed on a microchip and its detection sensitivity was characterized by pressure pumping KCl through the detector and monitoring the change in conductivity. Also, the topography of the device was characterized using scanning electron and optical microscopy images.

A novel computer-controlled mechanical syringe-pump is described which uses a piezoelectric actuator and a pivoted lever for amplification of the linear displacement of the piezo to deliver solvents free from pump pulsations at volumetric flow rates approaching 1 nanoliter per minute even at high loading levels (high output pressures). The piezo-pump was found to adequately deliver stable flow of solutions with loading pressures as high as $3.79 \times 10^5$ Pa (actual loading pressure at the piezo $= 3.41 \times 10^6$ Pa). Monitoring the flow stability using fluorescence indicated that the volume flow was fairly noise-free at pumping rates from 4-150 nL/min. A diffuser/nozzle system was fabricated which allowed automatic refilling of the syringe pump and was micromachined into PMMA using
X-ray lithography. The diffuser/nozzle system contained channels that were 50 µm in depth and tapered from 300 to 30 µm.

The modification of a PMMA surface was demonstrated and characterized by monitoring the contact angles of water and laser-induced fluorescence confocal microscopy. The surface was shown to be amenable to attachment of biomolecules. A Hae III restriction enzyme was immobilized on the surface of the PMMA and used for solid phase DNA digestion without affecting the activity of the enzyme. Also, 35-mer single-stranded oligonucleotides were immobilized to PMMA and hybridization experiments were performed which demonstrate the utility of the PMMA substrate for DNA microarrays applications.
Chapter 1

Microchip Capillary Electrophoresis Devices

1.1 Introduction

Capillary electrophoresis on microchips is emerging as a technology that will revolutionize chemical analysis. Channels can be patterned into substrates in parallel arrays and analysis times can be reduced with the application of higher separation field strengths, which should yield ultrahigh throughput for microchip capillary electrophoresis (μ-CE) devices. Also, these devices have the potential for sample preparation and analysis on one platform, additionally reducing reagent consumption which can be on the order of picoliters for μ-CE devices.

Microchip capillary electrophoresis devices stem from the concept of “miniaturized total analysis systems” (μ-TAS), which was first coined by Manz in 1990 [1]. Although in the 70s, the first instrument on-a-chip was developed by Terry et. al. [2], which consisted of a gas chromatographic system integrated on a silicon wafer. Integrated into the device was a sample injection valve and a 1.5 m long capillary separation column. The 200 μm wide column was isotropically etched to a depth of 30 μm and was covered and hermetically sealed with a Pyrex glass cover plate. A thermal conductivity detector (TCD) based on a nickel film resistor was fabricated on a separate silicon wafer and was later clamped to the column wafer. Unfortunately, the device did not generate much interest among other researchers since capillary GC was still a relatively new technique and researchers lacked the technological experience required to use such a device. Since
the introduction of the concept of $\mu$-TAS, there has been an avalanche of developments and discoveries that has triggered growth both in academic research and on a commercial level.

$\mu$-CE device fabrication technologies were developed in the semiconductor industry where the initial devices were fabricated on a silicon wafer using standard photolithography and subsequent wet chemical etching to produce microchannels for sample injection and capillary electrophoresis separation [3]. Electrokinetic pumping is typically used to transfer samples through the microchannels, with the magnitude of the pumping determined by the type of substrate material used. Glass [4-9], quartz [10-18], or plastics [19-28] can support electropumping with silicon proving to be problematic because of dielectric breakdown of the material when high field strengths are used [29-31]. Because standard microfabrication technology is used, mass production of microchip devices should be economically feasible. Although, factors for inexpensive production of microstructures such as; (1) cost of substrate material, (2) fabrication steps and (3) surface chemistry of the substrate need to be considered.

Since most $\mu$-CE devices have a comparatively large footprint, on the order of several cm$^2$, the cost of substrate material can become an important factor for high production. Polymers possess a major advantage in cost compared to typical silicate glasses. Poly(methyl methacrylate) (PMMA), for example, costs between 0.2 to 2 cents per cm$^2$ whereas the cost of silicate glasses can range from 10 to 40 cents per cm$^2$ depending on the grade of glass.
The microfabrication of the device can impart a significant influence on the cost as well. During the microlithography process required to develop the devices, many steps (cleaning, resist coating, exposure, development, etching) are involved. Many of these steps can be hazardous and costly (e.g. HF etching) when these devices must be fabricated using these steps serially as in the case of glass devices. Many times these steps can be eliminated by using plastic substrates where different molding technologies can be utilized to easily fabricate devices.

The surface chemistry of materials can pose problems when developing microfluidic devices as well. Many separations (e.g. DNA separations) must be performed using a sieving matrix, which can extrude from the channel if a significant electroosmotic flow (EOF) is present. Also, the EOF in glass channels has been shown to be greater than the electrophoretic mobility of DNA and thus needs to be reduced or eliminated for efficient separations to occur [32]. Additional steps must then be taken to reduce this EOF, such as surface coating with a silanizing reagent and attachment of a polymer overcoat, which typically reduces the usable life of the device because of degradation of the polymer overcoat with each electrophoresis run [32]. Whereas plastic devices have been shown to produce a significantly reduced EOF [24] and no surface treatment is necessary before performing the separation. This would not only increase the throughput of the completed device but also increase the usable life of the devices since there would be no coating to degrade over time.
In this chapter, a review of the current technologies used to develop \( \mu \)-CE devices is given and intended to give the reader a basic understanding of these processes. In addition, techniques used to characterize these devices in addition to fluidic pumping in \( \mu \)-CE devices will be discussed briefly.

## 1.2 Current Technologies for Developing \( \mu \)-CE Devices

The process of micromachining \( \mu \)-CE devices requires four or five general processing steps depending on the substrate chosen. In Figure 1.1 is shown a flow chart of these processes. Initially a film deposition process is necessary to deposit thin layers of metals or resists. Once a resist has been deposited, exposure of the resist (photolithography) is used to transfer a computer generated image of the pattern to the substrate. After the exposed resist is developed (removed), etching of the substrate is carried out to create a two-dimensional pattern in the substrate. For glass substrates, the device is assembled by thermally annealing a top plate, while for plastic materials a mold insert can be created, which can be used to effectively produce the devices using molding technologies before device assembly occurs.

### 1.2.1 Film Deposition

Film deposition processes include spin coating, thermal oxidation, physical vapor deposition (PVD) or chemical vapor deposition (CVD), whereas the most common forms used in micromachining are spin coating and PVD. Depending on the method of choice and the film being deposited, film thicknesses of a few
Figure 1.1 Flow chart demonstrating the micromachining processes involved in developing \( \mu \)-CE devices.
nanometers to a few micrometers can be observed. These films can consist of metals, inorganic oxides, and various polymeric materials.

Spin coating is a method that is commonly used to apply photoresists on planar substrates. The resists are applied by dropping the resist solution on the wafer, or substrate, and rotating the wafer on a spinning wheel at high speeds so that centrifugal forces push the excess solution off of the wafer and a thin film of about 10 $\mu$m thickness remains due to surface tension. The thickness of the film is related to the solution viscosity and rotation speed, which can be determined empirically.

PVD reactors may use a solid, liquid, or vapor raw material for deposition onto a substrate. Two of the most common forms of PVD are evaporation and sputtering. Sputtering is the preferred PVD method for several reasons, including a wider choice of target materials, better step coverage and adhesion to the substrate. Typically, sputtering consists of holding the target material (the material to be deposited) at a high negative potential and bombarding it with positive argon ions. The target material is sputtered away as neutral atoms by momentum transfer and condensed or deposited onto the substrate material placed at the anode in a vacuum. The amount of material sputtered, $W$, from the cathode is inversely proportional to the gas pressure, $P_T$, and the distance (d) between the anode-cathode interfaces;

$$W = \frac{kVi}{P_Td}$$  \hspace{1cm} (1.1)

with $V$ being the applied voltage, $k$ is a proportionality constant and $i$ the discharge current. The adhesion of sputtered films has been shown to be superior compared to
other deposition methods [33]. The main reason for the increased adhesion in sputtering is that the ejection energies of ions from the target are high ranging from 10 to 100 eV, allowing the ions to penetrate a substrate one to two atomic layers. One drawback to sputtering is the deposition rate. A typical deposition rate for sputtering is one atom layer per second, whereas evaporation rates of a thousand atomic layers per second are normally obtainable.

Thermal evaporation is a technique which is also used to apply thin films to wafers. It is based on the boiling off (or sublimating) of a heated material onto a substrate under vacuum. Typically, a metal is evaporated by passing a high current through a metal containment structure (e.g., a tungsten boat or filament) while the substrate to be coated is positioned above the containment structure. Evaporation rates of a few thousand atomic layers per second (≈ 0.1 to 1.0 μm/min) are commonly achieved, which is source dependent. Also, evaporation is able to deposit films of extreme purity and known structure, which can be of prime importance when developing electrochemical sensors.

1.2.2 Photoresist Processing (Photolithography)

Photolithography is the technique by which a computer generated pattern is transferred to a photosensitive material, called a photoresist, using a light source. Typically, for structures larger than 1 μm, visible light can be used to transfer the pattern. For submicron patterning, UV, X-ray, or e-beam photolithography is used. Ultimately, the parameter that limits the size of the structure is the wavelength of the light source used to transfer the pattern to the substrate. The resolution of the
projection system used to transfer the image to the resist is limited by optical diffraction, according to the Rayleigh equation;

\[ R = k_1 \lambda / NA \]  

(1.2)

where \( \lambda \) is the wavelength of the illuminating source, NA is the numerical aperture of the lens system and \( k_1 \) an empirically determined constant that depends on the photoresist sensitivity to the light used. The minimum feature size that can be obtained is typically determined by the wavelength (\( \lambda \)) of light that is used, although the theoretical limit set by optical diffraction is normally \( \lambda / 2 \). As a result, illuminating sources with shorter wavelengths such as soft X-rays (6 – 40 nm) are becoming more popular as feature sizes become increasingly smaller.

1.2.2.1 Photoresists

Photoresists are polymer materials that react with certain types of radiation to transfer images to substrates depending on the pattern of the optical mask. Regardless of the exposure method, there are certain criteria that a resist must possess to be viable for microfabrication, i.e., the ability to form good films, chemical and storage stability, fine feature resolution, significant property changes upon irradiation to facilitate development, and radiation sensitivity. A viable resist must exhibit excellent film formation after spinning, good development properties and sufficient thermal properties (i.e. high glass transition temperature, \( T_g \)) to facilitate small feature development. Polymers above their \( T_g \) are described as flexible, while those below there \( T_g \) are rigid. The effect of the \( T_g \) of the resist on feature aspect ratios is shown in Figure 1.2. The photoresist should have a \( T_g \) which
is higher than the typical post-processing temperatures to obtain structures with high aspect ratios. Low Tg resists tend to flow during high temperature processing steps and lose feature integrity. Photoresists are usually termed positive tone, where the solubility of the irradiated area increases, or negative tone, where the solubility decreases upon exposure. The coating characteristics and the developmental characteristics can normally be obtained through empirical studies and can be easily determined as long as the polymer exhibits a definite change in solubilities before and after irradiation. For example, in deep X-ray lithography, PMMA is used as the standard resist and acts as a positive tone resist. The photochemical reaction of PMMA is depicted in Figure 1.3. Upon interaction of the polymer with a photon, a main-chain radical is produced which undergoes rearrangement through β-scission to cleave the chain and generate an acyl-stabilized, tertiary radical where carbon monoxide, carbon dioxide and methyl and methoxyl radicals are generated. The cleavage of the chain decreases the molecular weight of the polymer, increasing its solubility in conventional developers.

1.2.3 Etching Processes

Etching entails transferring two-dimensional patterns defined on the wafer to either underlying films or the substrate itself. Etching processes are classified as wet or dry. Wet etching has a specificity for the substrate and not other films or coatings on the substrate, while dry etching is specified as those processes that are carried out in partially or fully ionized gases or X-rays synchrotron sources. Wet chemical etching provides a higher degree of selectivity than do dry
Figure 1.2 Post processing effects on resists as a function of $T_g$. The aspect ratio (ratio of height of structure to the width of the structure) is shown to decrease because of the fluidic nature of polymers when heated above their $T_g$. 

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Figure 1.3 Representation of interaction of a chain scission type polymer with a photon of light. The mechanism of the photoreaction of PMMA with X-rays is shown. The soft X-rays (7–8 Å) interact with the polymer through bond breakage in the polymer backbone creating a radical rearrangement in the main chain and generating several radical fragments. Taken from [34].

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etching techniques. Also, wet etching is normally faster than dry, with approximately several tens of microns etch depth per minute for isotropic etchants compared to 0.1 μm/min for dry etching techniques.

1.2.3.1 Wet Chemical Etching

μ-CE devices are commonly fabricated in glass substrates [4,5,7,8,10,12,13,15-17,35-72] because of the good optical properties and well-understood surface characteristics of glass. Microstructures on glass are normally fashioned using microfabrication procedures shown in Figure 1.4 [8,65,73,74]. Initially, a sacrificial layer of photoresist is spin-coated onto the glass substrate. Then, the pattern to be etched is transferred to the photoresist by UV exposure through an optical mask, which has an image of the pattern on it. The substrate is then baked at a temperature above the Tg of the photoresist to harden the exposed resist (positive photoresist). The unexposed photoresist is then dissolved with a developer solution revealing the glass substrate in the design of the transferred pattern. The glass substrate is now ready to be chemically etched (wet etching).

Hydrofluoric acid (HF) is the etchant that is typically used for glass substrates and can be prepared in solutions of HF/NH₄F, HF/HNO₃ and concentrated HF. The etching rate can be easily controlled by controlling the etching temperature and the HF concentration, which also allows for very reproducible structures. The rate can be monitored using a profilometer. A major disadvantage of wet chemical etching
Figure 1.4 Microfabrication process for glass μ-CE devices. Taken from [75]

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in glass substrates is the isotropic nature of the etch. Since the etch rate is the same horizontally as it is vertically, the features are usually very rounded and as such, high aspect ratio structures cannot be created using this method.

1.2.3.2 Dry Etching

1.2.3.2.1 Reactive Ion Etching

Reactive ion etching (RIE) is one of the most common forms of dry etching used in microfabrication of silicon-based devices. In this process (Figure 1.5), a plasma is produced between two electrodes using radio frequency gas discharge in a pressurized chamber (≤ 10 Pa). The silicon substrate is placed in contact with one of the electrodes and an ion sheath is formed at both surfaces because of the large mobility difference between electrons and ions in an electric field. When a potential is applied between the electrodes, the ions in the ion sheath are accelerated toward the substrate creating an etching reaction. Silicon is etched by RIE using a fluorine containing gaseous compound, such as carbon tetrafluoride (CF₄). The addition of oxygen to the composition increases the F⁺ concentration, which subsequently increases the etch rate of the process.

Manz and co-workers have used RIE to etch channels in μ-CE devices [10]. The researchers performed RIE with chlorine ions using an Applied Materials AME 8100 dry etching system. The etching rates were reported to be between 14 nm/min and 39 nm/min depending on the position of the substrate in the reaction chamber. Also, an aspect ratio, the ratio between the height and width of the structures, of about 5 was reported for their devices.
Figure 1.5 Schematic of an apparatus to perform reactive ion etching.
1.2.3.2.2 Laser Photoablation

Laser ablation is another common dry etching technique that is used for the fabrication of $\mu$-CE devices [20,21,76]. Laser ablation entails absorption of a high energy laser pulse to break covalent bonds in long chain polymers, which subsequently produces a shock wave that ejects decomposed polymer fragments from the ablated area (see Figure 1.6). Laser ablation sources normally consist of an eximer laser (ArF (193 nm), KrF (248 nm) to produce pulses at frequencies between 10 Hz and several kHz. Penetration depths depend upon the energy of the laser but are typically between a few hundred nanometers to 5 $\mu$m per laser pulse. Laser ablation can be used on several types of rigid polymeric materials including PMMA, polystyrene (PS), poly(carbonate) (PC), poly(ethylene terephthalate) (PET), cellulose acetate, and polyimide. Although, due to the longer wavelength of the radiation used, the feature size is limited compared to synchrotron X-ray etching. Roberts et al. reported the use of laser photoablation to develop $\mu$-CE devices [21]. The researchers used an excimer laser to project UV radiation (200 mJ/pulse at 193 nm, pulse rate = 10 – 50 Hz) through a 10:1 telescopic objective, then onto the polymer substrate. By moving the substrate under the excimer laser at a rate of 0.2 mm/s, channels with dimensions of 40 $\mu$m wide x 37 $\mu$m deep could be fabricated.

1.2.3.2.3 X-ray Synchrotron Lithography (LIGA)

*LIGA* is a German acronym for X-ray lithography (lithographie), electroplating (galvanoforming), and molding (abformtechnik). This process was
Figure 1.6 Processing steps used in UV laser ablation of polymeric devices. Taken from [21].
first introduced in 1975 by researchers at IBM to create very high aspect ratio features [77]. LIGA is a process which uses synchrotron radiation for photolithographic development of an X-ray resist, followed by electroplating to form a mold insert to replicate the devices inexpensively. X-ray lithography uses a synchrotron source to produce x-rays which interact with resists at high energies to form channels by degrading the polymer through bond breakage in the polymer backbone (Figure 1.3). The wavelength of the radiation is in the soft X-ray region, <1 nm [34]. Using X-ray synchrotron sources is advantageous over most other optical sources because of the reduced exposure time and limited geometrical distortion of the light source. These advantages stem from the fact that in synchrotron sources, the intensity of the flux is large and the radiation is collimated vertically and its divergence is small [34]. Additionally, synchrotron sources alleviate many of the problems associated with UV exposure methods simply because the wavelengths of X-rays are smaller than those of UV sources. Thus smaller features can be produced since effects from diffraction are limited according to the Rayleigh condition. Also, by using PMMA as a resist during X-ray lithography, very high aspect ratio structures can be formed. This is due to the fact that PMMA is a very rigid polymer with a $T_g$ that is higher than the post development process temperatures required in X-ray lithography (see Figure 1.2).

A schematic of the LIGA process is shown in Figure 1.7. An X-ray mask is created from an optical mask using optical proximity printing and the feature dimensions are defined by the optical resolution of the optical mask, which are typically on the
order of 2 - 5 μm but can be < 1 μm when using e-beam writing. Initially, a layer of deep UV resist is spin-coated onto an appropriate conductive substrate that is reasonably transparent to X-rays (i.e. a Kapton membrane coated with a gold plating base or a graphite wafer). The channel design is transferred to the deep UV resist by UV exposure through a standard optical quartz-chrome mask. The resist is then developed and, after a post bake to harden the exposed resist, X-ray absorber structures are fabricated by electroplating gold structures 5 - 10 μm in height onto the conducting areas of the substrate (i.e. areas where resist has been removed). A mold template is then made by exposing PMMA which has been annealed to a conducting substrate (e.g. titanium or stainless steel) through the X-ray mask. The exposed areas of the PMMA are then dissolved away (developed) to yield channels, where the depth of the channel is a function of the exposure time and the X-ray beam energy. Nickel is then electroplated in the channels and overplated to form a mold, which is a mirror image of the μ-CE device. High aspect ratio polymeric devices can then be created using a molding technique, such as injection molding or hot embossing [78,79].

In some cases a transfer mask maybe used to create single devices from X-ray exposure. In this case, the PMMA substrate is made conductive by applying a plating base (Au and Cr) directly on its surface. The resist is spun on top of the plating base and, at this point, the process is identical to that of developing the X-ray mask. After exposure and development of the substrate, the plating base is then simply peeled away leaving a PMMA substrate. The obvious disadvantage of this
Figure 1.7 Schematic representation of *LIGA* micromachining process.
process is that only one device is created for one exposure making this process very expensive and time consuming.

1.2.4 Device Molding

In order for devices to be fabricated using LIGA, a viable method for economically efficient replication needs to be used without remaking the primary structure. For molding plastics, a metal structure is formed by electroforming and serves as a mold insert which can be used successively without reverting back to direct optical exposure. Usually one of the four following molding methods is used to mass produce plastic devices: hot embossing, thermoplastic injection molding, casting or imprinting.

1.2.4.1 Hot Embossing

Hot embossing is currently the most widely used process for fabrication of polymer microfluidic devices [19,80,81]. The main reason being that it is such a simple and straightforward process. In Figure 1.8 is shown a schematic of the apparatus used for hot embossing. After a master mold is fabricated, it is placed in an alignment jig in the embossing system opposite the planar polymer substrate. The mold and the substrate are then heated in a vacuum chamber to a temperature above the $T_g$ of the polymer. The vacuum is used to prevent air bubbles from being trapped in small cavities causing irregularities in the molded substrate and to allow water vapor to be driven off. The mold is brought into contact with the polymer under a controlled force, which is typically several kN. Finally, the device is cooled below the $T_g$ while still applying the force. The force is then released and the
pattern is transferred to the polymeric substrate. The total temperature cycle should be minimized to decrease thermal-induced stresses in the material. Typical cycle times with PMMA as the substrate are 5 to 7 minutes.

Locascio and co-workers have described a method to hot emboss PMMA μ-CE devices from a silicon master [19]. A silicon master was etched resulting in raised channel structures. The PMMA and silicon master were placed in a heated press at 135°C for 5 min and the raised structure was transferred into the PMMA as an embossed channel. Using this method, the researchers reported that the channel dimensions were very reproducible with standard deviations being 2, 6, and 13 % for the depth, width at bottom of channel, and width at top of channel, respectively [19].

1.2.4.2 Thermoplastic Injection Molding

Thermoplastic injection molding (TIM) is the process of heating the polymer to a viscous free flowing form before introducing it to the mold and then hardening occurs by slowly cooling the insert. Figure 1.9 shows the cross section of an injection molding machine with the processing steps labeled. The process usually entails feeding the granular form of the polymer into a cylinder, which contains a heated screw. The polymer pellets start to melt at the screw and are injected into the mold insert using a hydraulic pump. The free-flowing polymer is then cooled below its $T_g$ to form the plastic device. As the pattern features become smaller, the polymer must be heated to its melting point to allow flow into the small
Figure 1.8 Diagram of a hot embossing machine used to micromachine polymeric devices.
structures of the mold insert. Because of the large temperature gradient involved in this process, thermal shrinkage can occur, which degrades the pattern features in the mold and substrate.

McCormick et al. [82] used injection molding to produce \( \mu \)-CE devices in an acrylic copolymer resin. A nickel mold was produced from a wet chemically etched silicon master. Subsequently, nickel "daughter" molds were produced from the nickel mold to injection mold devices. The feature topography of the molded devices were characterized by profilometer studies and showed low aspect ratios, which were due mainly to the isotropic wet chemical etch used to fabricate the silicon master. Although the features were degraded, the authors were able to mass produce \( \mu \)-CE devices inexpensively using this method.

1.2.4.3 Casting

Casting has found widespread use in the fabrication of \( \mu \)-CE devices because of its simplicity and low cost [26,27,83]. Casting is the process in which a polymer is poured over the molder and then cured. The polymer usually requires contact with the mold for several hours making this process not very amenable to mass production on a commercial level. The first use of casting to miniaturize a CE device was reported by Ekström et al. [84]. The researchers used a cast silicon rubber to form the microchannels and placed this between two glass plates for mechanical support and channel sealing. Recently, Whitesides and co-workers have demonstrated the fabrication of \( \mu \)m structures by casting poly(dimethyl siloxane)
Figure 1.9 Schematic representation of a machine for injection molding of plastics. Taken from [81].
The researchers created an elastomeric master by casting PDMS over a relief structure that was created using photolithography. One end of the elastomer master was cut and the master placed on a solid support. A drop of prepolymer was placed at the open end of the master and the channels filled in the master through capillary action. Once the channels were filled, the prepolymer was cured and the PDMS master removed. The cured polymer was then removed from the substrate with tweezers or by dissolving away the support.

### 1.2.4.4 Imprinting

Imprinting consists of using a material with a high tensile strength (e.g. metals) to transfer channels into a substrate of lesser tensile strength (e.g. polymers). This normally occurs by using a metal filament, such as a tungsten wire (diameter $\approx 10 - 100 \ \mu$m), which is placed between two pieces of plastic material and applying a force to the plastic pieces at a temperature above the $T_g$ of the plastic. The shape of the wire is transferred, or imprinted, into the material.

The main advantages of imprinting is that the equipment required is low cost compared to other microfabrication techniques and it can be accomplished in most analytical laboratories. While imprinting is a simple and economical technique, this method is constrained to simple channel designs, can only produce a few devices at a time and the channels suffer from low aspect ratios when compared to other micromanufacturing techniques [24,25,28].

Locascio et al. [19] described a wire-imprinting method in which two wires were used to create cross channels on two separate substrates. The researchers used
a Chromel wire with diameters ranging from 13 to 25 µm to imprint channels in PMMA. The method proved to be reproducible, but was constrained to simple linear channel designs. Chen et al. reported the use of wire-imprinting to create µ-CE devices in PMMA [22]. The authors used two 79 µm Chromel wires to produce channels with rounded dimensions and depths of ≈75 µm. The channel variations were reported to be less than 6% on different sites of the same chip and less than 10% from chip to chip using profilometer data [19].

1.2.5 Device Assembly

Bonding refers to the assembly of devices to form enclosed channels. Bonding usually leads to fusion of two planar substrates and a perfectly tight seal. Several techniques have been reported in the literature for bonding glass [47,65,85,86] and polymeric µ-CE devices [19-28]. The process typically involves the application of heat and pressure at controlled rates. In polymer devices, care must be taken not to damage the structures by the addition of pressure.

Glass substrates normally require high temperatures (≈ 600°C) over several hours for adequate bonding. Ramsey et al. [46] have reported a direct bonding technique for glass substrates. The process entailed hydrolyzing both the chip and the cover slip, then annealing them at 500°C. Polymer-based devices are typically bonded at the T_g of the material with the addition of a small amount of pressure (≈ 30 – 50 N/cm²) for short periods of time (≈ 8 – 10 min.). Although, some polymer materials can be sealed by exposure to a plasma to form covalent siloxane bonds [26]. Whitesides and co-workers [26] subjected a device fabricated in PDMS to a
plasma discharge source, which oxidized the surface of the device, most likely converting the surface $-\text{OSi(CH}_3\text{)}_2\text{O-}$ groups to $-\text{O}_n\text{Si(OH)}_{4-n}$. Once the surfaces were oxidized, they were placed in intimate contact, which the authors stated created a hermetically sealed device. Ford et al. [25] reported the development of $\mu$-CE devices fabricated in PMMA. They used a thermal bonding technique to hermetically seal a top plate of PMMA to an etched bottom plate of PMMA. The method consisted of heating both top and bottom on a hot plate to a temperature of 150°C for 5 min. The PMMA plates were then placed in an alignment jig (for alignment of buffer reservoirs) and placing a 50 lb copper weight on the plates. The entire assembly was then placed in a furnace at 180°C then immediately allowed to slowly cool to room temperature over a 2 hour period.

1.3 Topographical Characterization Tools

A very important aspect of micromachining $\mu$-CE devices is topography characterization. By assessing the device topography, information can be obtained about surface roughness and channel dimensions. Also, when developing devices it is imperative to know the coating thickness of metal or resist layers so that microlithography can be performed. Two of the most common techniques for topographical characterization are the profilometer and the scanning electron microscope (SEM).

The profilometer is a stylus instrument that is used to obtain a 2-dimensional (2-D) and sometimes 3-D topographical image of a planar surface. The components of profilometer system typically consist of a stylus (1 – 30 $\mu$m dia.), which is
physically attached to an optical or piezo-electric transducer or a linear variable differential transformer (LVDT) and is used to convert the vertical movement to an electrical signal which is amplified and recorded. The resolution of a profile system is mainly dependent on the stylus tip size, but also somewhat dependent on the A/D converter used when converting the electrical signal to digital. Typically, the vertical resolution is around 100 Å.

The SEM is an optical instrument which is used to obtain 2-D and 3-D images of devices. The SEM consists basically of three groups of components: (1) electron optical column, (2) vacuum system and (3) image display unit. The electron optical column consists of an electron gun, two or three magnetic lenses and two set of scanning coils. The electron gun emits an electron beam, which flows through the lenses and is focused onto the specimen surface. The scanning coils are placed in front of the final lens and cause the electron spot to be scanned across the substrate in a square raster fashion. The current that passes through the scanning coils are made to pass through deflection coils of a cathode ray tube (CRT) which produce an image on a screen. One disadvantage of using a SEM is that the substrate must be conductive. This usually requires sputtering a metal onto microdevices to ensure they are conductive. Typically, only 2-D qualitative information is obtained when using a SEM, while special 3-D quantitative techniques have been developed but not widely used [87,88]. Current commercial systems can acquire spatial resolutions of several nanometers, which is usually adequate for imaging microdevices with typical dimensions of a few microns.
1.4 Fluid Pumping in \( \mu \)-CE Devices

Fluid pumping in \( \mu \)-CE devices is typically performed through electrokinetic force, in which the intrinsic electroosmotic flow (EOF) generated by the surface charges of the substrate causes bulk flow of solvent. Positively charged ions in the buffer solution build near the wall forming what is called the electrical double layer and in an electric field, the positively charged layer is attracted toward the cathode causing a bulk flow of solvent toward the cathode. The potential difference in the double layer is referred to as the zeta (\( \zeta \)) potential. The EOF can be expressed through the equation:

\[
\nu_{EOF} = \frac{\varepsilon \zeta}{\eta} E, \tag{1.3}
\]

or

\[
\mu_{EOF} = \frac{\varepsilon \zeta}{\eta} \tag{1.4}
\]

where \( \nu_{EOF} \) is the velocity of the EOF (cm/s), \( \mu_{EOF} \) is EOF mobility (cm\(^2\)/Vs), \( \zeta \) is the zeta potential, \( \varepsilon \) is the dielectric constant of the solvent (C\(^2\)/Jcm) and \( \eta \) is the viscosity (Nsec/m). The \( \zeta \) is a function of the surface charge on the wall, the pH and the ionic strength of the buffer. The EOF has been very well characterized in glass and has been found to be approximately \( 9.24 \times 10^{-4} \) cm\(^2\)/Vs at very basic pH values (pH > 9.0) in a 10 mM borax buffer [89]. This EOF results from the high surface charges found on glass due to deprotonation of the silanol groups at basic pH values creating a negative charge along the walls and an EOF flow toward the
cathode and also creating a pH dependent EOF. Although, many polymers do not contain ionizable groups on the surface and have been shown to produce a much smaller EOF [23,24].

Ford et al. monitored the EOF in PMMA which had been machined through X-ray lithography and determined the EOF to be $1.2 \times 10^{-4} \text{ cm}^2/\text{Vs}$, with the direction of the EOF toward the cathode [24]. Additionally, they found the EOF to be independent of pH over a range of 3 to 11, which is in sharp contrast to the pH dependent EOF found in glass or fused silica.

Rossier and co-workers monitored the EOF on several UV photoablated polymers (PS, PC, cellulose acetate, and PET) [21]. The authors reported values between $2.31$ and $5.79 \times 10^{-4} \text{ cm}^2/\text{Vs}$, which was comparable to the EOF observed in glass or fused-silica substrates. The high EOF rates were attributed to the possibility of the incorporation of oxygen or nitrogen decomposition products when performing UV photoablation in an ambient atmosphere.

Locascio et al. has recently monitored the EOF in imprinted polystyrene fabricated microchannels [23]. They reported an EOF value of $1.8 \times 10^{-4} \text{ cm}^2/\text{Vs}$ and observed that the addition of different types of materials to the channel walls of the device can affect the EOF. For example, the authors observed that when the channels were filled with a protein, goat IgG antibodies, the EOF was reduced to $2.5 \times 10^{-5} \text{ cm}^2/\text{Vs}$, which is significantly lower than the EOF observed from the native polymer. The suppression of the EOF was attributed to adsorption of the protein to the walls of the microdevice.

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Additionally, Whitesides and co-workers have demonstrated EOF in PDMS microdevices [26]. Native PDMS exhibits a very small EOF value, while subjecting the PDMS device to oxidation in a plasma discharge changes the surface charge of the device, most likely converting the surface $-\text{OSi(CH}_3\text{)}_2\text{O}-$ groups to $-\text{O}_n\text{Si(OH)}_{4-n}$ and producing a substantially larger EOF.

1.5 References


Chapter 2
Detection Strategies for Micro-Capillary Electrophoresis

2.1 Introduction

Detection is a major concern in microelectrophoresis devices due to the reduced dimensions (typically 20 μm x 50 μm) associated with the planar integrated columns which results in ultra-small detection volumes. In order to obtain high separation efficiencies and minimize zone broadening, detection is preferably accomplished on column which can create challenges when designing a detection method for these devices. The contributions to total variance ($\sigma^2_{TOT}$) from detectors in capillary electrophoresis is given by the following:

$$\sigma^2_{DET} = \frac{l_d^2}{12}$$

(2.1)

where $l_d$ is the length of the detector cell. In microdevices, due to the reduced column dimensions, the detector cell length is reduced thus minimizing the contribution to total variance in a separation system allowing for more efficient separations.

The choice of detector is generally dependent on the analyte being investigated. The optimum detector should be nondestructive, selective, sensitive, cost efficient, responsive, produce a low detection limit and easily integrated into the microdevice. A number of different detection schemes have been explored in microdevices because of the large amount of variables involved in determining the proper detector for a system. Table 2.1 lists the various detection strategies that have been used in
conjunction with microelectrophoresis devices along with their typical detection
limits. As shown in the table, fluorescence detection is the most sensitive detection
scheme currently for μ-CE devices with detection limits reported to be ≈5.0 zmol
[1]. Also, fluorescence detection has been the most widely used scheme as
compared to traditional capillary electrophoresis where UV absorption is the most
popular. This is due to the fact that reducing the column dimensions reduces the
pathlength which has a direct effect on the sensitivity in UV absorbance detectors.
Another trend that is evident from the table is that the detectors that are more
universal, tend to yield the poorest detection sensitivity. For example, refractive
index detection is the most universal detection scheme, but gives the poorest
detection limits listed among the detection strategies.

This chapter will introduce the reader to the various detection schemes that have
been utilized in microelectrophoresis devices. A general theoretical description of
the technique will be discussed plus design considerations and current instrumental
approaches to microelectrophoresis detection implementing each method. This
chapter will be restricted to only those detection schemes, which have been
implemented in μ-CE devices.

2.2 Optical Detection Strategies

2.2.1 UV Absorbance Detection

UV detection is one of the most widely used detection techniques in analytical
chemistry. One of the main reasons being that most analytes possess a chromophore
that permits detection without the need for chemical derivatization prior to the
Table 2.1 Detection limits of various detection strategies applied in μ-CE

<table>
<thead>
<tr>
<th>Detection Strategy</th>
<th>Mass Detection Limit (amol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractive Index</td>
<td>$6.0 \times 10^5$ [2]</td>
</tr>
<tr>
<td>UV absorption</td>
<td>$7.2 \times 10^5$ [3]</td>
</tr>
<tr>
<td>Fluorescence$^{c,d}$</td>
<td>$5.0 \times 10^3$ [1]</td>
</tr>
<tr>
<td>Raman$^a$</td>
<td>$4.6 \times 10^2$ [4]$^a$</td>
</tr>
<tr>
<td>Electrochemical (amperometric)$^c$</td>
<td>$6.7 \times 10^1$ [5]</td>
</tr>
</tbody>
</table>

a Calculated based upon a 200 pL injection volume  
b Calculated based upon a 120 pL injection volume  
c Calculated based upon a 20 pL injection volume  
d Calculated based upon 300 bp ddDNA

This fact can also be detrimental because selectivity is lost when many components absorb UV radiation and often times can interfere or mask the component being analyzed. One of the principle problems associated with UV detection in small bore channels is that the optical path length is exceedingly small (ca. 50 μm) which inherently decreases the detection sensitivity for UV absorbance.

In UV detection, photons from a light source impinge upon a sample and, if the energy of the photon corresponds to the energy difference between electronic levels of the molecule, the energy of the photon is absorbed and promotes the electron from the ground state ($S_0$) to an excited electronic state ($S_1$). The analytical signal,
absorption (A), can be determined through the use of the classical Beer-Lambert law;

\[ A = \varepsilon b c \]  

(2.2)

where \( \varepsilon \) is the molar extinction coefficient, \( b \) refers to the optical path length and \( c \) is the concentration of the absorbing species.

Absorbance detectors can be used in micro-electrophoresis applications, but one must be concerned with the optical path length of the microfabricated channel, which can reduce detection sensitivity. Liang et al. [3] reported the microfabrication of a U-shaped detection cell in a planar glass substrate. The cell was 120-140 \( \mu \)m long parallel to the flow direction and detection limits of \( \sim 6 \) \( \mu \)M were estimated for hydrolyzed fluorescein isothiocyanate. Shown in Figure 2.1 is an optical micrograph of the U-cell. Single mode optical fibers were integrated into the device for excitation and collection of the light [3]. The authors noted that the U-cell gives at least a 10-fold increase in absorbance compared to absorbance paths perpendicular to the flow direction due to the increase in the path length.

2.2.2 Laser-induced Fluorescence Detection

Laser-induced fluorescence (LIF) is an attractive detection strategy because it is both selective and sensitive. The sensitivity of laser-induced fluorescence has been demonstrated recently at the single molecule level [6] demonstrating the tremendous sensitivity of LIF in microchip applications. The need for sensitive detection in microelectrophoresis devices becomes apparent when considering the reduced
Figure 2.1 Optical micrograph showing launch of 488 nm beam from lower fiber with the beam path illuminated by fluorescein at 520 nm (503-533 filter). Flow channel was 20 μm deep, with a 140 μm longitudinal optical path length. Taken from [3].
column diameters, column volumes and sample sizes, such as when attempting to sequence DNA [7-10]. The selectivity in LIF derives from the fact that there are a limited number of compounds that exhibit intrinsic fluorescence, especially in the visible and near-infrared regions of the spectrum. Therefore, samples which are comprised of complex matrices can be analyzed with minimal fluorescent interferences and little need for extensive sample cleanup. Although, the limited number of analytes which exhibit intrinsic fluorescence can be problematic in some cases. This problem can be overcome by the use of fluorescence derivatizing agents such as fluorescein isothiocyanate (FITC), which reacts very efficiently with primary amines and fluoresces in the visible [11-13].

The process of fluorescence and other processes which compete with fluorescence to depopulate the excited-singlet state are shown schematically in the Jablonski diagram shown in Figure 2.2. Upon absorption ($k_a$) of a photon of energy, the molecule goes from the ground state ($S_0$) to the first excited singlet state ($S_1$) then relaxes to an equilibrium excited state through a process called internal conversion ($k_{ic}$). The molecule relaxes to the ground state through intersystem crossing ($k_{isc}$) to the triplet state, nonradiative pathways ($k_{nr}$) or fluorescence ($k_f$). Once the molecule passes into the triplet state via intersystem crossing, it may return to the singlet ground state by phosphorescence ($k_p$) or another nonradiative pathway ($k_{nr}$). Measuring each of these rate constants experimentally allows the calculation of the fluorescence quantum efficiency ($\Phi_f$), which is defined as the probability of
the emission of a fluorescence photon once the molecule is in the excited state. The quantum efficiency is defined as:

\[ \Phi_f = \frac{k_f}{k_f + k_{ic} + k_{uc} + k_{nr}} \]  

(2.3)

where the denominator contains all of the rate constants which depopulate the excited state. Thus, the larger the fluorescence quantum efficiency, the lower the detection limit for a fluorescent probe.

The intensity of a fluorescence signal \( (I_f) \) (photons sec\(^{-1}\)) is defined by the following relation:

\[ I_f = 2.3I_0bC\Phi_f\varepsilon F(\theta)G(\lambda) \]  

(2.4)

where \( I_0 \) (photons sec\(^{-1}\)) is the incident photon flux, \( b \) (cm) is the pathlength, \( C \) (M) is the concentration of the fluorphore, \( \varepsilon \) (cm\(^{-1}\) M\(^{-1}\)) is the molar absorptivity of the fluorescent species, \( F(\theta) \) is the instrumental collection and transmission efficiency of the fluorescent photons and \( G(\lambda) \) is the photodetector quantum efficiency at a specified wavelength. One of the advantages of fluorescence is that by simply adjusting the excitation power \( (I_0) \), an increase in the fluorescence intensity maybe obtained until a saturation point is reached, where the intensity does not scale linearly with \( I_0 \). The saturation arises from the finite time the molecule spends in the excited state, which essentially is determined by the fluorescence lifetime \( (\tau_f) \).
Figure 2.2 A simplified Jablonski diagram which represents the fluorescence and nonfluorescence processes.
Fluorescence detection, in particular laser-induced fluorescence (LIF), has proven to be the most popular detection scheme for microchip applications [1,3,9,13-50]. The most common set-up for LIF detection on microchips uses a confocal detection system. Mathies and Woolley [1] described a LIF confocal system in 1994 which is shown in Figure 2.3. The apparatus used the 488 nm output of an Ar ion laser as the excitation source. The light was passed into the confocal microscope and reflected with a dichroic beam splitter onto a 40X microscope objective which focused the beam to an ≈ 10 μm spot within the electrophoresis channel. The fluorescence was then collected by the objective, passed through the dichroic beam splitter, filtered and focused onto a 400 μm confocal pinhole followed by detection with a photomultiplier tube. Using this detection scheme, φX174 Hae III restriction fragments were analyzed after intercalation with a visible fluorescent dye, thiazole orange [1]. The fragments were resolved in under 120 s using stacked or plugged injection methods, with the plugged injection method giving the higher efficiencies (1.3 × 10⁵ plates for the 234-bp peak) and the stacked injection method being more sensitive (50-pg/μL DNA). Liang et al. [3] also used their integrated cell for fiber optic fluorescence detection on a microchip device (See Figure 2.1). The authors used the output of an argon ion laser (488 nm, 5.86 mW) coupled to a single mode fiber for excitation. The output power of the fiber was 0.143 μW. A fluorescence detection limit of 3 nM fluorescein (~20,000 molecules) was reported.
2.2.3 Raman Detection

Raman spectroscopy not only provides quantitative information about a molecule but also structural information, which can lead to the identification of unknown components. Although, Raman detection is not as widely used for capillary electrophoresis applications due to lack of sensitivity. This is caused by the fact that this technique essentially detects scattering photons which can increase the noise unless a sufficient amount of filtering is used and also the intensity of the Raman bands are typically very weak. Raman spectroscopy produces a vibrational spectrum of a molecule that is very similar to that observed in infrared spectroscopy. While infrared is an absorption technique that requires the use of an infrared excitation source, Raman benefits from the fact that it is not an absorption technique so practically any wavelength of light can be used for excitation. In addition, in Raman detection the excitation sources can be tuned to an electronic transition (S_0 \rightarrow S_1) associated with the scattering components, and thus, the intensity of the Raman bands can be enhanced. Also, aqueous solvent interference is minimal in Raman compared to IR, which can be very advantageous as most capillary electrophoresis experiments are performed in predominately aqueous buffers. This is because water is a very polar molecule with a large dipole moment; thus the vibrational frequency is large while the polarity is small. The minimal amount of polarizability of a water molecule makes it a weak scatterer of light.

Normal and resonance Raman spectroscopic energy level diagrams are shown in Figure 2.4. In normal Raman spectroscopy, the energy of the photon lies below an
Figure 2.3 Schematic of the CE chip and the laser-excited, confocal fluorescence detection system. The size of the features in the channel intersection area is exaggerated, and only every third channel on the chip is shown. Taken from [1]
excited electronic level ($S_1$) and collides inelastically with the scattering molecule which causes a loss in energy of the photon. This loss in energy corresponds with a characteristic vibrational mode of the molecule. In resonance Raman spectroscopy, the incident photon, which strikes the molecule, possesses an energy that promotes the molecule to an excited state $S_1$ and then relaxes into an excited vibrational level of $S_0$. The main advantage of the resonance technique is that larger signal intensities are observed. Unfortunately, these bands tend to be obscured by the intense fluorescence of the molecule as most of the Raman bands correspond to the chromophoric region of the molecule.

In Raman spectroscopy there are typically two types of shifts that are observed, Stokes and anti-Stokes shifts. Stokes shifts are because the initial vibrational state of the molecule lies below that of the final state, thus the scattering photon has a lower energy than the incident photon. Whereas for anti-Stokes shifts, the opposite effect is observed and the scattering photon is of a higher energy than the incident photon. Typically, the width of these bands is between 1-100 cm$^{-1}$. In Raman spectroscopy, the band in which the initial and final vibrational states of the molecule are the same (elastic scattering) is called the Rayleigh band or the 0 energy shift band. The Rayleigh band can be on the order of 1000 times more intense than typical Raman bands. The Stokes and anti-Stokes bands are distributed symmetrically about the Rayleigh band with the Stokes bands typically being more intense than the anti-Stokes due to the fact that most molecules are in the lowest
Figure 2.4 Schematic representation of energy level diagrams depicting Normal Raman and resonance Raman transitions. The arrows represent: (1,2) excitation from ground vibrational levels; (3,4) Rayleigh scattering \((E=\hbar\nu)\); (5) Raman Stokes scattering \((E=\hbar\nu-\Delta E)\); (6) Raman anti-Stokes scattering \((E=\hbar\nu+\Delta E)\).
vibrational state of S_0 at room temperature. Information about the scattering molecule can be obtained from the position of the Stokes and anti-Stokes bands. Normal Raman spectroscopy has been demonstrated by Walker et al. [4] as a viable detector for microchip applications. Shown in Figure 2.5 is a diagram of their Raman detection system. The Raman microprobe consisted of a 2W, 532 nm NdYVO_4 laser which was focused onto the microchip with a 50X/0.8 numerical aperture objective. The data was collected at 8 cm^{-1} resolution at a rate of 2 - 5 spectra/s. The authors were able to separate and detect two common pesticides (paraquat and diquat) at working concentrations of 10^{-5} to 10^{-7} M [4]. Also, the 980 cm^{-1} Raman band of sulfate, the counter ion, was used as an internal reference to correct for any instrument variations.

2.2.4 Refractive Index Detection

Refractive index (RI) is considered the most universal detection strategy available because it does not depend on molecular absorption and any wavelength of light can be used for monitoring. The only requirement being that the analyte must have a different index of refraction than the solvent in which it is dissolved. RI detectors are reliable and unaffected by the flow velocity, while being highly temperature sensitive requiring stringent temperature control. The temperature dependence can be a major problem when implementing RI detection in CE because of the thermal gradient in the channel caused by Joule heating. The CE running conditions should be optimized to reduce Joule heating effects.
Figure 2.5 Diagram of Raman spectroscopy/microchip isotachophoresis system. The Raman system used the 532 nm output of a 2-W NdYVO$_4$ laser coupled to an upright microscope. A 50×/0.8 NA objective was used to transmit and collect the light. The collected Raman-scattered light was analyzed by an 85-mm focal length transmission spectrograph and imaged onto a charged coupled device (CCD). Taken from [4].
One of the most common types of RI detectors used in small bore capillaries is the deflection type, where a change in the RI of the solution inside the capillary causes refraction of the light rays entering the capillary. The angle of refraction of light at an interface can be calculated using Snell’s law;

\[ n_1 \sin \theta_1 = n_2 \sin \theta_2 \]  \hspace{1cm} (2.5)

where \( n_{1,2} \) are the refractive indices of the material at the interfaces and \( \theta_{1,2} \) are the angle of the beam with respect to the normal of the interface. In \( \mu \)-CE devices there are optical interfaces which separate the incident beam into three components (reflected, refracted and transmitted parts) that interfere in the far field and produce an interference pattern consisting of equally spaced fringes, which are normally detected using a photodiode array. As an analyte passes through the channel, a change in the RI occurs which induces a phase change in the probe beam resulting in a lateral shift of the fringe pattern.

The major sources of noise that are associated with RI detection in \( \mu \)-CE devices include; (1) thermal gradients in the capillary (Joule heating) and (2) fluctuations in the light source. Noise due to fluctuations in the light source can be minimized by using a dual beam method and splitting the beam and subtracting the reference. Micro-CE devices are inherently more stable than capillary columns because of the solid platform from which they are built, which should minimize noise typically observed in capillaries from movement caused by changes in electrical double layer. Also, the smaller internal diameters that can be produced in \( \mu \)-CE devices will
reduce the magnitude of Joule heating which should improve detector sensitivity [2].

Burggraf et al. has described a holographic-based refractive index detector for \( \mu \)-CE devices [2]. A holographic optical element (HOE) was used to produce an interference pattern with small diffraction effects so that smaller internal diameter channels could be utilized to reduce Joule heating effects. The researchers used an isotropically etched glass substrate with channel dimensions of 10 \( \mu \text{m} \) deep and 40 \( \mu \text{m} \) wide at the top and 20 \( \mu \text{m} \) wide at the bottom of the channel. The HOE was glued at an angle of 30° to a plastic pipe and attached to the end of a laser diode (\( \lambda = 670 \text{nm} \), 3 mW) used as the illumination source. The diode laser assembly was mounted on a motor controlled x-y translation stage for alignment purposes. The fringe pattern was imaged onto a photodiode array consisting of 4 pairs of diodes to monitor the four fringes observed from the channel. Using this device, the authors were able to separate and detect 3 carbohydrates (sucrose, N-acetylglucosamine and raffinose), reporting detection limits between 600 and 900 fmoles.

2.3 Electrochemical Detection

Electrochemical detectors are some of the most selective and sensitive detectors used to monitor chemical separations. The selectivity arises from the fact that they operate on the principles of oxidation or reduction of analytes at an electrode interface. This oxidation or reduction potential is different for each species and the selectivity can be controlled by changing the magnitude of the potential applied to the electrochemical cell. The sensitivity or the limit of detection for an
electrochemical measurement is the difference between the background signal and the sample signal for a given concentration. Normally in electrochemical measurements, the analytical response is measured against a null background, increasing the signal-to-noise ratio in the measurement. High sensitivity also comes from the ability to efficiently convert electroactive species and from the ability to measure small currents accurately. In this section, the discussion will be limited to amperometric detection due to the fact that it has been the only electrochemical detector that has been applied to micro-capillary electrophoresis devices.

Amperometric detection has been the only type of electrochemical detection implemented in μ-CE devices because of the ease of construction using standard microfabrication techniques and the sensitivity afforded [5,51]. Using this scheme, the current (nanoampere level) is measured at a controlled potential as a function of time. Typical phenomena that can affect amperometric detection behavior are slow kinetics, irreversible reactions and mass transfer. The cell potential, $E_{\text{cell}}$, can be defined by the Nemst equation:

$$E_{\text{cell}} = E^\circ - \frac{RT}{nF} \ln \frac{a_{\text{red}}}{a_{\text{ox}}}$$  \hspace{1cm} (2.6)

where $E^\circ$ is the standard electrode potential, $R$ is the molar gas constant, $T$ is the absolute temperature, $a_{\text{ox}}$ and $a_{\text{red}}$ are the activities of the oxidized and reduced forms of the analyte involved in the electrode reaction. The Nemst equation describes the potential $E_{\text{cell}}$ of an electrode system for an oxidation/reduction reaction.
Woolley et al. [51] were the first to report on the microfabrication of an electrochemical detector on a μ-CE device. The pattern of the working and counter electrode was defined photolithographically 30 μm beyond the end of the separation channel. A Ti adhesion layer (200-Å thick) was thermally deposited on a glass substrate and the Pt working and counter electrodes (2600-Å thickness) were sputtered over the Ti adhesion layer. They reported the use of this design as an amperometric detector for the indirect detection of DNA restriction fragments and PCR product sizing. The DNA fragments were intercalated with an electrochemically active reagent, Fe-(phen)$_3^{2+}$. The constant background current from free Fe-(phen)$_3^{2+}$ decreased when DNA- Fe-(phen)$_3^{2+}$ complexes migrated through the detection region which was indicated by negative peaks in the electropherogram. A mass detection limit (SNR = 2) of 28 zmol was determine for a DNA restriction fragment. In addition, the authors reported a mass detection limit (SNR = 2) of 66 amol for a neurotransmitter (dopamine) [51]. Wang and co-workers also designed an amperometric detector on a μ-CE device [5]. The authors sputtered a gold working electrode at the outlet of the separation channel, which had a thickness of about 200 nm. The sputtering was accomplished without the use of photolithographic placement of the electrode. All areas of the etched chip were covered with tape except the area to be sputtered (the outlet well), and the tape was simply removed after sputtering to reveal the working electrode with an area of 0.78 mm$^2$. A concentration limit of detection (SNR = 2) of 1 μM dopamine was reported, which compared favorably to previous work by Woolley et al. [51].
Rossier et al. developed a three electrode amperometric detector in polyethylene terephthalate (PET) using UV photoablation from an excimer laser (193 nm, 50 Hz @ 200 mJ/pulse) [52]. The working and counter electrode channels were etched 40 \( \mu \text{m} \) in width in a “face-to-face” configuration with 40 \( \mu \text{m} \) between (the width of the separation channel). The channels were filled with carbon ink and then cured at 70°C for 2h. A Ag/AgCl reference electrode was placed in the exit reservoir. Pulsed amperometry was performed using a controlled potentiostat in a two or three electrode system. The authors reported electrophoresis and detection of 2mM aminophenol using pulsed amperometry in a phosphate buffer. Additionally, they reported that work is in progress to improve the separation and detection efficiency of this device.

2.4 References


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Chapter 3

Conductivity Detection of Polymerase Chain Reaction Products Separated by Micro-Reverse-phase Chromatography

3.1 Introduction

The polymerase chain reaction has become a powerful tool for the rapid enzymatic amplification of specific DNA fragments (Figure 3.1). The PCR reaction can amplify genomic DNA exponentially using temperature cycles consisting of denaturation, annealing and extension and can provide sufficient amounts of material for subsequent analysis. Indeed, the PCR reaction has been successfully used to amplify target DNAs for mutation analysis, genetic mapping and sequencing. However, before subsequent analysis of the amplicon can occur, one must purify the PCR product to remove unwanted salts, primers, and enzymes. Numerous strategies have been used to perform purification of PCR-generated DNA. These include gel filtration [1], gel electrophoresis [2], ultrafiltration [3] and ion-exchange [4,5]. However, these processes require large amounts of solvents, can be time-consuming, plagued by low sample recoveries and often cannot be easily automated. In addition, the gel-based methods require the amplicon to be excised from the gel matrix following separation.

An attractive alternative to the aforementioned purification techniques for PCR products is a reverse-phase separation, which can readily purify PCR products with
1) Heat to separate strands
2) Cool; add synthetic oligonucleotide primers
3) Add thermostable DNA polymerase to catalyze 5' to 3' DNA synthesis

Repeat Steps 1 & 2

Step 3 is repeated

Figure 3.1 An illustration of the polymerase chain reaction (PCR) process.
high recoveries, typically exceeding 97% [6-9]. In addition, the use of reverse-phase chromatography is conducive to automation, making it amenable to implementation in high throughput DNA sequencing applications. For most reverse-phase separations of DNA amplified via PCR, an ion-pairing agent is used in conjunction with a packed-column containing a C_{18} stationary phase. The common ion pairing agents that have been used are quaternary ammonium salts, such as tetrabutylammonium phosphate or triethylammonium acetate.

Electrolytic conductivity is the ability of an electrolyte solution to conduct electricity between two electrodes in which an electric field is applied. In this system, Ohm's law, \( V=IR \), is obeyed and the magnitude of the current depends on the applied potential and inversely on the resistance between the two electrodes. Conductance (\( G \)) of a solution is related to the solution electrolytic resistance and measured in reciprocal ohms.

\[
G = \frac{1}{R} \tag{3.1}
\]

Specific conductance (\( k \)) relates the solution conductance to the cell dimensions,

\[
k = G \frac{L}{A} \tag{3.2}
\]

where \( A \) is the area of the electrodes in cm^2 and \( L \) is the distance between the electrodes in cm and thus \( k \) has the units of ohms \( \cdot \) cm^{-1}. The cell constant (\( K \)) is defined as \( L/A \) and has units of cm^{-1}. The equivalent conductance (\( \Lambda \)) relates the
specific conductance to the concentration of the analyte and is defined by the following equation:

\[ \Lambda = \frac{1000k}{C} \]  \hspace{1cm} (3.3)

where \( C \) is the concentration in equivalents per 1000 cm\(^3\). From equations 3.2 and 3.3 the relationship between conductance and conductance, \( G \) is derived;

\[ G = \frac{\Lambda C}{1000K} \]  \hspace{1cm} (3.4)

Electrolytic conductivity detectors consist of a cell, a readout meter and the electronics required to measure the conductance and vary the sensitivity settings (i.e. applied potential, resistor). The specific conductance can be calculated from the conductance if the cell dimensions are known; although, normally the conductance of a dilute solution of known specific conductance is measured and the cell constant is calculated. Once the cell constant is known, the specific conductance of other solutions can be measured and calculated using equation 3.4.

The principle operation of a conductivity cell is such that when an electric field is applied to two electrodes in an electrolyte solution, anions move toward the anode and cations toward the cathode. The concentration and velocities of the ions in bulk solution determine the resistance \((1/G)\), of the solution. Several factors determine the ionic mobilities such as the charge and size of the ion, the temperature and type of medium and the ionic concentration. The ionic velocities depend on the
magnitude of the potential that is applied to the cell. The potential can be constant or oscillating such as a sinusoidal or pulsed wave.

During cell operation, several phenomena can occur which can cause the effective cell potential to change. These phenomena are illustrated in Figure 3.2. Formation of a double layer capacitance can occur if the electrode is below the decomposition potential. When this happens, the solution layer immediately adjacent to the electrode will attract ions of opposite charge and a charge layer will be formed. This charged layer consists of two parts: (1) a thin inner layer, in which the concentration decreases linearly with distance from the electrode surface and (2) a more diffuse layer, in which the ionic concentration decrease is exponential. Formation of the charged double layer lowers the effective potential applied to the bulk electrolyte.

Electrolysis can occur when the applied potential is above the decomposition potential of the electrode or solution. During electrolysis, current flows across the electrode solution interface by oxidation at the anode and reduction at the cathode. This Faradaic impedance phenomena can be caused by depletion of ions at the electrode surface, or by slow electron transfer processes which changes the effective potential applied to the bulk electrolyte.

These phenomena that affect the cell potential can be eliminated through the use of alternating electrode potentials. Electrolysis can be eliminated by changing the sign of the applied potential, which reverses the direction of ion motion. As the
Figure 3.2 Diagram of the conductivity detector cell and the solution ion migration when a field is applied between the electrodes.
frequency is increased, the effects due to electrolysis are reduced or even eliminated and most of the current flow is delivered by capacitance formation; with an upper frequency limit being approximately 1 MHz. At frequencies above 1 MHz, the ions cease to move in the electric field and no current can be measured, although dipole orientation can still occur. The capacitance effects can be eliminated by either matching the cell capacitance in the electronic circuitry or by measuring the instantaneous current, which is the current measured when the potential is first applied and before the double-layer has formed. In some instances a bipolar pulsed technique can be used to eliminate the electrolysis and capacitance formation [10-12]. This technique uses two voltage pulses of equal but opposite polarity that are of short duration (~100 µs). The cell current is measured at exactly the end of the second pulse, which is an instantaneous current. Through pulsing and measuring an instantaneous cell current, this technique prevents electrolysis and capacitance formation and an accurate cell resistance is measured.

The use of conductivity detection has been explored in numerous applications, for example, the detection of metals and other ionic materials separated by liquid chromatography and/or capillary electrophoresis [11,13-19]. The pioneering work of Mikkers et al. [20] demonstrated the utility of conductivity to detect carboxylic acids using a "potential gradient" detector that essentially measured changes in conductance. Since that time, several different conductivity detector designs have been reported for a variety of separation platforms [11,16,21]. However, to-date, no reports have appeared describing the use of conductivity detection in reverse-phase
chromatography (RPC) for the separation and analysis of oligonucleotides, such as PCR products.

Conductivity can potentially offer several advantages compared to other common detection schemes used for DNA detection in conjunction with the micro-column separation methods, such as UV absorbance or fluorescence. UV absorbance suffers from poor detection limits, which have been reported to be on the order of $10^{-4}$-10$^{-5}$ M range for DNAs [22] when the separation is performed using micro-columns because of the reduced pathlength associated with the column. As stated previously, the cell constant is calculated from $L/A$, where $L$ is the distance between the electrode pair and $A$ is the electrode area. From this relation, a small electrode spacing will actually increase the sensitivity of the measurement for conductivity, making this detection strategy particularly attractive for miniaturized separation platforms. While fluorescence methods can demonstrate low limits of detection (LODs), the difficulty with the strategy is that staining or labeling of the oligonucleotide is performed prior to detection [23]. As a result, pre-sample processing is required and if the purification product is used for further analysis, destaining of the target is necessary as well. Since conductivity measures the bulk conductance of the solution, as long as the analyte has a conductance different from that of the carrier mobile phase solution, the DNA can be analyzed in its native state. In addition, the equipment required for implementation of conductivity can be much simpler and inexpensive compared to laser-induced fluorescence.
The work described in this manuscript outlines the development and operational characteristics of a novel, miniaturized PCR separation/purification system, which utilizes ion-pairing RPC for the separation with bipolar-pulsed conductivity detection. The separation was performed using a micro-column packed with a C18 stationary phase and a mobile phase comprised of 50/50 H2O/CH3CN with triethylammonium acetate as the ion pairing agent.

3.2 Experimental

3.2.1 Construction of the Conductivity Cell

A schematic of the conductivity cell is shown in Figure 3.3. The conductivity cell was constructed from Pt wires (Scientific Instrument Services, Ringoes, NJ), where each wire was 360 μm in diameter. Two pieces of Pt wire were cut to 2.5 cm in length and the end of the wire polished with fine grit sandpaper. The ends of the wires were inspected under a 20x stereomicroscope to insure proper polishing. After polishing, the wires were cleaned with isopropyl alcohol then inserted into opposite ends of a glass tee (Innovaquartz, Phoenix, AZ), which had an inside diameter of 365 μm. The ends of two pieces of 75 μm i.d. fused-silica capillary were then polished and a piece of 60 μm i.d. tungsten wire inserted into the capillaries connecting them. The capillaries were then inserted into the tee, where the tungsten wire served as an alignment tool for the Pt wires. The Pt wires were pushed against the tungsten wire and glued into place with Bond It adhesive (Scientific Instrument Services, Ringoes, NJ). The fused-silica capillaries were pushed against the Pt wire
and tacked into place. The tungsten wire was then simply pulled from the capillaries leaving a 60 μm spacing between the Pt wires.

The bipolar-pulsed technique for conductance measurements was used for obtaining the solution conductivity [10-12]. The electronics were controlled by a PC operating at 5 MHz. The bipolar pulse waveform was controlled by a 12 bit digital-to-analog (D/A) board (National Instruments, Austin, TX). A brief pulse (pulse width = 100 μs) of positive polarity was followed by a negative pulse of the same time duration and amplitude. The use of the bipolar pulse technique allows the ability to discriminate against the cell double layer charging current from the current flowing between the electrode pair when a fixed voltage is applied, which can be related to the solution conductivity through Ohm’s Law. The bipolar pulse height was controlled by the D/A output and was limited by the resolution of the D/A conversion. The frequency of the pulses (typically 5,000 Hz) was controlled by a timer on the D/A board. The timer output was connected to a standard digital input port. Each time the computer detected a rising edge from this input port it generated the bipolar pulse. The potential of one cell electrode was maintained at virtual ground while the potential of the other electrode was controlled by the generated waveform. The current, which was sampled at the end of the negative pulse, flowed through a feedback resistor and was processed by an amplifier and subsequently reported at an analog output pin. Software was written in LabView (National Instruments, Austin, TX).
Figure 3.3 Schematic of the conductivity cell.
3.2.2 Flow Injection Analysis (FIA)

A flow injection system was built in-house to characterize the conductivity detector. It consisted of a HPLC pump (Rainin Instruments, Woburn, MA) connected to a standard 75 μm i.d. fused-silica capillary (Polymicro Tecnologies, Inc., Phoenix, AZ) which was connected to the inlet of the glass tee that housed the conductivity detector. All flow injection studies were run at 5 μL/min flow rates. KCl standard solutions and Herring Sperm DNA (Gibco-BRL, Gaithersburg, MD) standard solutions were prepared in 18 MΩ doubly-distilled H₂O daily for characterization studies.

3.2.3 μ-RP-HPLC System

A schematic of the μ-RP-HPLC system is shown in Figure 3.4. A Rainin Rabbit HP pump (Rainin Instruments, Woburn, MA) was used to pump the mobile phase through the system. One end of a 15 cm, 300 μm i.d. reverse-phase Hypersil (C₁₈) column (ThermoQuest, Holliston, MA) was connected to a low volume injector (Valco Instruments, Houston, TX) which was connected to the high pressure mobile phase pump. The low volume injector allowed reproducible injection of 100 nL volumes of sample into the μ-RP-HPLC system.

The chromatography of the PCR product was carried out using ion-pairing reverse-phase chromatography with the mobile phase consisting of 50% acetonitrile, 50% H₂O (pH = 7.0) and 50 mM triethylammonium acetate (TEAA). The volumetric flow used was 7.5 μL/min and the conductivity cell was operated at a
frequency of 5,000 Hz with a 0.5V bias voltage applied between the electrode pair. The PCR mix was manually loaded into the low volume injector to inject sample into the flow stream of the μ-RP-HPLC system. Also, the PCR product was compared to a DNA low mass sizing ladder (Gibco-BRL, Gaithersburg, MD) that contained five fragments of 200, 400, 800, 1000 and 1200 bp. The sizing ladder was separated using the separation conditions described above. All resolution (R*) values were calculated based on the following formula:

\[
R_i = \frac{t_{r_{i+1}} - t_{r_i}}{w_{i+1} + w_i}
\]

where \( t_r \) is the retention time of the components (in units of time) and \( w_{i/2} \) is the width of the corresponding peaks at half height (in units of time).

3.2.4 PCR Amplification of λ-bacteriophage DNA

A 500-bp fragment of λ DNA (5.0 ng/μL) (Gibco-BRL, Gaithersburg, MD) was amplified by PCR in a Perkin Elmer 2400 series thermocycler (Foster City, CA). The PCR mixture contained 1 μL of λ DNA, 10 μL 1X PCR buffer (20 mM Tris-HCl, pH=8.4, 50 mM KCl), 2 μL dNTPs, 85 μL double-distilled water (ddH₂O), 1 μL PCR01 forward primer (1 μM, 5'-GATGAGTTCGTACAACTGG-3') and 1 μL of PCR02 reverse primer (1 μM, 5'-GGTTATCGAAATCAGCCACAGCGCC-3'). PCR was performed under "hot start" conditions, which entailed addition of 1 μL of the Thermus
Figure 3.4 Schematic of the $\mu$-RP-HPLC system.
aquaticus (Taq) DNA polymerase (Gibco-BRL, Gaithersburg, MD) after the reaction temperature reached 80°C, which ensured high fidelity during DNA amplification. Twenty-five and thirty PCR cycles were performed using the following program; 1) denature dsDNA at 94°C for 45 seconds; 2) anneal primer at 55°C for 30 seconds and; 3) extend primer at 72°C for 90 seconds.

3.2.5 PCR Amplicon sizing

Following amplification, sizing of the 500 base-pair PCR amplicons was accomplished by slab-gel electrophoresis using a 0.8% agarose gel. Shown in Figure 3.5 is the gel electropherogram of the PCR product along with the sizing ladder. The amplicons were run in parallel with a DNA low mass sizing ladder (Gibco-BRL, Gaithersburg, MD) to assure the integrity of the PCR reaction.

3.3 Results and Discussion

3.3.1 Characterization of the conductivity detector using FIA

In order to determine the analytical figures of merit of conductivity detection for DNA’s (linearity of response and concentration/mass limit of detection), experiments were performed using FIA. Various concentrations of KCl and DNA were injected into the system and the conductivity of the sample was measured against ddH_{2}O and 50 mM TEAA carrier solutions. The choice of carrier solution for the DNA measurements was based upon the fact that TEAA would serve as the ion-pairing mobile phase in the μ-RP-HPLC separation. It was important to measure the observed response against the mobile phase used for μ-RP-HPLC in these FIA studies, since the conductivity response results from a differential
Figure 3.5 Slab gel electropherogram of (A) 25 cycle and (B) 30 cycle PCR mixes run in parallel with a low DNA mass sizing ladder. The number of bases for each fragment in the sizing ladder is labeled on the side of each band. The sieving matrix (gel) consisted of 0.8% (w/v) agarose with ethidium bromide used as the staining dye. The running buffer consisted of 100 mM Tris-Borate-EDTA (TBE) pH = 8.3.
Figure 3.6 Calibration plots for KCl (•) and DNA (Δ). The plots were constructed by integrating the area under each FIA peak and averaging over five runs. The KCl was run using ddH$_2$O as the background carrier and the DNA was run using 50 mM TEAA as the background carrier.
measurement between the carrier solution and that of the sample. As such, the background conductivity will have a profound influence on the analytical signal with a higher background conductivity associated with the mobile phase degrading the signal-to-noise ratio (SNR) in the measurement. However, this must be balanced with the chromatographic needs, since the ion-pairing agent concentration will affect the resolution in the chromatography of the PCR-product. Calibration plots (see Figure 3.6) were constructed from the data for both KCl and DNA by integrating the area under each FIA peak and averaging over five runs. The concentration range for the DNA sample was from 0.50 – 2.50 mg/mL, while for KCl it was from 1.00 - 100 µg/mL. Each plot was fit to a straight line and the linear correlation coefficients were found to be 0.9971 for the DNA sample and 0.9991 for KCl. By extrapolating the plots to 0 concentration using a S/N = 3 criteria, the detection limits were calculated for DNA and KCl. A limit of detection (LOD) of 110 mg/L (330 nM) was calculated for the DNA sample, which is approximately 2 orders of magnitude lower than LODs reported for UV absorbance detection of dsDNAs using a 75 µm i.d. capillary column [22,24]. The LOD for KCl was found to be 34.6 µg /L (34.6 ppb). Taking into account the injection volume (100 nL), the mass detection limits were 11.0 ng and 3.46 pg for the DNA and KCl samples, respectively. The improved mass LOD for KCl results from the fact that its conductivity is significantly different from that of the carrier solution. For comparison to fluorescence, the mass detection limit of double stranded DNA's stained with mono-
intercalating dyes and separated by CE has been reported to be 20 fg [23]. While the mass detection limit obtained for the conductivity detector is not as good as fluorescence, conductivity is much easier to implement due to the fact that staining is not required and also, the instrumentation is significantly less complex. It should be noted that improvements in the mass LOD could also be realized by lowering the conductivity of the carrier solution. Therefore, if the counter ion concentration, TEAA in this case, could be reduced, improvements in the LOD could be achieved because the mode of detection is an indirect one. For indirect detection it has been shown that the concentration limit of detection is directly proportional to the counter ion concentration [25]; thus by reducing the counter ion concentration the concentration LOD is reduced.

3.3.2 Separation of PCR fragment

After amplification, conductivity detection of a 500-bp segment of λ DNA was tested using the conductivity cell. The amplification reaction was cycled 25 or 30 times and analyzed using ion-pair on a reverse-phase (C18) micro-column. The results of the PCR analyses are shown in the chromatograms of Figure 3.7. The primer set forms a primer-dimer product during amplification, which co-elutes in the unretained peak in this separation, which is also comprised of the buffer and metal ions found in the PCR reaction cocktail. This was determined by performing a null PCR reaction with all PCR ingredients thermal cycled in the absence of the target DNA and then, simply performing a separation on the μ-RP-HPLC system (data not shown). Indirect detection of the PCR amplicon was observed, which caused a
negative peak to occur when the amplicon passed the conductivity cell because the DNA had a lower conductivity than the background carrier (see Figures 3.7-B,C). Also, the PCR results were compared to a standard DNA ladder (Figure 3.7-A) for sizing purposes. The separation of the standard ladder was performed under the same conditions as that for the PCR amplicon. As can be seen in Figure 3.7-A, the 200/400 and 400/800 bp fragments were baseline resolved \((R \geq 1.5)\) with resolution values being 1.69 and 1.98, respectively. The 800/1000 and 1000/1200 bp fragments were only partially resolved with the resolution factors determined to be 1.13 and 1.01, respectively. It should also be noted that the elution time of the PCR product matched the appropriate fragment on the sizing ladder indicating the correct identity of this product (500-bp). Although the components of the ladder were not completely resolved, baseline resolution of the ladder could be accomplished by simply adjusting the running buffer composition as shown by previous work using ion-pairing chromatography [26]. However, changes in the ion pairing concentration will also affect the analysis (plate numbers, elution time, etc.) for the PCR amplicon.

Shown in Table 3.1 is a list of chromatographic and detection efficiencies for the PCR product. The concentration of the 500-bp \(\lambda\) DNA in each series of PCR reactions was estimated by interpolation of the calibration plot of concentration vs peak area for Herring Sperm DNA, assuming that the conductivity detector response is independent of fragment size. The estimated values of 1.8 mg/mL (25 cycles) and 2.1 mg/mL (30 cycles) corresponds to an increase of \(3.6 \times 10^6\) and \(4.2 \times 10^6\),
Figure 3.7 Chromatograms of (A) a standard low mass DNA ladder, (B) 25 cycles and (C) 30 cycles, 500 bp PCR product separated by μ-RP-HPLC. The mobile phase composition was 50/50 H₂O/Acetonitrile with 50mM TEAA (pH = 7.0). The injection volume was 100 nL and the flow rate was 7.5 μL/min. The bias-voltage applied to the conductivity cell was 0.5 V.
respectively, over the initial concentration of 0.5 ng/mL for the target. After accounting for the injection volume (100 nL), each injected sample contained $3.3 \times 10^{11}$ and $3.8 \times 10^{11}$ copies of the 500-bp PCR amplicon for the 25 and 30 PCR cycles, respectively. The theoretical copy number ($N$) for 25 PCR cycles can be calculated from $N = N_0 \times 2^n$, where $N_0$ is the starting copy number and $n$ is the number of PCR thermal cycles. For the conditions used for our PCR reactions, we would expect to see $1.53 \times 10^{11}$ copies in a 100 mL volume after 25 PCR cycles, in close agreement to the number obtained from the calibration plot. The reason that the copy number is not increased by 32-fold ($2^5$, where 5 is the difference in PCR cycle number between the two samples) for the 30 cycle PCR mix is that we are most likely operating on the plateau region of the amplification versus cycle number, where the copy number does not scale with the cycle number [27] due to deactivation of the polymerase enzyme either through heat deactivation or production of large amounts of DNA.

As can be seen from Table 3.1, the plate numbers were found to be 5,888 (25 thermal cycles) and 9,098 (30 thermal cycles), which were calculated assuming the peaks were Gaussian-shaped (i.e., no asymmetry). For the 30-thermal cycle case, the HETP value was determined to be $1.65 \times 10^{-3}$ cm, yielding a total variance ($\sigma_i^2$) of $2.48 \times 10^{-2}$ cm$^2$. Calculation of the variance arising from the conductivity detector alone produced a value of $1.08 \times 10^{-4}$ cm$^2$, only a 0.44% contribution to $\sigma_i^2$, indicating that the major contribution to the band broadening is kinetic effects.
associated with the column. The additional extra column variances, for example the variance arising from the injection volume or connecting tubing, were calculated to be <0.1% of the total variance. In addition, the resolution value between the amplicon peak and void volume, which consisted of the PCR cocktail reagents such as salts, dNTPs, primers and enzyme, was found to be 11.33, with a capacity factor of 2.5 for the PCR amplicon. The large resolution factor observed for this separation will allow for the efficient isolation of the amplicon for potential downstream processing applications, such as sequencing. Removal of excess PCR reagents is necessary to obtain high quality sequencing reads for both slab and capillary gel separations [28,29].

3.4 Conclusions

The feasibility of performing separation and/or purification of PCR products in a \( \mu \)-RP-HPLC format using ion-pairing agents with conductivity detection has been demonstrated. One of the advantages of \( \mu \)-RP-HPLC is that very low sample volumes and mobile phase volumes compared to conventional PCR separation/purification methods are required. For example, during the ~4 minute run used to isolate the PCR amplicon in this work, only 30.00 µL of mobile phase was consumed (\( F_v = 7.5 \) µL/min). In addition, we have demonstrated the utility of conductivity detection for the monitoring of oligonucleotides in chromatographic applications. Since the analytical response is produced from a difference in conductivity between the carrier solution and the sample, no staining of the sample
was required. Therefore, the detection process is non-destructive and allows the
target to be readily accessible for further analysis without significant post-run
processing. Furthermore, this detection scheme is very amenable to miniaturization
since the response sensitivity is improved when the electrode spacing is reduced
compared to UV absorbance, which shows poor SNR in micro-columns due to the
pathlength dependent signal response. This will make conductivity appealing for
micro-column separation platforms, such as micro-chip electrophoresis.

Table 3.1. Chromatographic efficiencies, LOD's and amplification numbers for PCR
amplified λ DNA.

<table>
<thead>
<tr>
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<th>25 PCR Cycles</th>
<th>30 PCR Cycles</th>
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<td>k'</td>
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<td>2.51</td>
</tr>
<tr>
<td>Plate Numbers (N)</td>
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<td>9098</td>
</tr>
<tr>
<td>Mass of DNA Detected (ng)</td>
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<tr>
<td>No. of DNA Copies (× 10^{11})</td>
<td>3.3</td>
<td>3.8</td>
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</tbody>
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3.5 References


Chapter 4
Piezoelectric Mechanical Pump with Nanoliter per Minute Pulse-free Flow Delivery for Pressure Pumping in Micro-Channels

4.1 Introduction

There is currently a pressing need for mechanical pumping devices that can deliver small volumes of materials (liquids or gases) at controlled rates with volumetric delivery rates in the low to sub-nanoliter per minute range. Not only is it necessary to possess the ability to pump at low volumetric flow rates, but also the pump should be able to operate effectively when the pressure drop is large. For example, in the biomedical area, small and compact pumps can be used for administering drugs for the treatment of such diseases as diabetes. In analytical chemistry, pumps which can deliver low volumetric flow rates can be used for micro-liquid chromatography [1], and micro-dialysis [2].

Recently, several research groups have focused on the development of miniaturized chemical analysis systems that can perform several different analytical techniques, such as separation via electrophoresis or chromatography, sample preparation and finally, sample detection [3-18]. These devices consist of a series of channels with dimensions in the range of 20-40 \( \mu \)m lithographically fabricated in glass or plastic materials. While most of the micro-fluidics in these devices have been accomplished via electropumping produced by electroosmotic flows generated in these micro-devices when fabricated in glass substrates, mechanical pumps will need to be developed

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which can be used in devices where the magnitude of the electroosmotic flow is insufficient to allow facile pumping. For example, when predominately organic run buffers are used in the micro-electrophoretic format, the magnitude of the electroosmotic flow maybe insufficient to allow facile electro-pumping [19]. In addition, we have developed miniaturized chemical analysis systems in Plexiglas (PMMA, polymethylmethacrylate) using X-ray lithography and determined that the small electroosmotic flow generated in these devices will require an external pumping source in some cases to move fluids at reasonable linear velocities [20].

Since these miniaturized chemical analysis systems possess ultra-small channels, micro-pumps must be developed which can deliver fluids at very low volumetric flow rates to obtain reasonable linear velocities. In Figure 5.1 is shown the calculated volumetric flow rate versus linear velocity through circular tubes with various diameters (10, 20 and 50 μm ids). As can be seen, to obtain a linear velocity of 0.01 cm/s in a 10 μm id channel, a volumetric flow rate of 450 pL/min is required. Another concern associated with pumping liquids through narrow bore channels is the pressure drop created by the small diameter and as such, the micro-pump should be able to adequately drive solutions through these tubes when operated with high loading pressures. In Figure 4.1 is also shown the pressure drop across a micro-tube generated under different linear flow velocities. For example, a micro-tube possessing an id of 10 μm with a total length of only 5 cm and pumping water (viscosity = 1 cP) at a linear velocity of 0.01 cm/s results in a pressure drop of approximately 450 Pa. Therefore,
Figure 4.1 Volume flow rate (solid lines, closed symbols) and pressure drop (dashed lines, open symbols) as a function of linear flow velocity. The volume flow rate and pressure drop were calculated assuming that pumping was occurring through a circular capillary tube of id 10 μm (circles), 20 μm (triangles) and 50 μm (squares) with a length of 10 cm. The solution viscosity was assumed to be 1 cP.
one can see that micro-fluidic pumping in small id channels requires a mechanical pump that can operate reasonably well at high load pressures and also demonstrate stable flows at low volumetric rates.

Micro-mechanical pumps can also find applications in systems where it becomes necessary to transfer small volumes (picoliter to nanoliter) of material into micro-reactors for sample preparation utilized in miniaturized chemical analysis systems. An example is a genetic analysis system we are currently constructing which involves the restriction digestion of DNA samples in an enzyme micro-reactor possessing a total volume of approximately 20-30 nL and transferring this minute sample to an electrophoresis separation channel for fractionation. Since the handling of nL volumes of samples can be problematic, the enzyme-reactor is incorporated on-line with the electrophoresis separation device. Prior to separation, intact DNA molecules are subjected to enzyme catalyzed fragmentation. Based on our experience, the required residence time of the DNA sample in the micro-reactor to achieve exhaustive digestion ranges from 5 to 10 minutes. Therefore, with a micro-reactor possessing a volume of 30 nL, a volumetric flow rate of 3-6 nL/min is required to obtain the required residence time.

Several groups have constructed micro-pumps which are based upon a reciprocating or peristaltic mode of operation and have used piezo-actuators to produce the pumping action [21-25]. In most cases, these pumps use a bimorph which bends when a voltage is applied to the piezo. By inserting valves into the device, the pump can continuously
operate with the volumetric pumping rate determined by the frequency of the driving voltage to the pump chamber. Unfortunately, many of these can operate only at low loading pressures and pump at volume flow rates in the \( \mu \text{L/min} \) range. Also, since the pumping action is based on a reciprocating process, pump pulsations may be evident which could present problems when used in particular applications, for example in micro-chromatographic systems where the detector is sensitive to such noise sources.

In this paper, we will describe the construction and operational characteristics of a computer controlled mechanical pump constructed from a piezoelectric actuator (PA), pivot lever and micro-syringe (see Figure 4.2). By applying a voltage to the piezoelectric, the crystal can be expanded pushing the plunger of the micro-syringe causing fluid displacement. The expansion of the PA is amplified by means of the pivoted lever, which provides a higher volume displacement per pump stroke. The volumetric flow rate is controlled by specifying the slope on the driving voltage ramp to the PA. Due to the precise positioning capabilities of the PA, low nL/min volumetric flow rates can be generated with this device making it appropriate for fluid pumping in miniaturized chemical analysis systems. In addition, we will discuss the micro-fabrication of a diffuser/nozzle system which would allow automatic refilling of the syringe pump without requiring disconnecting the pump from the supply source and the chemical analysis system to which it is interfaced [26]. Recently, several groups have shown that the diffuser/nozzle geometry is a viable choice for valve-less operation in micro-mechanical-based pumps using reciprocating type action [26-30]. The
Figure 4.2 Schematic representation of the piezo-driven micro-syringe pump.
diffuser/nozzle system was micromachined in poly(methylmethacrylate) (PMMA) using X-ray lithography. The PMMA-based diffuser/nozzle was interfaced to the syringe pump using small diameter fused silica capillary tubes with o.d.s of 50 \( \mu \)m and i.d.s of 20 \( \mu \)m.

4.2 Experimental

4.2.1 Construction of PA pump

In Figure 4.2 is shown a schematic of the micro-pump. All components were mounted on a Plexiglas stage (14.0 x 8.0 x 1.3 cm). The expansion of the PA (model P-178.50, from Polytec PI Inc, Costa Mesa, CA) was controlled by a computer (Gateway 2000, P5-120) equipped with a 12-bit analog output voltage (0 to ±10 V, DC) from a Multi-function I/O board (model AT-MIO-16XE-50, National Instruments, Austin, Texas). The maximum linear displacement for this piezo was stated to be 80 \( \mu \)m by the manufacturer when a voltage of -1000V (negative polarity) was applied from a high voltage power source (model K10N, from Emco High Voltage, Sutter Creek, CA) which was driven by the output of the DAC. With 4096 (12-bit) step resolution over 10 V (2.44 mV/step), the minimum linear displacement of the PA was 19.5 nm/step (assuming a total linear expansion of 80 \( \mu \)m). The piezo-expansion was amplified using an in-house constructed lever (5.8 x 1.5 x 0.64 cm) and a pivot. The lever sits horizontally on a 0.8 mm thick 2.5 mm i.d., 12.0 mm o.d. copper disk that surrounds the pivot and can move without touching any part of the Plexiglas stage. The PA was placed in a rectangular slot (8.1 x 1.8 x 0.4 cm) made on the Plexiglas stage.
such that the center of the front face of the PA-head was butted against the tip of one arm (A-1, see Figure 4.2) of the lever. As shown in the figure, the distance between A-1 and the center of the pivot was 5.0 mm, whereas the distance between the center of the pivot and the second arm (A-2) of the level was 50.0 mm. A spring (S-1) maintained contact between A-1 and the PA-head. A micro-syringe (gas-tight, model RN80230, from Hamilton, Reno, NV) made of glass was situated horizontally on the Plexiglas stage and mounted (at a right angle with respect to the long axis of the lever) onto a drilled slot in this stage. Another spring (S-2) maintained contact between A-2 of the lever and the syringe plunger. The syringe was connected directly to a fused silica capillary (Polymicro Technologies, Phoenix, Arizona) with a 2.0 cm long 0.010” i.d. polyvinyl chloride (PVC) tubing (Elkay Products, Shrewsbury, MA).

4.2.2 Measurement of absolute expansion of PA

In order to measure the absolute expansion of the PA and subsequent amplification by the lever, one end of a short piece of fused silica capillary (20 cm long, 150 μm od) was attached to the PA-head and A-2. The respective expansions were determined by focusing the other end of the capillary under a stereomicroscope (100X magnification, Optiphot-2 microscope, Nikon, Japan) equipped with a CCD-video camera and monitored by measuring the movement of this capillary on a calibrated TV screen. The small size of the capillary (low mass) minimized the loading back pressure on the PA.

4.2.3 Measurement of absolute flow rates

To measure the absolute linear fluid velocity under pump operation (applied voltage), the micro-syringe was filled with a suspension of 0.0005% uniform latex
micro-beads (0.203 μm polystyrene micro-spheres, Duke Scientific Corp, Palo Alto, CA) in water. Fused silica capillaries of various lengths were connected to the syringe and movement of the latex beads through a small segment of the capillary window was monitored under the microscope. A window for optical viewing was made by removing about a 1.0 cm portion of the polyimide wall-coating situated about 20 cm from the capillary inlet. The entire capillary was filled with the bead-solution manually from the syringe. Care was taken to avoid hydrostatic flows generated by a height difference between the two ends of the capillary causing siphoning action. A timer was used to record the time necessary for the beads to travel through the known viewing segment of the capillary when various voltage ramps were applied to the PA. Since beads were chosen randomly throughout the internal diameter of the capillary tube, the stated flow velocities represent the average linear flow rate of the parabolic flow profile produced by this pressure driven system.

4.2.4 Laser-induced fluorescence determination of flow stability

The flow stability was determined using laser-induced fluorescence (LIF) signals by flowing a solution of 50 nM fluorophoric dye (IR-144, from Kodak Chemicals, Rochester, NY). Due to the photobleaching experienced by a fluorogenic dye when flowing into a focused laser beam with high irradiance, the fluorescence signal becomes a sensitive probe of flow rate [31,32]. LIF was performed using a 750 nm diode laser (GaAlAs) as the excitation source. This beam was focused onto a 75 cm long multimode glass fiber (50 μm core, 125 μm cladding, 0.30 NA, from 3M) with a 20X
microscope objective. The distal end of the fiber was situated in close proximity to the observation window of a 10 cm long (100 μm i.d., 365 μm od) fused silica capillary which was affixed onto a home-made Plexiglas capillary holder. The fluorescence was collected at right angles (with respect to the laser light) with a 40X (0.85 NA) microscope objective and imaged onto a slit serving as a spatial filter to reduce the amount of scattered photons generated at the air/glass and glass/liquid interfaces of the capillary from reaching the photon transducer. The fluorescence was further isolated from the scattering photons by a 780 nm (±10 nm) bandpass filter and a 780 nm long pass filter and finally, focused onto the photoactive area of the detector (single photon avalanche detector, EG&G Optoelectronics, Vaudrieulle, Canada) with another microscope objective (20X). The fused silica capillary was filled with a 50 nM solution of IR-144 fluorescent dye solution (Kodak Chemicals, Rochester, NY) and connected to the pump.

4.2.5 Construction of diffuser/nozzle system

The principle operational modes of the diffuser/nozzle system is depicted in Figure 4.3-A. There are basically two modes of operation, pump supply and pump delivery. During pump supply, the plunger of the syringe pump is retracted, causing sample to fill the pump chamber. During the pump delivery mode, the sample in the pump chamber is delivered to the chemical analysis device. The proper direction of fluid flow during the appropriate pump cycle is determined by two elements, a diffuser and nozzle, which have different pressure drops across them causing maximum flow from
one port (diffuser) and minimizing the flow in the other (nozzle). For optimal performance, the pressure drop across the diffuser should be much less compared to the nozzle so that the majority of the solution movement occurs preferentially through the diffuser. The pressure drop across the diffuser ($\Delta P_d$) and nozzle ($\Delta P_n$) can be calculated from [26];

$$\Delta P_d = \frac{\rho v^2}{2} \xi_d$$  \hspace{1cm} (4.1)

$$\Delta P_n = \frac{\rho v^2}{2} \xi_n$$  \hspace{1cm} (4.2)

where $\rho$ is the density of the solution, $v$ is the linear flow velocity through the narrowest part of the diffuser or nozzle element and $\xi$ is the pressure loss coefficient. To achieve efficient filling of the pump chamber $\xi_d/\xi_n > 1$, which can be accomplished by minimizing the pressure loss coefficient associated with the diffuser [33]. The pressure loss coefficients depend on a number of design parameters associated with the diffuser and the general topology of the diffuser (flat-walled versus conical). For a flat-walled diffuser, the minimum pressure loss occurs in a flow regime classified as transitory stall, where flow separation occurs at the wall of the diffuser. The physical dimensions of the diffuser which must be considered in order to operate in this regime, are $2\theta$ and the ratio, $L/d$, where these dimensions are defined in Figure 4.3-A. In the present case, $L/d = 41$ and $2\theta = 8^\circ$, which put the diffuser in the transitory stall regime.
Figure 4.3 (A) Operational modes of diffuser/nozzle system. The arrows represent the direction of fluid flow and the magnitude of fluid flow through the device during operation. (B) Scanning electron micrograph of the diffuser/nozzle system micromachined in PMMA. The channels were 50 μm deep.
The required channels were machined into the PMMA substrate using X-ray lithography following procedures previously described [20,34-36]. Briefly, an optical mask containing the required device topography was situated on a 5 cm square piece of PMMA which was coated with a 5 nm layer of Au/Cr, which served as a plating base, and a positive resist. After UV exposure, the resist was developed and a thick overlayer of Au (3 μm) was applied electrolytically to the developed areas to serve as the X-ray absorber during exposure. After the required Au layer was applied to the device, the remaining resist was removed and the device was placed in the X-ray beam and exposed to soft X-rays at our Center for Advanced Microstructure and Device facility. After exposure, the PMMA substrate was developed to remove exposed PMMA and then, after thorough cleaning, another piece of PMMA was thermally bonded to the diffuser/nozzle device. In Figure 4.3-B is shown an SEM micrograph of the micromachined PMMA. The depth of the channels was found to be 50 μm and the narrowest portion of the diffuser/nozzle was 30 μm with the widest part being 300 μm. Due to the ability to machine in PMMA using X-rays with high aspect ratios, the channel topology in the diffuser/nozzle was considered to be flat-walled and not conical.

After sealing the PMMA top sheet to the diffuser/nozzle, small diameter capillary tubes were inserted into the three ports of the diffuser/nozzle, one interfaced to the syringe pump, the other to the sample supply and the final port to the chemical analysis system. The capillary tubes possessed an i.d. of 20 μm and an o.d. of 40 μm. These
capillary tubes were inserted into the narrow PMMA channels by placing the tube on an XYZ micropositioner and then carefully inserting the capillary tube into the PMMA channel using an optical microscope for visual inspection. Once the capillary tube had been properly inserted into the device, it was sealed to it using epoxy.

4.3 Results and Discussion

4.3.1 Piezo-pump characteristics

The PA used in the fabrication of the pump was made from a thin-layered ceramic stack which allowed greater expansion. The translator expands between the casing and the magnetic top piece (PA-head). This particular piezo-stack is expected to produce a linear expansion of 80 µm and operate with a maximum pushing force of 2,000 N (pressure = 6.37 x 10^6 Pa for head area of 3.14 cm^2). Our microscopic observations revealed that the maximum expansion of the PA was 72 µm at an applied voltage of -1000 V when approximately 68.9 Pa (0.01 PSI) back pressure was loaded onto the syringe. This amounts to a 10% loss in linear displacement when little or no load was applied against the PA. The main purpose of using the pivoted lever was to amplify the PA expansion in order to permit higher volume displacements per pump stroke. The output displacement of the single-lever expansion unit was determined to be 648 µm which results in an expansion gain of 9.0.

4.3.2 Expansion and amplification of PA-movement during voltage ramp

The absolute expansion of the piezoelectric actuator and its amplification by the pivoted lever (with no load) was monitored as a function of a linear voltage ramp. A
plot of the observed PA and PA/lever linear displacement with respect to the progression of the voltage ramp is shown in Figure 4.4. In both the amplified and non-amplified linear displacements, no expansion was observed until the applied voltage reached \(\sim -100\) V. As can be seen from this plot, the displacement of the PA was fairly linear with applied voltage except at low applied voltages \((-100\) to \(-300\) V) and then at the high end of the voltage ramp as well \((-800\) to \(-1000\) V). This type of expansion profile can be expected due to hysteresis effects from this type of piezoelectric translator, particularly when operated without a position sensor. To obtain a linear expansion of the PA, a nonlinear voltage ramp can be used to compensate for these effects. It can also be observed from Figure 4.4 that the amplified displacement by the lever tracked the displacement of the PA without amplification fairly well with nonlinearities at the high and low ends of the applied voltage ramp. Assuming a 9X amplification factor by the lever, it can be seen from this plot that the amplification is less than 9 below an applied voltage of \(-200\) V and then exceeds 9 above an applied voltage of \(-600\) V.

4.3.3 Pump flow profile during voltage ramp

The volumetric flow rate (nl/min) is basically a function of two parameters; the slope of the applied voltage ramp (V/s) and the diameter of the micro-syringe. Since we used a linear voltage ramp, the pivoted lever amplified expansion was expected to be dependent on the absolute expansion behavior of the PA and the load pressure as well.
Figure 4.4 Linear displacement of piezo-head, non-amplified (squares) and amplified (circles), versus applied voltage. The linear displacement was determined by placing a short piece of capillary tube against the PA head and then monitoring the movement of the capillary tube under a stereomicroscope with a video screen that was calibrated.
The flow rate of latex beads was followed in a 2.56 mm long window of a 30 cm long 50 μm i.d. fused silica capillary (the viewing window was in the middle of the capillary). Based on our microscopic studies, no pumping action was seen until the voltage reached approximately -100 V with a load pressure (ΔP) of 0.22 PSI (1.5 x 10³ Pa) which was calculated using the expression:

$$\Delta P = \frac{8LQ\eta}{\pi R^4}$$  \hspace{1cm} (4.3)

where L is the length of the capillary tube (m); Q is the volume flow rate (m³/s); η is the solution viscosity (Pa·s); and R is the radius of the capillary (m). In this case, the solution viscosity was assumed to be near that of pure water (1 cP or 0.001 Pa·s). It should also be noted that due to the amplification by the pivoted lever, this load pressure is back amplified to the PA so that the actual load pressure at the PA head is 1.98 PSI (1.35 x 10⁴ Pa). In addition, this load pressure was calculated at the highest investigated volume flow rate (47.8 nL/min) and is expected to decrease at the lower volume flow rates. The observed flow patterns at three ramp speeds are shown in Figure 4.5. Qualitatively, we did not notice any pulsing movement of the microbeads resulting from pump steps in the ramp-speed range of 0.25 to 4.4 V/s in this 50 μm i.d. capillary.

However, in these experiments we did observe the beads close to the wall moving slower than those in the center of the capillary due to the parabolic nature of the laminar flow. As is apparent from Figure 4.5, the volume flow rate requires a fixed time period.
Figure 4.5 Volume flow rate versus applied voltage to the amplified PA head. The linear velocity was determined by observing the movement of the micro-beads in water moving through a 30 cm x 50 μm id capillary tube using a stereomicroscope. The volume flow rate was calculated by multiplying the observed linear velocity by the cross-sectional area of the capillary tube.
(applied voltage) to reach a constant value with the duration dependent upon the ramp rate (i.e., load pressure). For example, at a ramp rate of 1.96 V/s, the volume flow rate does not become constant until an applied voltage of ~ -400 V is reached, whereas in the case of a voltage ramp of 0.49 V/s, the volume flow rate reaches a constant value at an applied voltage of approximately -150 V. As shown in the graph inset of Figure 4.5, the volume flow rate was found to be linear (r = 0.9998) with the applied linear voltage ramp over the range investigated (0.49-1.96 V/s).

4.3.4 Effect of applied back-pressure

The dependence of liquid flow rate on applied back pressure at different voltage ramps was determined from microscopic experiments. A 2.0 m long 20 μm i.d. fused silica capillary was connected to the pump and filled with a latex bead solution. The liquid was pumped through the capillary at different flow rates which was controlled by varying the slope of the applied voltage ramp to the PA. This operation was repeated after reducing the length of the capillary by 0.3 m increments. The observed flow rates increased linearly with the slope of the voltage ramps for this series of capillaries which is illustrated in Figure 4.6-A. If the load pressure did not exert a perturbation on the volume flow rate, then these lines should exhibit similar slopes, independent of tube length. However, as can be seen from this graph, deviations are observed at high pumping rates (large pressure drops) for different lengths of capillaries indicating that the volume flow rate will show some dependence on load pressure. The effect of pressure-drop across the capillary on the observed flow rate is presented in
Figure 4.6-B in which the pressure was calculated from equation (3). As shown in the
Figure, the volumetric flow rate decreased with increasing pressure drop. Given a fixed
diameter and length of capillary, the magnitude of this effect (slope of volume flow rate
versus load pressure) increases with increasing pumping rate since the load pressure
also depends on the volume flow as well.

These effects can easily be rationalized based upon the fact that the PA can be
considered to act as an elastic body with a given stiffness and a changing load during
the expansion. The load pressure changes during the expansion since the flow velocity
increases during each voltage step, which results in reductions of expansion under load
conditions ($\Delta L$). This loss can be determined from the expression;

$$\Delta L = L_0 \frac{c_T}{c_T + c_S}$$  \hspace{1cm} (4.4)

where $L_0$ is the linear displacement under no load conditions ($72 \mu m$); $c_T$ (N/\(\mu m\)) is the
stiffness of the PA (45 N/\(\mu m\)) and; $c_S$ (N/\(\mu m\)) is the stiffness or spring constant of the
load. For a capillary of 20 \(\mu m\) id and a length of 2.0 m and a pump volume flow rate
of $\sim 47 \text{nL/min}$, the pressure drop at the PA head is 513 PSI (57 PSI x 9, where 9 is the
amplification factor of the pivoted lever) or $3.54 \times 10^6 \text{Pa}$. This results in a value of $c_S$
$= 15.4 \text{N/\(\mu m\)}$. Under these load conditions, the actual linear expansion is 54 \(\mu m\) or 13.6
nm/step (12-bit DAC) compared to 17.6 nm/step under no-load conditions. At a
driving voltage ramp of 2.44 V/s, a volume flow rate with this load was calculated to
be 55.5 nL/min which compared favorably to the observed 47.8 nL/min volume flow

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Figure 4.6 (A) Volume flow rate as a function of ramp speed (Vs⁻¹) for 20 μm id tubes of various lengths. The lengths of the capillary tubes were changed in 0.3 m increments so as to alter the load pressure on the PA head. (B) Volume flow rate versus load pressure at five different ramp speeds (Vs⁻¹). If the volume flow rate was not dependent on load pressure, then the slopes of these plots should be zero. The load pressure shown here does not account for the amplification factor associated with the pivoted lever. Therefore, the actual load pressure at the PA head should be multiplied by nine. The volume flow rate was calculated using the same procedure as described in Fig. 4.4.
rate measured under these load conditions (see Figure 4.6-B). Under no-load operation, the volume flow rate expected would have been 74.6 nL/min.

**4.3.5 Flow stability**

In order to examine the stability of the pump when operated under low volume flow rates, a dye solution was pumped through a 100 μm id capillary tube and the fluorescence monitored during the driving voltage ramp at three different ramp rates. When the dye molecules travel through an intense Gaussian laser beam, the integrated fluorescence intensity depends on the photoalteration parameter (F), which was determined using the expression [31];

\[
F = \frac{P \Phi_d \sigma}{\pi^2 \omega \nu}
\]  

(4.5)

where P is the average laser irradiance (photons/s); \( \Phi_d \) is the dye photodestruction quantum efficiency, defined as the probability that a molecule photodegrades once in the excited state; \( \sigma \) is the absorption cross section (cm\(^2\)); \( \omega \) is the 1/e\(^2\) laser beam radius (cm) and \( \nu \) is the linear flow velocity of the fluorescent dye molecule (cm/s). As can be seen from this expression, F depends inversely on the linear flow velocity and as such, will affect the integrated fluorescence intensity. Therefore, monitoring the fluorescence intensity will be a sensitive indicator of flow fluctuations produced by the pump. However, it should be pointed out that only under the conditions of 0.1 \( \leq F \leq 100 \) does the integrated fluorescence depend directly on the photoalteration parameter. When F < 0.1, no dye bleaches during its travel through the beam and when F > 100,
all dye molecules are immediately bleached upon entering the sampling volume. In the present case for the dye used in these experiments (IR-144); $P = 1.88 \times 10^{16}$ photons/s (5 mW at 750 nm); $\sigma = 2.4 \times 10^{-16}$ cm$^2$; $\omega = 25 \times 10^{-4}$ cm; $\Phi_d = 9 \times 10^{-7}$ and; with $v$ varied from $1.9 \times 10^{-5}$ cm/s to $8.2 \times 10^{-4}$ cm/s resulted in a photoalteration parameter which ranged from 47 to 1.1. The fluorescence intensity as a function of three different volumetric flow rates is displayed in Figure 4.7. As can be seen, the average intensity is seen to be a function of the flow rate, indicating that the photoalteration parameter is within the range where the fluorescence intensity does depend upon the linear flow velocity. Careful inspection of the data when the pump has reached a level where the average fluorescence intensity is constant demonstrates the lack of large fluctuations in the intensity which could arise from pulsations in the pumping action. However, there is some noise superimposed on these traces, most of which arises from Poisson noise (shot noise) in the counting experiment. This is particularly evident at the very low pumping rate (9.18 nL/min) where the average fluorescence intensity is low and the degree of photobleaching is high ($F = 47$).

4.4 Conclusions

We have fabricated a micro-syringe pump which consisted of a piezo-pusher and pivoted lever for amplifying the displacement of the piezoelectric. This pump can deliver volume flow rates in the low nL/min range, even under the conditions of high loading pressures where the peristaltic pumps may display difficulties. Since the pump will operate under high loads, it will be an important device for micro-fluidic
Figure 4.7 Pump stability at three different volume flow rates. The flow stability was determined by monitoring the fluorescence produced by the dye IR-144. The fluorescence was excited with 5 mW of laser power at 750 nm. The fluorescent dye was dissolved in methanol at a concentration of 50 nM.
applications where solutions must be pumped through narrow bore channels. In addition, the low volume flow rates that are achievable will allow manipulation of fluids in narrow channels which require long residence times, such as in the micro-chromatographic techniques or micro-based flow injection analysis. Another advantage associated with the present device is that pump-dependent noise resulting from pulsations is absent. However, a difficulty associated with this pump in its present format is the limited volume it can deliver per pump stroke. At a pump volume of 560 nL and a volume flow rate of 9.2 nL/min, it can effectively operate for 60 min before requiring to be refilled, but at a flow rate of 368 nL/min, the pump can only operate for 1.5 min. Another potential problem is the ruggedness of the device due to the need for the sophisticated pivoted/lever system. An alternative format for amplifying the linear displacement of the piezoelectric could reduce this difficulty, for example the implementation of a pulley system. In order to refill the pump automatically without requiring disconnecting the pump from the chemical analysis system, a diffuser/nozzle device was micromachined into PMMA to create a low volume pump chamber and channel network to allow ease of use. The micromachined diffuser/nozzle contains no moving parts and can allow directing flows in hydrodynamically driven systems. However, it should be pointed out that during operation of the diffuser/nozzle for pump refilling, discontinuities in the flow do result.
4.5 References


Chapter 5

Functionalization of Poly(methyl methacrylate) for Applications in Micro-Analytical Systems

5.1 Introduction

Over the past decade there has been an increasing interest in miniature bioanalytical systems which has been motivated in large part by a substantial interest in miniaturized total analysis systems (\(\mu\)-TAS) [1-5]. \(\mu\)-TAS are devices that can perform many of the fundamental functions of analytical chemistry and biochemistry laboratories, such as sample preparation, separation, and detection, on a small integrated platform. These systems are based on microfluidic channels on the order of 10 to 50 \(\mu\)m etched into substrates using photolithography. Using this technology, microfluidic components such as pumps, valves and detectors can be fabricated and integrated into \(\mu\)-TAS [6-10]. Despite the very appealing developments thus far, a number of critical issues such as overall cost, support material, detection schemes, fluid handling and injection still need to be addressed for these \(\mu\)-TAS to be widely accepted.

Initial approaches to fabricate these miniaturized systems using silicon wafer technology were hampered by the poor electrical characteristics of the silica material for electrophoretic experiments [11]. Although, glass and quartz can also be micromachined using similar photolithographic techniques and have superior electrical properties for micro-electrophoresis applications [8,9,12-70]. Glass is very amenable to spectroscopic detection schemes such as laser-induced
fluorescence [12,14,17,20,26,28,50,51,54,63,65,67,70-81] because of its favorable optical properties. Also, the dielectric breakdown and bulk resistivity are sufficiently high to allow high electric fields to be applied during CE separations allowing for faster analysis times. Plus, the surface modification methods for glass are well characterized allowing one to easily modify the channel walls of glass microdevices. However, the fabrication of glass microchips can be rather labor-intensive since an annealing step to cover the chip typically consists of 600°C for several hours and also, glass chips are not amenable to mass production by molding techniques which drastically increases the cost per device.

In the past few years, polymers have become attractive materials for fabricating microfluidic systems [7,75,76,80,82-87]. Poly(methylmethacrylate) (PMMA) in particular, is rugged, inexpensive, optically transparent, and amenable to photolithography using an X-ray synchrotron source [75,76,80]. PMMA is well-suited for working with aqueous, biological samples while it dissolves or swells in many non-polar organic solvents and thus may be difficult to use when using organic solvents, such as required for capillary electrochromatography. In addition, one would ultimately prefer a surface that is amenable to attachment of biomolecules through simple chemistry to create devices such as DNA digestion reactors or microarray devices. Also, in some instances, an EOF would be needed to transport analytes through reaction channels at controlled rates, as in the case of

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Figure 5.1 Electroosmotic profiles for PMMA and fused silica taken as a function of the buffer pH. The EOF's were measured by monitoring the migration time of a neutral near-IR fluorescent dye which was obtained from Li-COR (Lincoln, NE) with the appropriate applied electric field strength and buffer solution. Taken from [76].
DNA digestion reactors. Unfortunately, the EOF in PMMA is significantly smaller than that of glass and is not pH dependent (Figure 5.1) [76]. Therefore, mechanical pumps may be required to shuttle liquids through the device at reasonable rates or modification of the surface can be envisioned to support a larger EOF.

Fluid pumping in μ-CE devices is typically performed through electrokinetic forces, in which the intrinsic electroosmotic flow (EOF) generated by the surface charges of the substrate causes bulk flow of solvent. Positively charged ions in the buffer solution build near the wall forming what is called the electrical double layer and in an electric field, the positively charged layer is attracted toward the cathode causing a bulk flow of solvent toward the cathode. The potential difference in the double layer is referred to as the zeta (ζ) potential. The EOF can be expressed through the equation:

\[ v_{EOF} = \frac{\varepsilon \zeta}{\eta} E, \]  

(5.1)

or

\[ \mu_{EOF} = \frac{\varepsilon \zeta}{\eta} \]  

(5.2)

where \( v_{EOF} \) is the velocity of the EOF (cm/s), \( \mu_{EOF} \) is EOF mobility (cm²/V·s), \( \zeta \) is the zeta potential, \( \varepsilon \) is the dielectric constant of the solvent (C²/J·cm) and \( \eta \) is the viscosity (N·sec/m). The \( \zeta \) is a function of the surface charge on the wall, the pH and the ionic strength of the buffer. The EOF has been very well characterized in glass and has been found to be approximately \( 9.24 \times 10^{-4} \) cm²/V·s at very basic pH.

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values (pH > 9.0) with a 10 mM borax buffer [4]. This EOF results from the high
surface charges found on glass due to deprotonation of the silanol groups at basic
pH values creating a negative charge along the walls and an EOF flow toward the
cathode and also creating a pH dependent EOF. Although, many polymers do not
contain ionizable groups on the surface and have been shown to produce a much
smaller EOF [76,88]. Functionalization of the PMMA surface with ionizable
groups, such as amines, can lead to an increase in the surface charges, thus
increasing the EOF in these devices.

The PMMA surface was functionalized using a variation of the method
developed by Karandikar et al. where a diamine molecule was covalently attached
to the surface creating an amine-terminated PMMA surface [89]. By using simple
chemistry that has been developed for coupling biological molecules to amines [90],
DNAs and restriction enzymes can be covalently attached to the PMMA surface to
create simple bioreactors and microarrays.

In this chapter, a method will be described for chemically modifying the surface
of PMMA substrates and the utility of this method will be demonstrated through
several bioanalytical applications. Characterization of the surface was accomplished
using contact angle measurements and laser-induced fluorescence confocal
microscopy. The usefulness of this modification method will be demonstrated by
modifying PMMA beads and attaching restriction enzymes to perform solid-phase
DNA digestion and modifying PMMA sheets to perform DNA hybridization
studies. In addition, EOF measurements will be made on amine-modified surfaces.
5.2 Experimental

5.2.1 Functionalization Scheme for PMMA

The procedure for functionalizing PMMA was similar to that of Karandikar et al. with slight modifications (see Scheme 5.1) [89]. A 100 ml three-neck round bottom flask along with a stir bar was purged with dry nitrogen for twenty minutes prior to and during the reaction. Ethylene diamine (50 mmol) was then purged for 10 minutes before dropwise addition of N-butyl lithium (2.5 mmol in 10 ml hexane) under nitrogen at room temperature. After the reaction turned blue (30 min), an aliquot of the reaction mixture was transferred to a nitrogen purged vessel containing the PMMA to be functionalized. The reaction mixture was cannulated into the vessel and allowed to react with the PMMA for a maximum of 10 minutes. Any further reaction time discolored the PMMA. After 10 minutes, the PMMA reaction was quenched with water and the PMMA was rinsed with ddH₂O. The success of the reaction was monitored via contact angle measurements.

5.2.2 Contact Angle Measurements

Contact angle measurements were used to measure the wettability of the surface upon reaction with a lithiated diamine. The contact angle measurements were carried out at ambient temperature using ddH₂O. A droplet of 10 μL of ddH₂O was placed on the surface of the PMMA and imaged using a VCA 2000 Contact Angle System. The image was analyzed using the commercial software and the contact angle for the water-surface interface was calculated. Contact measurements were performed on both pristine (unmodified) and modified PMMA.
Scheme 5.1 Reaction scheme for (1) the formation of N-lithio ethylene diamine and (2) poly(methyl methacrylate-co-N-(2-amino ethyl)-methacrylamide).
5.2.3 Confocal Fluorescence Microscopy Images

In order to demonstrate the ability to label the amine terminated PMMA, a fluorescein isothiocyanate (FITC) fluorescent dye was conjugated to the surface and the fluorescence signal from the surface was monitored. After the PMMA surface was functionalized with the diamine, the sheets were cleaned by doubly rinsing with 2-propanol (IPA, Fisher Scientific, Fair Lawn, NJ) and 18 MΩ ddH₂O, then drying under nitrogen. The sheets were placed in the conjugation solution which consisted of 1 µM FITC, 1% IPA/99% H₂O, 10 mM Borate (pH = 9.2). The samples were held in the dark on a slow shake for 23 hours then triply rinsed with 18 MΩ ddH₂O, IPA and 18 MΩ ddH₂O and finally dried under nitrogen.

Two-dimensional confocal fluorescence images were collected using an Odyssey XL Confocal Laser Scanning Microscope (Noran Instruments, Middleton, WI) which was connected to an Argon ion laser (Coherent Lasers, Palo Alto, CA) for excitation at 488 nm. The fluorescence emission was collected with a 100X microscope objective then spectrally filtered using a 515 nm long pass and spatially filtered with a 10 µm slit before being detected with a charge-coupled device (CCD). One-hundred Z-series images were collected at a rate of 0.10 µm per image and each image was a jump average of 32 frames.

5.2.4 DNA Digestion Protocol

The procedure for derivatizing the surface of the PMMA prior to immobilization of the enzyme was similar to that described by Locascio-Brown et al. with slight modifications (Scheme 5.2) [90]. Approximately 0.1 g of spherically shaped
PMMA particles (6 μm diameter, Bangs Laboratory, Fishers, IN) was placed in a snap-cap vial to which a 5% (v/v) aqueous solution of glutaraldehyde (GA) was added and the mixture shaken continuously for 4 hours at room temperature. The GA-derivatized PMMA had a characteristic pink color. The supernatant was discarded and residual glutaraldehyde reagent was removed from the PMMA by extensive water washes. The PMMA was stored in de-ionized water at 4 °C until used.

For attachment of the restriction enzyme to the GA derivatized PMMA particles, approximately 9 μL (~0.00944 g) of the PMMA slurry was added to a snap-cap vial using a Gilson pipette. The reaction buffer (63 μL) was added to the slurry followed by addition of Hae III enzyme solution (18 μL, 900 U). The reaction mixture was kept on ice for about three hours with occasional mixing by gentle tapping. The reaction vessel was then incubated in a 37°C water bath for approximately 4 hours with occasional mixing and then placed on an ice water bath overnight. The following day, the vial was centrifuged and the supernatant fluid was removed. The resultant immobilized enzyme was washed three times with 200 μL aliquots of the phosphate buffer and once with 500 μL of water and stored in 30% BSA (bovine serum albumin). This BSA solution was found to preserve the activity of the enzyme until required for use.

5.2.5 CE Conditions

CE was performed using a Beckman P/ACE 5000 Series instrument (Beckman Instruments, Fullerton, CA) equipped with a 488-Ar ion laser. The fluorescence
was filtered through a 520 nm bandpass filter then imaged onto a photomultiplier tube. All separations were performed in 100 mM tris-borate-EDTA (TBE) (pH=8.3) and sieved using a 1% hydroxyethylcellulose (HEC) matrix. The capillary was a DB-17 nonpolar coated capillary (J&W Scientific, Folsom, CA) (50 μm i.d., 365 μm o.d.) The length was 27 cm with a window for detection 20 cm from the injection end. The samples were electrokinetically injected for 3 sec at -10 kV and separation was performed at a field strength of 296 V/cm. DNA/HaeIII restriction enzyme fragments (0.025 mg/mL) were stained with Yo-Pro-1 iodide (Molecular Probes, Eugene, OR) (500 ng/mL) fluorescence staining dye for detection.

5.2.6 DNA Hybridization on PMMA

Oligonucleotide hybridization experiments were performed on PMMA sheets (Goodfellow) cut into 20 x 50 mm rectangles. Before preparation, the PMMA slides were soaked in 10% isopropanol (v/v) for thirty minutes, rinsed with ddH₂O, and dried with compressed air. In order to derivatize the PMMA, the sheets were reacted with a diamine using Scheme 5.1. The treated sheet was then washed with ddH₂O. The PMMA sheet was then placed in the 5% glutaraldehyde solution for five hours. After treatment, the sheets were rinsed with ddH₂O. The derivatized sheet was then dried with compressed air and placed under a vacuum until use.

The oligonucleotide utilized was 5’-amino modified with a sequence of 5’-NH₂-
TTTTTTTTTTTGTGTTTTACAACGTCGTG-3’ with the complementary probe being a dye-labeled (IRD800) 19mer with a sequence of 5’-CACGACGTTGTAAAACGAC-3’. These oligonucleotides were placed in a 5 mM
Scheme 5.2 Reaction scheme for the attachment of the *Hae* III enzyme to 6 μm amine-terminated PMMA beads.
Scheme 5.3 Radiolabeling of a 34-mer ssDNA using terminal transferase enzyme. The labeling was accomplished with an α-32P ddATP to ensure the addition of only one radiolabel per ssDNA.
solution in HCO\textsubscript{3}/CO\textsubscript{3} buffer at a pH of 9.02. The DNAs were reacted to GA-
derivatized PMMA according to Scheme 5.2. After completion of the reaction, the
sheet was soaked in 1\% NH\textsubscript{3}•H\textsubscript{2}O for one minute and subsequently rinsed with
ddH\textsubscript{2}O. After drying with compressed air, the dye-oligonucleotide solution (100
nM dye-oligonucleotide, 5X saline sodium phosphate EDTA (SSPE), and 0.5%
SDS) was spotted onto the PMMA in blank as well as derivatized areas. The
hybridization reaction took place at 30° C in darkness for 30 minutes.

5.2.7 Determination of DNA Surface Coverage

In order to determine the amount of DNA attached to the surface of the amine
terminated PMMA, a \textsuperscript{32}P-labeled oligonucleotide was attached to the surface and
scintillation counting was performed. The reaction for the terminal tranferase
addition of the \textsuperscript{32}P-ddATP labeled ssDNA is shown in Scheme 5.3. The 3‘ end of a
5‘-amino modified oligonucleotide (5‘-NH\textsubscript{2}-TTTTTTTTTTTTTGTGTTTTACAGTGCTG-3‘) was labeled with a [\textalpha-\textsuperscript{32}P]ddATP using a 3‘-end labeling kit (Amersham–Pharmacia Biotech, 5000
Ci/mmol, Arlington Heights, IL). Purification of the labeled product was performed
with a Microspin G-25 column (Amersham–Pharmacia Biotech, Arlington Heights,
IL). The 35mer oligo was then covalently attached to the PMMA surface using
procedures described above. One microliter of a 420 nM oligonucleotide solution
was applied to 9 x 9 mm glutaric dialdehyde-derivatized PMMA piece at 37°C for 2
h. The PMMA surface was washed five fold with 1\% NH\textsubscript{4}OH followed by water.
Scintillation counting was then performed with a Beckman LS6000 scintillation
counter (Beckman Instrument Co., Fullerton, CA) and the surface concentration was calculated from the measurements.

5.3 Results

5.3.1 Contact Angle and LIF Confocal Microscopy Results

Contact angle ($\theta_a$) measurements are very sensitive to the surface structure and composition and have been used to monitor changes in monolayer composition [91,92]. In this study, contact angle measurements were used to determine the successful modification of a PMMA surface (see Scheme 5.1). Contact angle measurements for pristine and amine-modified PMMA are shown in Figure 5.2. For pristine PMMA, the methyl ester terminated surface is somewhat hydrophobic ($\theta_a (\text{H}_2\text{O}) = 76^\circ$). After reaction with a lithiated amine, the PMMA surface became more hydrophilic ($\theta_a (\text{H}_2\text{O}) = 45^\circ$), which suggests that the surface was now terminated with an amine functionality.

To correlate the contact angle studies with the change in composition of the surface, LIF confocal microscopy studies were performed. LIF confocal microscopy is a very sensitive method for detecting fluorescence on surfaces due to the fact that the signal-to-noise ratio is enhanced by reducing the background through the use of a pinhole (confocal imaging). The pinhole acts as a spatial filter, which removes much of the out of focus light. In this experiment, the surface of pristine and amine-terminated PMMA was reacted with FITC then monitored using LIF confocal microscopy with excitation from the 488 nm line of an argon ion laser. The results of the experiments are shown in Figure 5.3. In Figure 5.3-A, pristine
PMMA was reacted with FITC and shows no fluorescence upon excitation indicating that no reaction occurred since the isothiocyanate (SCN) labeling group of FITC is reactive toward primary amine functionalities and not methyl esters. In Figure 5.3-B, fluorescence from the FITC molecules can be observed which suggests that the FITC molecules were covalently attached through SCN linkages on the surface of the PMMA. The dark areas observed in Figure 5.3-B occur because of poor surface labeling of amine in those areas which could be caused by improper handling of the PMMA.

5.3.2 Solid Phase DNA Digestion on PMMA Surface

In order to show the application of functionalized PMMA beads to DNA digestion, the \textit{Hae} III restriction enzyme was covalently attached through a glutaric dialdehyde linkage to PMMA surfaces. The \textit{Hae} III restriction enzyme recognizes a four base sequence in double-strand DNA and cuts between G (guanidine) and C (cytosine) residues within this sequence. Digestion of the double-stranded DNA phage, \(\Phi X174-RF\) (5386 bp), with \textit{Hae} III produces 11 fragments and is commonly used as a standard for size determinations of linear, double stranded DNA from 72 to 1353 bp in length. In order to verify coupling of the restriction endonuclease to the functionalized PMMA and to determine if the enzyme would retain activity when attached to the PMMA surface, the digestion fragments produced were identified by their sizes using capillary electrophoresis. Shown in Figure 5.4 are the free solution and immobilized digests of \(\Phi X174-RF\) phage. The digestion buffer in each case (free solution and immobilized) was identical. From Figure 5.4, there is
Figure 5.2 Contact angle measurements of unmodified and amine terminated PMMA.
Figure 5.3 Laser-induced fluorescence confocal microscope images of (A) unmodified PMMA and (B) amine-terminated PMMA labeled with FITC. Excitation occurred at 488 nm with detection at 515 nm.
Figure 5.4 Capillary electrophoresis (CE) separations of (A) a free solution and (B) a PMMA immobilized enzyme digest of φX174-RF DNA (0.025 μg/μl). Laser-induced fluorescence detection was performed using the 488 nm output of an Argon ion laser and detection accomplished at 520 nm using a Beckman P/ACE 5010 CE instrument. Run conditions: 296V/cm, 27 cm L, 20 cm Ld; Inj. cond.: -10kV/3s; Run buffer was composed of 1%HEC in 100mM TBE (pH=8.3) with Yo-Pro1 (500 ng/mL) as the intercalating dye.
no detectable difference in the DNA digestion patterns which indicates that the recognition sequence for the enzyme was unaffected by immobilization to PMMA. The migration time for the free solution digest (Figure 5.4-A) was consistently slower than that of the immobilized case and can be explained by the fact that the free solution digest buffer is much more viscous than the CE running buffer. The change in viscosity could retard the migration of the fragments yielding longer analysis times.

5.3.3 Oligonucleotide Surface Concentration Determinations

The concentration of surface-bound oligonucleotides was calculated using $^{32}$P scintillation counting. The 35-mer ssDNAs were radioactively labeled with a $^{32}$P ddATP according to the reaction scheme shown in Scheme 5.2. Results from the scintillation counting experiments are shown in Table 5.1. Initially, $2.1 \times 10^{-13}$ moles of ssDNA were reacted with $2.1 \times 10^{-13}$ moles of $^{32}$P ddATP and 10 units of the terminal transferase enzyme in a cacodylate buffer in 50 μL total volume. After purification with a spin column, scintillation measurements were used to assess the efficiency of the labeling reaction. One μL of the purified, labeled reagent was added to 2 mL of scintillation cocktail and counted in triplicate. From the counting results, the labeling efficiency was determined to be 52% (± 3%) assuming no loss of oligonucleotides during purification. One μL of radioactive-labeled oligo was then reacted with the GA-functionalized PMMA surface and placed in 2 mL of the scintillation cocktail and counting experiments performed in triplicate. As shown in
Table 5.1 Results from Terminal transferase reaction and GA-functionalized PMMA surface labeling.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (moles)</th>
<th># of Molecules (36-mer DNA)</th>
<th>Labeling Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{32}$P Labeled-oligo</td>
<td>$2.18 \times 10^{-13}$</td>
<td>7000</td>
<td>0.52 (± 0.03)</td>
</tr>
<tr>
<td>Surface labeled oligo</td>
<td>$3.31 \times 10^{-22}/\mu m^2$</td>
<td>400/\mu m (± 25.0)</td>
<td>----</td>
</tr>
</tbody>
</table>

Table 1, the surface coverage of the DNA, after accounting for labeling efficiency, was determined to be 400 molecules per $\mu m^2$ ($3.31 \times 10^{-14}$ moles/cm$^2$) compared to $\approx 100$ pmol/cm$^2$ for comparable DNAs hybridized onto glass [93]. The decrease in oligo surface concentration in the PMMA case could be caused by several factors including; (1) lower concentration of surface functional groups (methyl esters), (2) poor reaction efficiency of lithiathed diamines to the methyl esters, (3) poor reaction efficiency of the GA to the amines or (4) poor labeling efficiency of oligos to GA terminated surface. Efforts to determine which of the variables is the limiting factor are ongoing.

5.3.4 Discussion of Hybridization Experiments

The DNA microarray is a high throughput technique to gain information about gene function, in which thousands of DNA samples are immobilized onto a microscope slide then hybridized to the complementary DNA (cDNA) in a manner that is very similar to the northern or Southern blot [94,95]. An ideal support for microarrays should; (i) allow effective covalent attachment of DNA to the surface, (ii) withstand high temperatures and washes at high ionic strength, (iii) be non-
porous to reduce hybridization volume and retain fast hybridization kinetics and (iv) have a low fluorescence background or auto-fluorescence to reduce noise. Typically, these microarrays are fabricated on glass or nylon supports [94,96-98].

PMMA has many of the same advantages as glass supports; in addition, it has been shown that the fluorescence background is reduced when using PMMA compared to glass slides when detection is accomplished in the near-infrared region [Waddell et. al., manuscript in preparation]. Finally, using our functionalization strategy, we can easily immobilize oligonucleotide strands to the surface of PMMA for analysis using fluorescence wavelength or lifetime discrimination.

In order to show the utility of using the PMMA functionalization scheme to create DNA microarrays, an amine-terminated synthetic oligonucleotide was attached to the surface of a PMMA sheet. Next, an IRD800 labeled DNA was hybridized to the functionalized PMMA sheet and the detection was accomplished using a fluorescence scanning microscope built in-house [Waddell et. al., manuscript in preparation]. The fluorescence scan of DNA hybridization is shown in Figure 5.5. The surface concentration of the oligonucleotide fragments was determined to be 400 molecules per $\mu$m$^2$ from scintillation measurements. Using that measurement, the number of DNAs sampled was estimated to be $\approx 7.6 \times 10^3$. This equates to a limit of detection of $\approx 4$ copies of DNA at a SNR = 3. Also, by scanning the spotted area after denaturation of the hybridized DNA, no noticeable increase in the background noise was observed due to residual DNAs adsorbing to
Figure 5.5 Fluorescence scan of DNA hybridization. Fluorescent dye (IRD800) terminated DNA was hybridized to complementary strand 35mer DNA attached to the GA derivatized PMMA surface. The excitation source was the 780 nm output of a Ti:Sapphire laser (1mW, CW mode) with detection at 825 nm using a single photon avalanche diode (SPAD). After the initial hybridization scan the DNA was denatured and the surface was probed for residual DNAs. A second hybridization was performed and scanned in the same manner.
the surface of the PMMA. In addition, the spot could be reprobed by performing a second hybridization assay with very little loss in signal.

5.4 Conclusion

The ability to create an amine-terminated PMMA surface for biological applications was demonstrated in this chapter. The surface characteristics were monitored using contact angle measurements and LIF confocal laser microscopy. Also, the utility of such a functionalized system was demonstrated through the attachment of biomolecules to the functionalized surface of the PMMA. A DNA restriction digestion enzyme (*Hae* III) was immobilized to the surface of the PMMA through a glutaric dialdehyde linkage and was shown to adequately digest a DNA phage (φX174-RF) while removing the enzyme from the ensuing electrophoresis separation.

Single-stranded oligonucleotides were then immobilized onto a functionalized PMMA surface in an order to demonstrate the usefulness of this method for developing DNA microarrays. The ssDNA was immobilized then hybridized to a fluorescently labeled complementary ssDNA and detected using near-infrared (NIR) fluorescence. The surface concentration of the immobilized oligo was determined to be 400 molecules, which equates to a detection limit of ≈ 4 copies for the spotted DNA.

This method will be very useful when developing microfluidic applications with PMMA substrates, such as μ-CE devices. Devices can be fabricated and then simply functionalized before the assembly of the completed device.
5.5 References


6.1 Introduction

Recently, there has been considerable interest in microfabrication of capillary electrophoresis devices, in which the sample handing and detection devices are integrated onto one platform [1-8]. To date, the majority of microchip capillary electrophoresis (μ-CE) applications have chosen laser-induced fluorescence (LIF) as the detection strategy [7,9-26]. While LIF is a sensitive detection method for μ-CE devices, with single-molecule detection recently being demonstrated on a chip using LIF [27], the instrumentation required is bulky and detection is limited to compounds which intrinsically fluoresce or are amenable to fluorescence derivatization. Limited work has been explored in miniaturizing detection schemes for on-chip applications [19,28-30]. Electrochemical detection would easily lend itself as a sensitive detection scheme for μ-CE. Electrochemical detection would eliminate the need for tedious alignment of optical systems and requires minimal cost and power to implement. Electrochemical detection has already proven to be a valuable scheme for conventional capillary electrophoresis providing LODs ~6.6 amol [29]. Woolley et al. [28] reported on μ-CE devices with integrated amperometric detection, based on photolithographic placement of the working electrode at the end of the exit channel. Recently, Wang et al. [29] demonstrated electrochemical detection on μ-CE devices by sputtering the working electrode directly onto the channel outlet.
In this chapter, the miniaturization and characterization of a planar conductivity detector on a µ-CE device is described. The conductivity cell was micromachined using LIGA wafer processing in poly(methyl methacrylate). This method demonstrates a simple route for developing a conductivity detector from miniature wires used in microelectronic devices and eliminates the need for deposition of electrodes photolithographically or by sputtering methods. The analytical performance of the conductivity detector is demonstrated by analysis of KCl.

6.2 Experimental

6.2.1 Apparatus

The PMMA microchip was fabricated using LIGA wafer processing. A Kapton X-ray mask was made using standard photolithographic procedures previously published [13]. A mold template was created by annealing PMMA to a 5 x 5 cm titanium wafer. The PMMA was then fly-cut to the desired thickness (≈ 65 µm). The mold template was then exposed through the X-ray mask using a synchrotron source (CAMD, Baton Rouge, LA). The exposed PMMA was developed, which created the mirror image of the mold insert. The mold insert was created by electroplating Ni (Fisher Scientific, Houston, TX) in the exposed channels and overplating Ni to create a backbone for the mold insert. Hot embossing of PMMA was employed to develop the PMMA microchip devices using the device mold. The device mold and the PMMA square were placed on the stainless steel platens of a mechanical press. The temperature of the top platen (PMMA) was held at 106°C.
while the temperature of the bottom platen (mold insert) was held at 160°C while a total mass of 125 kg/ cm\(^2\) was applied for 5 minutes.

The topographical layout of the chip is shown in Figure 6.1. The device contains four fluid wells (A, B, C, D), where A and B are the buffer wells and C and D are the sample and waste wells, respectively. The channel between points A and B defines a 4 cm separation channel (60 μm wide × 65 μm deep), where the effective length is 3 cm (injection to detection). In the inset (F) is shown a blow-up of the conductivity cell, where 1 and 2 are the widths of the channel in the separation (60 μm) region and detection (30 μm) region. The platinum wires (60 μm × 30 μm) were inserted into the device under a 40X stereomicroscope. An optical micrograph of the assembled conductivity cell is shown in Figure 6.2. The distance between the platinum wires is approximately 30 μm. An exact distance was difficult to calculate due to the fact that the channel dimensions in the conductivity cell were distorted because of problems with the mold insert. The depths of the channels were determined by the use of a Tencor P-11 surface profilometer (KLA-Tencor, San Jose, CA) equipped with a 12.5 μm tip.

6.2.2 Chemicals

Eighteen MΩ distilled H\(_2\)O was used for all dilutions of metals and buffer solutions and was filtered through a 0.45 μm filter (Gelman Acrodisc). The electrophoresis buffer consisted of an acetate buffer (15mM, pH 4.7). All metal chlorides were 99+% grade and were obtained from Aldrich Chemical Co. (Milwaukee, WI).
Figure 6.1 Topographical layout of the \(\mu\)-CE chip. A, B, C and D are fluid wells (1 mm dia.) where A and B are buffer wells and C and D are the sample and waste wells, respectively. The total length of the separation column (60 \(\mu\)m wide \(\times\) 65 \(\mu\)m deep) is 4 cm, and the effective length is 3 cm. E denotes the conductivity detector (CD), where F is a magnified view of the CD cell. The electrode channels are 60 \(\times\) 65 \(\mu\)m and the space between the electrodes is 30 \(\mu\)m.
Figure 6.2 Optical micrograph of the conductivity cell. The Pt wires (30 μm x 60 μm) were inserted into the channels then sealed under a 1mm thick PMMA top. The dimensions of the separation channels in the conductivity detector are 65 μm deep x 30 μm wide.
6.2.3 Conductivity Protocol

Conductivity data was collected as in Chapter 3 using the bipolar-pulsed technique. The pulse frequency was 5000 Hz and the feedback resistor was 10 MΩ. The cell potential was set at 500 mV for all measurements. Various concentrations of potassium chloride dissolved in 50 mM sodium acetate were pressure pumped through the channels of the μ-CE device using a Harvard Apparatus 22 syringe pump at a rate of 10 μL/min. Before each measurement, the flow was allowed to equilibrate for 10 minutes. Three measurements of 100 data points were made at each concentration and a calibration plot was created from an average of the three measurements at each concentration.

6.3 Results and Discussion

6.3.1 Characterization of the μ-CE device

One of the primary advantages of development of the conductivity detector on-chip is the simplicity of the design and construction. Also, the detection is performed on column unlike most capillary electrophoresis designs using conductivity detection, which can increase the efficiency of the separation process by reducing extra column effects and reducing the detector cell volume [31-39]. Scanning electron microscopy (SEM) images of the conductivity (CD) cell and various other regions on the mold insert are shown in Figure 6.3. In 6.3-A is shown an image of the CD cell where the wire channel is 60 μm wide × 65 μm deep and the separation channel narrows from 60 μm to 30 μm in the CD cell to decrease the spacing
between the electrodes. A 190x magnification of the CD cell is shown in Figure 6.3-E, where the damage to the separation channel can be clearly seen in the boxed region. The damage was caused by mishandling of the mold insert during development. Although the detector region was damaged, flow could be established, while the device bonding was weak in the region of the CD cell because of the non-uniform surface. Figure 6.3-D shows an image of the surface of the mold insert (i.e. the bottom of the channel). As one can see, the surface roughness is high which can decrease efficiencies in \( \mu \)-CE separations by creating eddy diffusion in the pits. The roughness by the surface oxidation of the Ti wafer used as the plating base. This was necessary to improve the adhesion of the PMMA to the Ti plate. For future development, this issue can be resolved by polishing the surface after electroplating.

In Figure 6.4 is shown SEM images of silver coated embossed PMMA-devices. Figure 6.4-A shows a low magnification (50x) image of the CD cell. Close inspection (Figures 6.4-B, C) reveals that the defects observed in the mold insert were manifested in the embossed PMMA device, indicating that replication errors during embossing were minimal. Also from the figures, the rounded channel sidewalls are observed. This is a function of the embossing process. Typically, hot embossing is performed in a heated press under a vacuum where the mold insert and the PMMA are placed in separate alignment jigs. The jigs serve two purposes, (1) alignment of the substrates and (2) separation of the substrates when the force is released so that no unnecessary force is placed on the features of the plastic device. In this case, no alignment jigs were used and the substrates were
Figure 6.3 Scanning electron micrographs of the Ni mold insert. The micrographs show conductivity cell (A,E), the injection area (B), a fluid well (C), and an electrical contact area for the conductivity detector (D). Also, in (D) is shown the surface roughness of the mold insert.
separated after removal from the press mechanically, thus causing forces to be applied perpendicular to the wall creating the rounded side-walls. In Figure 6.4-D the surface roughness from the mold insert is observed on the PMMA device in the bottom of a fluid well.

6.3.2 Conductivity Measurements On-a-chip

In order to determine the analytical figures of merit of the miniaturized conductivity detector (linearity of response and concentration limit of detection), experiments were performed by pressure pumping various concentrations of KCl through the conductivity cell. In conductivity, the response results from a differential measurement between the buffer solution and that of the sample and the background will have a profound influence on the SNR. It is imperative that background conductivity associated with the buffer solution be reduced so that the signal-to-noise ratio is increased. This can be accomplished by taking into account the equivalent ionic conductances of the ions that comprise the buffer solution. A table of equivalent ionic conductances for several common ions is shown in Table 6.1. The effect of the equivalent ionic conductances on the conductance (G) can be calculated by using the following equation;

\[ G = \frac{(\lambda_+ + \lambda_-)C}{1000K} \]

where \( \lambda_+ \) and \( \lambda_- \) are the equivalent conductances of the cationic and ionic species (\( \mu \Omega^{-1}\text{-cm}^{-2}\text{-equiv}^{-1} \)), C is the concentration of the sample in equivalents (M) and K is
Figure 6.4 Scanning electron micrographs of the hot embossed PMMA. A thin layer of Ag has been sputtered over the PMMA to ensure that it will be conductive. The images show the conductivity detector (cd) cell (A, B, and C) at different magnifications and a fluid well (D). The surface roughness can also be seen in D.
the cell constant (cm$^{-1}$). In this case, a 50 mM sodium acetate buffer was chosen. From Table 6.1, the equivalent ionic conductances are given to be 50 (Na$^+$) and 41 (CH$_3$CO$_2^-$) for the buffer system chosen and by taking into account the cell constant (L/A) where L is the distance between the electrodes (cm) and A is the area of the electrodes (cm$^2$). A cell constant of 167 cm$^{-1}$ was calculated for this system which leads to a predicted G value of 2.72 $\times$ 10$^{-5}$ $\mu$Ω$^{-1}$ compared to an actual value of 154 cm$^{-1}$. A common practice to measure the cell constant is to measure the conductance of a dilute solution of known specific conductance (i.e. 1 mM KCl) then calculate the cell constant from the following equation;

$$ k = G K $$

(6.2)

where k is the specific conductance (Ω$^{-1}$), G is the conductance and K is the cell constant. The specific conductance (k) can be calculated from the following equation;

$$ k = \frac{C(\lambda_+ + \lambda_-)}{1000} $$

(6.3)

Shown in Figure 6.5 is a calibration plot for pressure pumped KCl. Five data points were taken in triplicate over one order of magnitude with a linear correlation coefficient of 0.9951. The concentration limit of detection was calculated to be 5 ppm (SNR=3), which is approximately 2 orders of magnitude less than the LOD for the LC conductivity cell reported in Chapter 3. The difference in the two detectors is the change in the cell constant, which is caused by the significant decrease in the area

154
Table 6.1 Limiting equivalent ionic conductances for several common anions and cations in aqueous solution at 25°C [40].

<table>
<thead>
<tr>
<th>Anions</th>
<th>λ_</th>
<th>Cations</th>
<th>λ_+</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH^-</td>
<td>198</td>
<td>H^+</td>
<td>350</td>
</tr>
<tr>
<td>F^-</td>
<td>54</td>
<td>Li^+</td>
<td>39</td>
</tr>
<tr>
<td>Cl^-</td>
<td>76</td>
<td>Na^+</td>
<td>50</td>
</tr>
<tr>
<td>Br^-</td>
<td>78</td>
<td>K^+</td>
<td>74</td>
</tr>
<tr>
<td>I^-</td>
<td>77</td>
<td>NH_4^+</td>
<td>73</td>
</tr>
<tr>
<td>NO_3^-</td>
<td>71</td>
<td>Mg^{2+}</td>
<td>53</td>
</tr>
<tr>
<td>HCO_3^-</td>
<td>45</td>
<td>Ca^{2+}</td>
<td>60</td>
</tr>
<tr>
<td>Formate</td>
<td>55</td>
<td>Sr^{2+}</td>
<td>59</td>
</tr>
<tr>
<td>Acetate</td>
<td>41</td>
<td>Ba^{2+}</td>
<td>64</td>
</tr>
<tr>
<td>Propionate</td>
<td>36</td>
<td>Zn^{2+}</td>
<td>53</td>
</tr>
<tr>
<td>Benzoate</td>
<td>32</td>
<td>Hg^{2+}</td>
<td>53</td>
</tr>
</tbody>
</table>
Figure 6.5 Calibration plot for KCl. The plots was constructed by pressure pumping KCl through the conductivity cell averaging the signal over three runs. The KCl was dissolved in a 50 mM sodium acetate (pH=5.0).
between the two electrodes with the area for the μ-CE CD cell being $1.80 \times 10^{-5}$ cm$^2$ and $1.05 \times 10^{-3}$ cm$^2$ for the LC CD cell and the electrode spacing with the electrode spacing being $30 \, \mu$m and $60 \, \mu$m for the μ-CE and LCCD cells, respectively.

6.4 Conclusions

A PMMA μ-CE device was fabricated with an integrated conductivity detector. The topography of the device was monitored using a SEM and optical micrographs. Fluids were pressure pumped through the device channels and the analytical merits of the conductivity detector were tested by pressure pumping KCl though the CD cell. A concentration limit of detection was determined to be 5 ppm (SNR=3) ($R=0.9951$).

6.5 References


[34] F. Foret, M. Deml, V. Kahle, P. Bocek, Electrophoresis 7 (1986) 430.


Chapter 7
Summary and Future Work

7.1 Summary

In Chapter 1, a review of the current technologies used to develop \( \mu \)-CE devices was given and intended to give the reader a basic understanding of these processes. Also, techniques used to characterize these devices in addition to fluidic pumping in \( \mu \)-CE devices was discussed.

In Chapter 2, the reader was introduced to the various detection schemes that have been utilized in microelectrophoresis devices. A general theoretical description of several techniques were discussed and design considerations and current instrumental approaches to microelectrophoresis detection implementing each scheme were also discussed. The chapter was restricted to only those detection schemes which have been implemented in \( \mu \)-CE devices.

In Chapter 3, the application of \( \mu \)-reverse-phase high-pressure liquid chromatography (\( \mu \)-RP-HPLC) for the separation and/or purification of polymerase chain reaction (PCR) products with detection accomplished using a miniaturized conductivity detector was described. The conductivity detector used two Pt wires and a bipolar waveform applied to the electrode pair from which the conductivity of the solution could be measured. In the mobile phase used for the \( \mu \)-RP-HPLC separation of the PCR product, the mass detection limit for Herring sperm DNA using conductivity was found to be 11 ng. Efficient separation of the amplified PCR amplicon from the other reagents present in the PCR cocktail was achieved in
less than 4 minutes with a capacity factor of 2.5 and separation efficiency of $9.1 \times 10^3$ plates. The separation was carried out using reverse phase ion-pair chromatography with a triethylammonium acetate ion-pairing agent.

In Chapter 4, a novel computer-controlled mechanical syringe-pump was described. The pump uses a piezoelectric actuator and a pivoted lever for amplification of the linear displacement of the piezo to deliver solvents free from pump pulsations at volumetric flow rates approaching one nanoliter per minute even at high loading pressures. The flow patterns could be programmed by controlling the voltage waveform to the piezo-actuator to produce a linear displacement of 72 µm. By using the pivoted lever, a nine-fold amplification of the piezo-expansion was achieved producing a total linear displacement of 648 µm. When a gas-tight glass syringe with a 1.0 mm diameter was interfaced to the piezo pump, the total volume delivered in a single pump stroke was 511 nL. While the pumping profile was governed by the expansion behavior of the piezoelectric actuator, the flow-rate was slightly affected by the loading pressure on the pump as well. The piezo-pump was found to adequately deliver stable flow of solutions with loading pressures as high as $3.79 \times 10^5$ Pa (actual loading pressure at the piezo = $3.41 \times 10^6$ Pa). Monitoring the flow stability using fluorescence indicated that the volume flow was fairly noise-free at pumping rates from 4-150 nL/min. Below the volume flow rate of 4 nL/min, the pump exhibited extensive noise characteristics due to the step resolution of the DAC driving the piezo actuator. A diffuser/nozzle system was fabricated which allowed automatic refilling of the syringe pump and
was micromachined in Plexiglas (PMMA) using X-ray lithography. The diffuser/nozzle system contained channels that were 50 μm in depth and tapered from 300 to 30 μm. The diffuser/nozzle system was interfaced to the syringe pump by connecting conventional capillary tubes to the PMMA-based diffuser/nozzle, the piezo-pump and the chemical analysis system.

In Chapter 5, the ability to create an amine-terminated PMMA surface for surface modification/functionalization was demonstrated. The surface characteristics were monitored using contact angle measurements and LIF confocal laser microscopy. Also, the utility of such a functionalized system was demonstrated through the attachment of biomolecules to the functionalized surface of PMMA. A DNA restriction digestion enzyme (Hae III) was immobilized to the surface of the PMMA through a glutaric dialdehyde linkage and was shown to adequately digest a DNA phage (φX174-RF) while removing the enzyme from the ensuing electrophoresis separation. Single-stranded oligonucleotides were immobilized onto the functionalized PMMA surface in an order to demonstrate the usefulness of this method for developing DNA microarrays. The ssDNA was immobilized then hybridized to a fluorescently labeled complementary ssDNA and detected using NIR fluorescence detection. The surface concentration of the immobilized oligo was determined to be 400 molecules which equates to a detection limit of ≈ 4 copies for the spotted DNA.

In Chapter 6, a PMMA μ-CE device was demonstrated with an integrated conductivity detector. The topography of the device was monitored using SEM and
optical micrographs. Fluids were pressure pumped through the device channels and
the analytical merits of the conductivity detector were tested by pressure pumping
KCl through the CD cell. A concentration limit of detection was determined to be
5 ppm (SNR=3) (R=0.9951).

7.2 Future Work

Future work should include studying the effects of electrode placement on
detection sensitivity for conductivity detection in a μ-CE device. For conductivity
detection to be a viable screening method for environmental or biological
applications, detection limits must be sub-part-per-million to regulatory
requirements. There are several ways to increase the detection sensitivity for
conductivity measurements; (1) increase the potential between the electrodes, and
(2) decrease the L/A ratio (the ratio between the distance (L) between the electrodes
and the sampling area (A) of the electrodes).

Also, the attachment of C18-thiocyanate terminated molecules to the amine
functionalized PMMA surface could be used in reverse phase ion-pairing
separations of DNA on a microchip, which can be integrated with the conductivity
detector cell. Henry et al have shown that C18-thiocyanate terminated molecules
can easily be attached to amine functionalized PMMA [Henry et al., manuscript in
preparation]. This procedure can be employed to immobilize C18 groups inside
narrow channels (<20 μm i.d.) for open tubular reverse phase separations.

Microposts can be constructed inside microfluidic channels using high aspect
ratio micromachining (HARMS), a subset of LIGA micromachining, which can act as
a solid support to attach C18 groups for reverse phase capillary electrophoresis (CEC) separation of DNAs coupled with conductivity detection. The CEC separation eliminates the need for using gel matrices to purify simple mixtures such as PCR reactions. Also, enzymes can be attached to the microposts using the PMMA functionalization chemistry from Chapter 5 so that microelectrophoresis devices can be created with integrated enzyme digestion chambers, which can be applied to both DNA and RNA applications.
Vita

Christopher S. McWhorter was born in Mineral Wells, Texas on March 9, 1971. He is the middle of three children (Skeet, Scott, and Neely) born to Larry and Carole McWhorter. He attended Matty Akin and Lucy Webb elementary schools from 1976-1985. He attended Greenville Christian School from 1985-1989. There he was a member and 4-year letterman on the varsity football, basketball, cross-country and track teams. He graduated from Greenville Christian School in 1989.

In the fall of 1989, he entered Delta State University in Cleveland, Mississippi. He received a bachelor of science degree in chemistry in the Spring of 1994. In the Fall of 1994, he entered the graduate program in the Department of Chemistry at Louisiana State University in Baton Rouge, Louisiana. He is currently a candidate for the degree of Doctor of Philosophy in analytical chemistry. Upon graduation, he plans to begin his career as a post doctoral research chemist with Westinghouse Savannah River Site in Aiken, South Carolina.
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Major Field: Chemistry

Title of Dissertation: Development of Miniaturized Devices Using X-Ray Microlithography in Poly (Methyl Methacrylate) for Chemical Monitoring and Microfluidic Applications

Approved:

Major Professor and Chairman

Dean of the Graduate School

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