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Fabrication of Polymeric Microfluidic Devices for Protein Analysis

Jikun Liu
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FABRICATION OF POLYMERIC MICROFLUIDIC DEVICES FOR
PROTEIN ANALYSIS

by

Jikun Liu

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry and Biochemistry
Brigham Young University

April 2006
of a dissertation submitted by

Jikun Liu

This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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As chair of the candidate’s graduate committee, I have read the dissertation of Jikun Liu in its final form and have found that (1) its format, citation, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrated materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

FABRICATION OF POLYMERIC MICROFLUIDIC DEVICES FOR PROTEIN ANALYSIS

Jikun Liu
Department of Chemistry and Biochemistry
Doctor of Philosophy

2-Bromoisobutyryl bromide was immobilized on poly(methyl methacrylate) (PMMA) substrates activated using an oxygen plasma. Atom-transfer radical polymerization was then performed to graft poly(ethylene glycol) (PEG) on the PMMA surface. PMMA micro capillary electrophoresis (µCE) devices made with the covalently modified surfaces exhibited substantially reduced electroosmotic flow and nonspecific adsorption of proteins. Both column efficiency and migration time reproducibility were one order of magnitude better with derivatized PMMA µCE devices compared to untreated versions. Fast, reproducible, and efficient separations of proteins and peptides were demonstrated using the PEG-grafted PMMA µCE chips. All analyses were completed in less than 60 seconds, and separation efficiencies as high as $5.3 \times 10^4$ plates for a 3.5-cm long separation channel were obtained.

A surface reactive acrylic polymer, poly(glycidyl methacrylate-co-methyl methacrylate) (PGMAMMA), was synthesized and evaluated for suitability as a substrate for fabrication of microfluidic devices for chemical analysis. This polymer has good
thermal and optical properties, and is mechanically robust. A key advantage of this polymeric material is that the surface can be easily modified to control inertness and electroosmotic flow using a variety of chemical procedures. In this work, the procedures for aminolysis and photografting of linear polyacrylamide on microchannel surfaces in PGMAMMA substrates were developed, and the performance of the resultant µCE devices was demonstrated for the separation of amino acids, peptides, and proteins. Separation efficiencies as high as $4.6 \times 10^4$ plates for a 3.5-cm long separation channel were obtained.

Finally, a novel approach was developed to integrate a buffer ion permeable membrane in a PGMAMMA micro electric field gradient focusing (µEFGF) device. Using the µEFGF device, green fluorescent protein (GFP) was concentrated 4000-fold. Separation of GFP and R-phycoerythrin (R-PE), and selective elution of GFP from a protein mixture containing GFP, FITC-labeled casein, and FITC-labeled hemoglobin were also demonstrated. It was found that the volume and concentration of buffer and presence of carboxylic acid impurities in the membrane, which control the conductivity and ion transport properties of the membrane, strongly affected the behavior of the µEFGF device.
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1 INTRODUCTION

1.1 Polymeric Micro-Total-Analysis Systems

1.1.1 Introduction to Micro-Total-Analysis Systems

Micro-total-analysis systems (µTAS) are microfabricated fluidic analyzers incorporating sample pretreatment, separation, and detection subsystems. Since concept establishment by Manz et al. in 1990, explosive progress has been achieved in the area of µTAS, and many applications have emerged in fields covering chemistry, biology, physics, environmental science, forensics, medicine, defense, and space exploration.\textsuperscript{1-3}

In a microfabricated analytical system, the transport of molecules is dominated by diffusion. If we express Fick’s first law of diffusion

\[ J = -D \nabla C \]  \hspace{1cm} (1.1)

in another format, we obtain

\[ t = \frac{l^2}{D} \]  \hspace{1cm} (1.2)

where \( J \) is the flux of individual molecules, \( D \) is the diffusion coefficient, \( \nabla \) is the Laplace operator, \( C \) is the concentration, \( t \) is the diffusion time, and \( l \) is the transport distance. It can be learned from Equation 1.2 that as the dimensions of the system decrease, a quadratic decrease in diffusion time will be achieved; in other words, achievement of diffusion-related equilibria in a microfabricated system should be fast.\textsuperscript{4}

When an external electric field is used to move analytes in the fluidic system, Joule heat will be generated, which limits the maximum electric field, \( E_{\text{max}} \), one can apply.\textsuperscript{5} According to Equation 1.3,

\[ E_{\text{max}} = \frac{1}{h} \sqrt{\frac{2}{\pi \lambda c}} \]  \hspace{1cm} (1.3)
where $E_{\text{max}}$ is the maximum electric field strength, $h$ is the channel height or capillary diameter, $\lambda$ is the molar conductivity of the buffer, and $c$ is the buffer concentration, by decreasing the channel dimensions, higher potentials can be applied.\(^{5}\) As a result, higher electrophoretic separation efficiency and shorter analysis time should be obtained in microfabricated electrophoresis devices.

In pressure-driven microfabricated fluidic systems, where the channel dimensions are on the micrometer scale, the flow is within the laminar flow region. According to the total dispersion equation

$$D_T = D + \frac{v^2d^2}{192D}$$

(1.4)

where $D_T$ is the total dispersion of a solute, $v$ is the velocity of the bulk flow, and $d$ is the hydraulic diameter, which is expressed as

$$d = \frac{2hw}{h+w},$$

(1.5)

where $h$ is the depth and $w$ is the width of the fluidic channel,\(^{6}\) as the channel dimensions are reduced, the dispersion of analytes will decrease until it reaches a value equal to the analyte diffusion coefficient. Consequently, the peak width will decrease and the performance of the pressure-driven separation system will be improved. Furthermore, microfabricated analytical systems consume less sample and reagents; therefore, less waste is generated. The most fascinating feature of µTAS is that multiple functions including sample pretreatment, separation, and detection can be incorporated within a single device.\(^{7,8}\) Furthermore, high-throughput analysis can be realized using arrays containing parallel µTAS units.\(^{9,10}\)
1.1.2 Fabrication of Microfluidic Devices Using Inorganic Materials

**Inorganic materials.** Many microfabricated fluidic analyzers or microfluidic devices were fabricated using inorganic materials including silicon,\textsuperscript{11-13} glass,\textsuperscript{14-16} and quartz.\textsuperscript{17-19} Among these three materials, silicon is not transparent to visible or ultraviolet (UV) light; in addition, the breakdown voltage of silicon is relatively low.\textsuperscript{20} As a result, microfluidic devices fabricated completely in silicon are rare, while silicon-glass hybrid microfluidic devices can be found quite often.\textsuperscript{12, 13} In comparison, glass has good optical, mechanical, electrically insulating and thermal properties; moreover, the surface chemistries of glass have been well-established. Therefore, glass has become the dominant inorganic material in microfluidic device fabrication. Quartz or fused quartz is an extremely pure form of silicon dioxide and it has superior physical and optical properties over other inorganic materials for microfabrication. Unfortunately, due to its high cost and difficult fabrication procedures, quartz is not widely utilized. Nonetheless, in some special circumstances, for example, where UV detection (200 nm ~ 300 nm) is required, quartz must be used.

**Fabrication of inorganic microfluidic devices.** Inorganic microfluidic devices are fabricated using a process consisting of pretreatment, standard photolithography, etching, and bonding (Figure 1.1). Before fabrication, the substrates are thoroughly cleaned using mixtures such as NH\textsubscript{3}/H\textsubscript{2}O\textsubscript{2}, H\textsubscript{2}SO\textsubscript{4}/H\textsubscript{2}O\textsubscript{2}, or NH\textsubscript{4}F/ HF.\textsuperscript{21} Then, sacrificial materials or etch masks are attached to the substrate surface [Figure 1.1(2)]. Common sacrificial materials include Cr/Au,\textsuperscript{15, 19, 22} amorphous Si,\textsuperscript{23, 24} and SiO\textsubscript{2}.\textsuperscript{25-27} In standard photolithography, a thin film of photo-sensitive photoresist is first spin-coated onto the top of the sacrificial material layer [Figure 1.1(3)]. Next, a high-resolution photomask,
which is a square glass plate bearing a thin patterned metal film or a piece of transparency printed with a high-resolution pattern, is placed on top of the photoresist/sacrificial material-coated substrate. UV radiation is then used to project the image of the pattern from the photo-mask onto the photoresist layer [Figure 1.1(4)]. After immersion of the exposed substrate in a developing solution for a certain period of time, the pattern will appear on the photoresist layer [Figure 1.1(5)]. In the following step, the sacrificial material unprotected by the photoresist is removed with an etchant, which exposes the substrate underneath [Figure 1.1(6a) or (6b)] for further etching [Figure 1.1(7a) or (7b)]. To finish device fabrication, the remaining sacrificial layer is stripped using etchant [Figure 1.1(8a) or (8b)] and a blank substrate is bonded to the patterned one to enclose the microstructures [Figure 1.1(9)].

Depending on the types of photoresist used, different fabrication results will be obtained in the photolithography step. When a positive photoresist is coated on the substrate, the UV-exposed portion of the photoresist will be dissolved by the positive photoresist developing solution, while the photoresist covered by the dark region of the pattern is unaffected by the UV radiation and stays attached to the substrate [Figure 1.1(5a)]. On the contrary, if a negative photoresist is used, the exposed portion of the photoresist becomes insoluble in the negative photoresist developing solution, whereas the unexposed region is removed by the solution, and, a reversed pattern emerges on the substrate after development [Figure 1.1(5b)].

The pattern transferred to the substrate directly affects the final product. As shown in Figure 1.1, when the same photomask is used, patterning using a positive
Figure 1.1. Fabrication of inorganic microfluidic devices.
photoresist gives rise to a recessed structure; however, a protruded structure results when a negative photoresist is used.

**Etching of inorganic materials.** Several etchants have been used to etch inorganic materials. Concentrated KOH solution is a typical anisotropic etchant for silicon,\textsuperscript{25-27} which preferentially attacks the $\langle 1\ 0\ 0 \rangle$ plane of silicon, resulting in the sidewalls forming an angle of $54.74^\circ$ with the top surface. Silicon can also be etched using HNA solution, a mixture containing HF, HNO$_3$, and CH$_3$COOH. HNA is an isotropic etchant, which produces rounded sidewalls and corners. Other than wet chemical etching, which can only produce microstructures with low aspect ratios (depth/width), dry etching techniques, by which substrates are etched in reactive gases, can produce geometric objects with high aspect ratios and very complex shapes in silicon substrates.\textsuperscript{28} Reactive ion etching (RIE) is one of the dry etching techniques and has been applied to the fabrication of microfluidic devices.\textsuperscript{31} To etch glass and quartz, HF-containing isotropic etchants such as HF/HNO$_3$,\textsuperscript{15,22} HF/NH$_4$F,\textsuperscript{16,19} and HF/HCl\textsuperscript{29,30} can be used. Etching glass using concentrated HF was also reported.\textsuperscript{23,24} Quartz is more difficult to etch with HF-containing etchants and the resulting aspect ratios are lower than those of glass; however, high-aspect-ratio quartz microchannels can be fabricated using RIE.\textsuperscript{17}

**Bonding of inorganic substrates.** Bonding of substrates is essential to enclose fluidic channels in most microfluidic fabrication processes. Thermal bonding is the most popular bonding method for inorganic microfluidic devices. Before bonding, glass substrates are immersed in hot, concentrated H$_2$SO$_4$ or H$_2$SO$_4$/H$_2$O$_2$ solution to remove residue and generate silanol groups on the surface. After the substrates are brought into
contact, pressure is applied to hold them together. The bonding process is often performed at an elevated temperature for a certain period of time to ensure the formation of siloxane bonds between silanol groups. Although room temperature bonding is possible for glass substrates, most glass microfluidic devices are bonded at temperatures within the range of 500 ~ 700°C, depending on the type of glass. The bonding of quartz substrates is more difficult and very high temperatures (~1100°C) must be used. To bond silicon to glass, electric-field-assisted thermal bonding or anodic bonding is usually employed. During the bonding process, a voltage ranging from 200 ~ 1000 V is applied to the substrates and the bonding temperature is between 180 ~ 500°C. In addition to thermal bonding, adhesives are also used to bond the inorganic substrates, which can significantly reduce the bonding temperature and minimize deformation of microchannels.

**Thin-film technique.** Recently, a novel microfabrication technique for inorganic microfluidic devices was developed. The technique is based on thin-film micromachining as illustrated in Figure 1.2. First, the substrate is thoroughly cleaned. A layer of silicon dioxide or silicon nitride is then deposited on the top of the substrate using plasma-enhanced chemical vapor deposition (PECVD) [Figure 1.2(1) and (2)]. In the following step, a composite sacrificial layer containing an aluminum and a photoresist layer is attached to the substrate and photolithographically patterned [Figure 1.2(3) and (4)]. Steps 1 to 5 are similar to those in traditional microfabrication; however, in step 6, PECVD is employed to deposit a layer of silicon dioxide or silicon nitride to enclose the sacrificial layer. In step 7, the sacrificial layer is removed using both aluminum etchant and photoresist developer and a hollow channel is obtained.
Figure 1.2. Fabrication of microfluidic devices using the thin-film technique.
Compared to traditional microfabrication, the thin-film technique has apparent advantages. First, substrate bonding, which is very tedious and demanding, is unnecessary in the thin-film fabrication technique. Since the microchannels are fabricated using PECVD, physical and chemical properties of the channels can be varied by selecting different materials for vapor deposition. Furthermore, multilayer cross-over fluidic channels can be directly constructed using the thin-film technique, which facilitates the fabrication of complex microfluidic systems.

Microfluidic devices fabricated from inorganic materials have shown great potential; however, it does not appear to be economical to make disposable clinical diagnostic microdevices using inorganic materials. The fabrication process must be performed in a clean room, and expensive facilities such as aligners and chemical vapor deposition systems must be used for the fabrication of every device. Furthermore, dangerous chemicals must be used in almost every step, which raises concerns about safety and waste disposal.

1.1.3. Fabrication of Microfluidic Devices Using Polymeric Materials

Polymeric materials. In response to the disadvantages of inorganic microfluidic devices, research groups in both academia and industry have been exploring alternative materials suitable for microfabrication. To date, many commodity polymers have been investigated for the fabrication of microfluidic devices. The most commonly used polymeric materials for microfabrication include polydimethylsiloxane (PDMS),\textsuperscript{41-43} poly(methyl methacrylate) (PMMA),\textsuperscript{44-46} polystyrene (PS),\textsuperscript{47,48} polycarbonate (PC),\textsuperscript{49,50} polyethylene terephthalate (PET/PETG),\textsuperscript{51,52} polyimide (PI),\textsuperscript{53,54} and polycycloolefin.
Because of their diverse properties, different fabrication methods must be used for different polymeric materials. Basically, the polymer microfabrication methods can be categorized into two families, i.e., replication methods and direct methods.58

**Template fabrication.** In replication methods, templates or molds are utilized to produce polymeric replicas. Since microchannels are the main structures of microfluidic devices, their templates have protruding patterns on the substrate surface. Although in some simple cases where only straight channels are required, thin metal wires can be used as templates,60, 61 the fabrication of the templates still relies on traditional microfabrication and electroplating techniques. The materials for templates can be rigid inorganic materials including silicon25-27, 60 and metal,31 or polymeric materials such as polyetheretherketone (PEEK)62 and SU-8.63

Methods for the fabrication of silicon templates are the same as those for fabricating inorganic microfluidic devices. When metal templates are required, electroplating can be used. An example of the fabrication of a metal template is shown in Figure 1.3. First, the pattern of the microfluidic device is fabricated in a silicon substrate using traditional microfabrication; and then, a thin metal seed layer (aluminum) is deposited on the silicon substrate using an evaporator or a sputterer. Next,
electroplating is used to grow a thick metal layer (nickel or nickel alloy) from the seed layer. To release the metal template, the silicon wafer and the seed layer are removed using concentrated KOH solution at an elevated temperature.\textsuperscript{31}

LIGA, which is a German acronym for lithography (lithographie), electroplating (galvaniformung), and molding (abformung), is a complex microfabrication technique that can be used to produce high-aspect-ratio templates.\textsuperscript{28} The first step of LIGA is to use standard photolithography and electroplating to transfer a device pattern from a standard optical photo-mask to an X-ray mask. Following the mask fabrication step, a thick-layer of PMMA is attached to a metal support, and then, exposed to X-rays with an X-ray mask placed on the top of the PMMA layer. After the exposed portion of PMMA is dissolved in a special developer solution, nickel is electroplated on the exposed area, and the unexposed PMMA is removed using organic solvents.\textsuperscript{28, 65} Compared to traditional microfabrication approaches, LIGA is an expensive and laborious process; however, it can produce high-quality templates. In addition, polymeric, metal, and even ceramic microdevices can be made using this technique.

Because of its good mechanical strength, high optical transparency, and chemical inertness, SU-8 can be directly used to fabricate high-aspect-ratio templates\textsuperscript{63} or microfluidic devices\textsuperscript{64} using standard photolithography. It should be mentioned that SU-8 microstructures are fabricated directly on the top of silicon or glass substrates; therefore, etching is not required.

**Hot embossing.** Hot embossing is one of the most widely used replication methods to fabricate polymeric microfluidic devices.\textsuperscript{25-27, 60, 65} The polymeric materials compatible with this process are thermoplastics such as PMMA, PS, PET, PC, and PCOC.
Generally, during hot embossing, the polymeric substrate is softened by heating it above its glass transition temperature ($T_g$) [Figure 1.4(1)]. Next, the substrate is brought into contact with a template, and then, the pattern on the template is embossed into the softened substrate using pressure [Figure 1.4(2)]. Afterwards, the template-substrate assembly is cooled to a releasing temperature, which is below the $T_g$ but higher than room temperature, and the applied pressure is removed to released the substrate from the template [Figure 1.4(3)]. The use of vacuum throughout the process can eliminate trapping of air bubbles between the softened polymer substrate and the template. Also, during releasing of the template, thermally induced stresses in the substrate should be minimized; otherwise, replication defects are formed in the polymeric substrates.\textsuperscript{58}

With softer thermoplastics such as PET and poly(vinyl chloride) (PVC), the embossing process can be performed at room temperature. Harder plastic substrates such as PMMA and PC can also be patterned using room-temperature embossing method.\textsuperscript{59} However, high-quality metal templates must be used because silicon or glass templates are readily broken in the process.

\textbf{Figure 1.4. Fabrication of polymeric microfluidic devices using hot embossing.}
**Injection molding.** Injection molding is another commonly used polymer machining technique for thermoplastic materials.\textsuperscript{66} During fabrication, polymer pellets are fed into an injection molding machine and melted at an elevated temperature. The liquid plastic is then injected into an evacuated cavity (with a template installed inside) under high pressure to form replicas. The replicas are then ejected from the cavity by reducing the temperature, and the machine is ready for the next production cycle.

In comparison to hot embossing, the cycle time for injection molding is shorter, which is preferred in mass production. In addition, elements such as optical fibers can be easily integrated into the plastic substrate during the fabrication process. However, the process time and temperature should be carefully controlled to prevent replication deviations. Moreover, since high temperature and pressure are used, high-quality metal templates are usually required in the injection molding process.

**Casting.** Unlike hot embossing and injection molding, which require special fabrication facilities and careful control of conditions, casting or soft lithography\textsuperscript{67} is a relatively simple and versatile technique. Very complex three-dimensional microfluidic devices, microvalves, and micropumps can be readily fabricated.\textsuperscript{68-73}

Silicone rubber, especially PDMS (Corning Sylgard 184), is the major polymeric material used in this technique. In a typical casting process, liquid PDMS is mixed with a curing agent, and poured into the template. The liquid is then cured at either room temperature or at elevated temperature. As a result, the patterns on the template are transferred to the solidified PDMS elastomer. Because the fabrication conditions of casting are mild, metal templates are not necessary. Softer materials such as SU-8 and even PDMS can be employed to fabricate templates.
Besides PDMS, other polymeric materials can also be used in casting. Cross-linked photo-curable perfluoropolyether, an elastomer that can withstand organic solvents, was employed to fabricate microfluidic devices using the casting approach.\(^7\) Although hot embossing and injection molding are the most popular fabrication methods for thermoplastics, e.g., PMMA, casting can be used to fabricate microfluidic devices such as capillary electrophoresis microchips.\(^5\) Due to its special properties, thermoset polymeric materials are difficult to micromachine using hot embossing and injection molding, whereas, casting can deal with these materials. Recently, Fiorini et al.\(^6\) used casting to successfully fabricate thermoset polyester microfluidic devices.

**Laser micromachining.** In contrast to replication methods, direct methods do not rely on the use of templates. Microstructures can be fabricated directly into the polymeric substrates. Laser micromachining or laser ablation is one of these methods. UV excimer lasers\(^7,\) and CO\(_2\) infrared lasers are two commonly used laser sources in microfabrication.\(^7,\) In the fabrication process, polymeric substrates are positioned on a computer-controlled X-Y motorized stage and, then, a laser beam is focused at the substrate surface using an optical system. By moving the stage with a computer aided design (CAD) program, complex microchannel patterns can be fabricated in the substrates.

UV excimer lasers, which include ArF (193 nm) and KrF lasers (248 nm), are operated in the pulsed mode with pulses of nanosecond duration. In comparison, a CO\(_2\) laser has a wavelength of 10.6 \(\mu\)m and operates continuously. UV excimer lasers provide shorter wavelengths than a CO\(_2\) laser; during the UV photoablation process, chemical bonds are cleaved photochemically without creating excessive amounts of heat. A CO\(_2\)
laser creates microchannels mainly by the photothermal effect, which readily deforms the polymeric microstructure. Therefore, UV excimer lasers can generate smaller features than CO₂ lasers. Generally, UV excimer lasers can create microchannels with dimensions smaller than 100 µm, and the channels usually have straight sidewalls, whereas, CO₂ lasers often produce larger channels (>150 µm) whose cross sections have Gaussian-like profiles. Since UV lasers can induce chemical reactions at polymer surfaces, surface modification of the microchannels can be performed simultaneously during the fabrication process.

A wide range of commodity polymers can be machined using laser micromachining. Since photomasks and templates are not necessary in this fabrication technique, device design can be changed rapidly during the prototyping stage. Nonetheless, laser micromachining is a sequential process, which limits its applicability to mass production. Furthermore, channels generated using both UV excimer lasers and CO₂ lasers always have greater surface roughness than those fabricated using hot embossing, injection molding, and casting. Finally, it should be mentioned that the ejected polymer residues or decomposed compounds produced during laser ablation may re-deposit on the surface of polymeric substrates and, hence, vary their local surface properties.

**Microfluidic tectonics.** Unlike the microfabrication methods described above, microfluidic tectonics (µFT) is a novel approach that combines photosynthesis of polymeric materials, photolithography, and laminar flow in the fabrication process. Briefly, in a typical µFT process, a cartridge with fluidic connections is prepared first and, then, a monomer solution containing a photoinitiator is filled into the cartridge chamber,
for which the chamber height defines the depth of the resulting microchannels [Figure 1.5(1)]. After a photomask is positioned on top of the cartridge [Figure 1.5(2)], UV radiation (usually 300 ~ 400 nm) is used to polymerize the monomers, and the unmasked areas become solidified to form the channel walls [Figure 1.5(3)]. Following the photopolymerization step, unreacted solution is flushed out of the channel to obtain a final polymeric microfluidic device [Figure 1.5(4)].

In a µFT process, templates are not required, and all microstructures form simultaneously in a very short period of time. In addition, since channels are fabricated directly inside a cartridge, bonding is not necessary. Moreover, by using different photomasks and multi-step exposure, very complex structures can be readily made. When laminar flow is utilized, membranes and metal wire can be directly fabricated in the microchannels.\textsuperscript{84, 85} It is remarkable that with this technique, microstructures including sensors, valves, and pumps can be fabricated from very fragile polymeric materials such
as stimuli-responsive hydrogels, which can be used to fabricate biomimetic microfluidic systems.\textsuperscript{86}

The monomers suitable for \textmu FT must have low shrinkage and fast reaction rates during polymerization. During photolithography, diffraction of UV radiation at the edge of the opaque patterns in a photo-mask will cause the projected images to become blurred and, thus, induce partial polymerization in regions close to the pattern edge. In addition, diffusion of free radicals through the liquid phase may also initiate unwanted polymerization. As a result, the resolution of \textmu FT is inferior to traditional photolithography techniques, and it is difficult to fabricate features smaller than 100 micrometers.

\textbf{SU-8 photolithography.} Besides patterning, negative photoresist SU-8 is suitable for the fabrication of polymeric microfluidic devices since it has high thermal stability, good mechanical strength, good optical transparency and chemical resistance. To fabricate SU-8 microfluidic devices, first, a layer of SU-8 is spin-coated on a glass wafer. After dehydration by baking, the SU-8 layer is exposed to UV radiation. Another SU-8 layer is then spin-coated on the top of the first SU-8 layer, baked, and patterned using standard photolithography. The thickness of this layer defines the depth of the microchannels. To enclose the pattern, a glass wafer with a spin-coated SU-8 layer is attached to the top of the patterned SU-8, and the whole assembly is subjected to further UV exposure and high-temperature baking to bond the three SU-8 layers.\textsuperscript{87, 88}

\textbf{Thermal bonding.} Thermal bonding or thermal fusion is the most widely-used approach for sealing microfluidic patterns made in thermoplastics.\textsuperscript{44-50, 55-57} To bond a patterned substrate to a blank substrate using this method, a clamp or hydraulic press is
used to hold the two pieces together. At an elevated temperature around the glass transition temperature ($T_g$) of the substrate, pressure is applied to the assembly. After a period of time, the temperature is lowered and the bonded microdevice is released from the clamp.

It should be mentioned that since bonding depends on the intermolecular interactions between the contact polymer layers, this bonding method cannot provide high bonding strength for polymeric substrates, and delamination often occurs. Moreover, since thermal bonding is performed at a temperature close to $T_g$, channel deformation always happens, which makes this technique unsuitable for sealing microstructures with very low aspect-ratios and small dimensions.

**Solvent bonding.** Alternatively, organic solvents that can dissolve the polymeric substrates can be used in microdevice bonding.\textsuperscript{31, 89, 90} As shown in Figure 1.6, two approaches may be used to bond polymeric substrates using organic solvents. In approach A, a thin layer of solvent is spin-coated on a blank substrate [Figure 1.6(A1)], and, then, a patterned substrate is quickly brought into tight contact with the blank [Figure 1.6(A2)]. After a period of time, the solvent partially dissolves the polymer at the contact surface, and the flexible polymer chains in both substrates infiltrate into each other and entangle when an external pressure is applied. As a result, strong intermolecular interactions are established between the two substrates [Figure 1.6(A3)].\textsuperscript{89} In approach B, the solvent is spin-coated on a rigid glass or silicon wafer. The patterned polymer substrate is then pressed onto the solvent-coated substrate [Figure 1.6(B1)] to wet the contact surface [Figure 1.6(B2)]. The bonding is finished by pressing a blank substrate onto the patterned substrate [Figure 1.6(B3) and (B4)].\textsuperscript{31}
Solvent bonding is performed at room temperature and can provide very high bonding strength; however, the organic solvents used should have moderate solubility in the polymer substrates, and their volatilities should not be high. In addition, the amount of solvent coated on the substrate surface should be very carefully controlled; otherwise, too much polymer may dissolve and flow into the channels, which can block the channels.

![Diagram of solvent bonding process](image)

**Figure 1.6. Solvent bonding of polymeric substrates.**

To protect the microchannels from polymer flowing into them during solvent bonding, phase-changing sacrificial materials such as wax can be introduced into the channel before applying the organic solvent. The patterned substrate is then wetted by the solvent, and a blank is pressed tightly against the patterned substrate for the specified period of time. After bonding, the wax is removed from the microchannels at elevated temperature. Fabrication of PMMA capillary electrophoresis microfluidic devices ($\mu$CE) has been successfully demonstrated using this approach.\(^9^0\)
**Adhesive bonding.** The procedures for adhesive bonding are the same as for solvent bonding. However, unlike solvents, which can be absorbed inside the polymer matrix, adhesives will stay on the substrate surface after bonding and, thus, the thickness of the adhesive layer must be small enough to prevent the adhesive from flowing into and blocking the channels. If route A [Figure 1.6(A)] is used, the enclosed channel will have one side coated with adhesive. Therefore, adhesives with similar surface properties to the substrate must be selected.\(^{46}\) When route B [Figure 1.6(B)] is adopted, adhesives with different surface properties from the substrate can be used since the surface area of the exposed adhesive layer in the enclosed channel is very small.\(^{91}\)

**Resin-gas injection bonding.** Recently, a novel bonding technique, resin-gas injection, was reported.\(^{92}\) In the first step of this method, patterned and blank substrates are held together [Figure 1.7(1)]. A monomer solution containing 2-hydroxyethyl methacrylate (HEMA) and 2,2’-dimethoxy-2-phenylacetophenone (DMPA) is then introduced into the microchannel [Figure 1.7(2)]. After the monomer solution fills the gap between the patterned and blank substrate through capillary action, nitrogen gas or vacuum is employed to remove the solution in the channel [Figure 1.7(3)]. Finally, the

![Figure 1.7. Resin-gas injection bonding.](image-url)
HEMA is cured using UV radiation, which bonds the substrates together [Figure 1.7(4)].

A unique feature of the resin-gas injection bonding method is that surface modification and bonding are performed simultaneously. However, it should be mentioned that the monomer layer coated on the channel wall should be uniform and stable, which affects the performance of the microdevice.

**Chemical bonding.** In chemical bonding or permanent bonding, polymeric substrates are bonded through chemical bonds formed at the contact surface. Because various polymeric materials are used in microfabrication, specific chemistries must be used in the chemical bonding process.

PDMS not only bonds to glass, silicon, and itself through intermolecular interactions, but it can also bond to these substrates covalently. To chemically bond PDMS to other substrates, a low power O₂ plasma is used to treat both the PDMS and the blank substrates for 1 min. Following O₂ plasma treatment, the PDMS is quickly pressed onto the blank, and the substrates become bonded chemically after several minutes.⁴²,⁴³ During O₂ plasma treatment, siloxane bonds in the substrates are cleaved by the plasma. If the substrates are brought into contact in less than 30 s, covalent bonds will form between the broken bonds in both substrates.

Commercially available PDMS preparation kits have two parts. The first part contains PDMS polymers bearing vinyl groups and a platinum catalyst, while the second part contains a cross-linker with silicon hydride (Si-H) groups. In the second chemical bonding method for PDMS, the patterned and blank substrates are prepared so that one piece has excess vinyl groups, while the other has excess Si-H groups. When the two substrates are brought into contact and baked at an elevated temperature, the vinyl groups
react with the Si-H groups at the contact surface to form covalent bonds as a result of the platinum catalyst.  

To bond a patterned SU-8 substrate, uncured SU-8 is spin-coated on a piece of glass and the glass is then attached to the patterned substrate. Permanent bonding forms when the temporarily attached substrates are subjected to UV exposure and high-temperature baking. During this process, epoxy groups in the polymer side-chains are activated by a photo-generated acid (HSbF₆), which form covalent bonds with other epoxy rings nearby at elevated temperature. This cationic polymerization happens not only in bulk, but also at the contact surface, thereby forming covalent bonds between the SU-8 surfaces.

The bonding of thermoset polyester (TPE) substrates is also chemical bonding. To prepare TPE substrates, styrene, an unsaturated polyester resin (Polylite), a photoinitiator (DMPA), and methyl ethyl ketone peroxide (MEKP) catalyst are mixed together. After casting the reaction solution onto a template, UV radiation is used to cure the pre-polymer and pattern a TPE substrate. Blank pieces of TPE are also prepared using the same approach. Next, the patterned substrate is temporarily bonded to a blank substrate, and subjected to UV radiation for a short period of time. Finally, the assembly is heated and the unsaturated polyester backbones in both TPE substrates are covalently linked under catalysis by MEKP.
1.2 Surface Modification of Polymeric Microdevices

1.2.1 Introduction

When dimensions of fluidic systems decrease, the surface-to-volume ratio increases dramatically. As a result, the influence of surface properties on the performance of microfluidic devices becomes significant. Surface properties of polymeric materials are determined by their composition. Furthermore, commodity polymeric materials contain additives such as fillers, heat stabilizers, plasticizers, antioxidants, and UV stabilizers. It is very difficult to predict surface phenomena of the polymeric materials just from the properties of the main polymers without considering the additives.

The surfaces of polymeric materials are usually charged when in contact with buffer solutions, which may be caused by adsorbed buffer ions or ionizable functionalities present on the surface. However, the density of the charged sites is usually low and they are not homogeneously distributed on the surface of the polymeric materials; therefore, an uneven zeta potential results. According to the Helmholtz-Smoluchowski equation

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

(1.6)

where \( \nu_{EOF} \) is the velocity of the electroosmotic flow, \( E \) represents the electric field strength, \( \varepsilon \) stands for the permittivity of the solution, \( \zeta \) is the zeta potential, and \( \eta \) is the viscosity of the solution, a non-uniform electroosmotic flow results if an electric field is established in the polymeric microchannel, which leads to deterioration of the resolution of electrophoretic separations. Furthermore, surfaces of most polymeric materials are not inert to biological samples such as proteins and cells. Electrostatic interaction, hydrophobic interaction, hydrogen bonding, and/or other types of interactions tend to
attract these species to the surface \(^93\) and, thus negatively affect analysis or processing of biological samples. Usually, surface modification is necessary to prevent unfavorable surface effects from decreasing the performance of the microfluidic devices. As pointed out by Professor Stellan Hjertén of Uppsala University, “one must find simple methods to eliminate adsorption onto the walls of the channels. This is not a simple problem, especially when the sample is protein-based and the chip is made from plastic, the most widely used material.” \(^94\)

### 1.2.2 Dynamic Coating

Dynamic coating is a simple and fast surface modification technique. In a typical dynamic coating process, surface-active compounds or surface modifiers are introduced into the separation buffer. The buffer is then forced through the channel at a constant speed. The coating materials are adsorbed onto the channel surface through physical interactions. Surface modifiers that can be used in analyses using polymeric microfluidic devices include charged compounds, neutral polymers, surfactants, and nanoparticles.

High molecular weight charged polymers such as polybrene (PB) and dextran sulfate (DS) were employed to manipulate EOF, \(^95\) or suppress adsorption of neutral analytes in PDMS microchannels. \(^96\) Sodium polystyrene sulfonate (PSS) and poly(allylamine hydrochloride) (PAH) were also used to passivate PET and PS microchannel surfaces; however, when PSS-PAH coated µCE devices were used in electrophoresis experiments, obvious peak tailing was observed, which could be caused by hydrophobic interactions of analytes with the polyelectrolyte backbones. \(^97\) In comparison, low-molecular-weight charged compounds such as mono-, di-, and
triethylamines did not effectively decrease the adsorption of biomolecules such as oligosaccharides on PMMA microchannels, which may be ascribed to insufficient coverage of the channel surface.\textsuperscript{98}

To reduce adsorption of saccharides, proteins, and DNA, neutral hydrophilic polymers have been used to coat polymeric channels. It was reported that poly(ethylene glycol) (PEG), hydroxyethylcellulose (HEC), hydroxypropyl methylcellulose (HPMC), and methylcellulose (MC) improved the separation of oligosaccharides labeled with 8-aminopyrene-1,3,6-trisulfonate in a PMMA \(\mu\)CE device.\textsuperscript{98} Xu et al. coated a PMMA \(\mu\)CE microchip with a mixture of low-viscosity HPMC, mannitol, glucose, and glycerol, and separated double-stranded DNA (dsDNA) fragments using the coated chip.\textsuperscript{99} Separation of dsDNA fragments was also realized using a PMMA \(\mu\)CE device coated with poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) (PEG-PPG-PEG) copolymer.\textsuperscript{100} A 15-s protein separation was demonstrated using a poly(N,N'-dimethylacrylamide) (PDMA)-coated \(\mu\)CE device,\textsuperscript{101} and isoelectric focusing of native fluorescent proteins was performed in a MC-coated PDMS microchip.\textsuperscript{102}

Since surfactants typically have hydrophobic alkyl components, they can attach to polymeric channel surfaces through hydrophobic interactions, while their hydrophilic/ionic moieties extend outward and change the surface properties of the channel walls. Cationic, anionic, and nonionic surfactants including tetrabutylammonium chloride (TBAC), cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium chloride (DTAC), sodium dodecyl sulfate (SDS), Brij 35, Brij 76, and Brij 78 were utilized to coat microchannels for increasing hydrophilicity, manipulating EOF, or separating DNA fragments and proteins.\textsuperscript{98, 100, 103-105}
Nanoparticles are usually used together with charged polymers or neutral hydrophilic polymers. Gold nanoparticles (GNP) were applied to poly(diallyldimethyl- ammonium chloride) coated glass µCE devices to improve the resolution of aminophenol isomers. Recently, a multi-layer coating containing GNP, poly(ethylene oxide) (PEO), and poly(vinyl pyrrolidone) (PVP) was applied to PMMA µCE chips, and high-efficiency separations of DNA digests were demonstrated. It was found that GNP increased the viscosity and stability of the coating, which contributed to the improvement in µCE performance.

In the separation of lipoproteins, Ping et al. found that HPMC and MC could not suppress the adsorption of the proteins, while SDS or CTAB successfully reduced the adsorption through electrostatic repulsion, but a strong EOF was generated in the surfactant-coated channel, which hindered the electromigration of the lipoproteins to the detection window. Therefore, a surfactant such as SDS or CTAB and a neutral hydrophilic polymer such as HPMC, MC, or PEO were finally used to passivate the PMMA microchannel to obtain a satisfactory separation.

Among the surface modification techniques, dynamic coating is the most convenient to perform. However, it is not permanent and analytes can compete for active sites on the surface. To solve this problem, surface modifiers must be added to the separation buffer. Unfortunately, dynamic surface modifiers can be detrimental in many applications that require coupling of a mass spectrometer or a miniaturized chemical reactor. Moreover, caution must be taken to minimize denaturing or even destruction of protein-based analytes by surface modifiers. Therefore, permanent surface modification is preferred.
1.2.3 Permanent Modification of Polymeric Materials

Permanent modification of polymer surfaces involves using specific chemical reactions to directly modify the chemical composition of the surface, or employing high-energy sources such as radiation, flame, corona, plasma, electron beam or ion beam to activate and alter the chemical structure of the surface layer.\textsuperscript{109, 110}

**Modification of PDMS surfaces.** PDMS is the most popular polymeric material for microfabrication in academia because of fabrication ease, simple bonding methods, high replication fidelity and low cost. Unfortunately, PDMS has an extremely hydrophobic surface, which makes it unfavorable in bioassays since analytes such as proteins can readily adsorb onto the channel walls through strong hydrophobic interaction. Furthermore, it is difficult to introduce aqueous solutions into PDMS microchannels due to this hydrophobicity. Besides dynamic coating, permanent surface modification has also been employed to modify PDMS surfaces.

Oxygen plasma treatment is a fast and simple surface modification technique for PDMS. During the plasma treatment, the surface of PDMS is subjected to high energy sources including electrons, radiation, ions, and radicals, which attack the polymer backbone and form Si-O-Si and Si-O-H structures on the PDMS surface.\textsuperscript{42} As a result, the hydrophobicity is greatly decreased.\textsuperscript{111} However, the hydrophilic nature of oxygen plasma-treated PDMS surfaces disappears after a period of time. According to the observation of Duffy et al.,\textsuperscript{42} the useful lifetime for reliable quantitative analysis using the oxidized channel is approximately 3 h. This hydrophobicity recovery is most likely caused by migration of PDMS chains through cracks in the modified layer to the surface.\textsuperscript{112}
A glass-like layer was created on a PDMS substrate using a sol-gel method. Briefly, a PDMS µCE device without access holes was fabricated using soft lithography and the whole microchip was soaked in tetraethyl orthosilicate liquid, which caused the substrate to swell. After 30 min, tetraethyl orthosilicate infiltrated the PDMS matrix and reached the channel surface. Subsequently, the chip was rinsed and placed in an aqueous solution containing ethylamine for 15 h. To finish the modification, the microchip was removed from the amine solution, rinsed, and heated to 95°C for 1 h. Finally, access holes were created using a cork borer. During the heating process, a condensation reaction (1.7) occurred with catalysis by ethylamine and nanometer-sized SiO₂ particles

\[
\text{Si(OR)}_4 + \text{H}_2\text{O} \xrightarrow{\text{Base}} \text{SiO}_2 + 4\text{ROH}
\]

formed within the PDMS polymer matrix and on the channel surface. It was found that in comparison to the original PDMS microchannels, both hydrophobicity and adsorption of hydrophobic analytes on the surface were decreased, while EOF and separation efficiency of fluorescein-derivatized amino acids were increased. However, since proteins could still adsorb on the treated PDMS surface, which had both hydrophobic and negatively charged patches, it was necessary to anchor protein-resistant species on the surface to prevent adsorption of proteins.

Various functionalities can be immobilized on the glass-like layer on the PDMS surface by silanization. Alkyltrichlorosilanes were linked to oxygen plasma-modified PDMS substrates by Ferguson et al. Using a similar strategy, Papra et al. grafted 2-[methoxy(polyethyleneoxy)propyl] trimethoxysilane and poly(ethylene glycol) di(triethoxy)silane on oxygen plasma-treated PDMS substrates to increase their protein resistance. Xiao et al. immobilized 1-trichlorosilyl-2-(p-chloromethylphenyl)ethane
(TCE) on a UV/O₃-oxidized PDMS substrate for further grafting of polyacrylamide on the polymer surface.

Recently, a microfabricated two-dimensional IEF-CE/CGE PDMS device was reported by Wang et al.¹¹⁷ To prevent protein adsorption, they used an oxygen plasma to activate the microchannel first, and 3-methacryloxypropyl trimethoxysilane (MPTS) was grafted onto the oxygen plasma-activated PDMS channel surface. Next, a monomer solution containing acrylamide, ammonium persulfate and tetramethylethylenediamine was prepared and introduced into the microchannels. The reaction was allowed to proceed for a period of time, during which polyacrylamide was grafted on the methacryl layer. To increase the protein resistance, methylcellulose was dynamically coated onto the polyacrylamide-grafted microchannel. It was mentioned that microchannels treated with this protocol had better resistance to protein adsorption than those only dynamically coated with methylcellulose.

It is well-known that UV radiation can directly generate free radicals on polymer surfaces, and these UV-induced radicals can further initiate polymerization. Hu et al.¹¹⁸,¹¹⁹ exploited this technique to graft various hydrophilic homopolymers or copolymers onto PDMS surfaces using monomers including acrylic acid (AA), acrylamide (AAm), N,N’-dimethylacrylamide, 2-hydroxyethyl acrylate, 2-methacryloxyethyltrimethylammonium chloride, poly(ethylene glycol) monomethoxyl acrylate (PEGMA), and poly(ethylene glycol) diacrylate (DiPEG). During this UV-initiated surface modification, PDMS substrates were immersed in a reaction solution containing sodium periodate, benzyl alcohol, and a monomer. Sodium periodate was used as an oxygen scavenger while benzyl alcohol was used to facilitate chain termination,
which limited polymerization in the bulk solution and, thus, greatly diminished the viscosity of the aqueous solution and enhanced diffusion of more reactive monomers or polymers to the PDMS surface. After a period of time, the substrates were taken out of the reaction solution and rinsed with distilled water to remove polymer residue or unreacted monomer. It was observed that the graft density for all monomers increased with irradiation time and AA had the highest graft density. In addition, the strength and direction of EOF could be tuned by grafting different monomers on the PDMS channel surface.

Hu et al.\textsuperscript{120} observed that the one-step UV-activated graft polymerization method\textsuperscript{118, 119} required a relatively long irradiation time, and the channel was easily clogged with nascent polymers formed in solution during the in-channel surface modification. Furthermore, it was very difficult to achieve a reproducible surface coating using the one-step method. Therefore, an alternative strategy, “surface-directed” graft polymerization, was utilized.\textsuperscript{120, 121} In this approach, first, a free radical initiator such as 2,2’-azobisisobutyronitrile (AIBN, thermal initiator) or benzophenone (BP, photoinitiator) was dissolved in an organic solvent and a PDMS substrate was immersed in this solution. The initiator adsorbed on the surface or infiltrated PDMS layers near the surface. Next, the polymeric substrate was removed from the solution, dried, and immersed in an aqueous solution of hydrophilic monomer without initiator. When heat or UV radiation was used to activate the adsorbed initiator to generate free radicals, polymer chains formed on the PDMS surface. An advantage of this method is that the adsorbed initiator accelerated the rate of formation of polymer on the surface relative to that in solution. Therefore, polymers mainly formed on the surface instead of in bulk solution, which
greatly decreased the chance of channel clogging. Moreover, this permanent surface modification technique can be readily scaled up. Using UV-mediated “surface-directed” graft polymerization, a copolymer consisting of PEGMA, AA, and DiPEG (20:1:1) was covalently attached to the channel surface of a PDMS μCE chip and an electrophoretic separation of two peptides was obtained. Unfortunately, in all of the reports from Hu et al., only peptide separations were shown. Electrophoretic separation of proteins was not demonstrated using the PEGMA- or PEGMA-AA-DiPEG-grafted microchips.

A high energy plasma such as an Ar-H₂ microwave plasma can directly generate a large number of free radicals by cleaving PDMS backbones on the surface. If monomers are present, the radicals at the cleaved sites will initiate polymerization. He et al. successfully grafted polyacrylonitrile onto a PDMS stamp using this plasma-assisted polymerization. The water contact angle of the PDMS was 28 ± 12° after grafting, while the original water contact angle of the PDMS was 100 ± 5°. Additionally, the hydrophilicity of the PDMS was stable and lasted at least one month.

Cerium (IV)-catalyzed polymerization is suitable for alcohols; the simplified initiation mechanism was proposed to be

\[
\text{Ce(IV)} + \text{RCH}_2\text{OH} \rightarrow \text{Ce(III)} + \text{H}^+ + \text{RCH}_2\text{O} \cdot
\]  

(1.8)

It is interesting that silanol groups can also be initiated to generate silanol radicals following the same mechanism. To modify a PDMS surface using this method, Slentz et al. first used an oxygen plasma to oxidize the PDMS substrates, and immediately soaked the substrates in a reaction solution containing a monomer, ammonium cerium nitrate, and nitric acid. The reaction was allowed to proceed for a period of time and
polymer chains were grafted onto the PDMS surface. Cerium (IV)-catalyzed polymerization was used to graft homopolymers or copolymers of AA, 2-acrylamido-2-methylpropanesulfonic acid, stearyl methacrylate, vinylsulfonic acid, and 4-styrenesulfonic acid on microfabricated PDMS capillary electrochromatography (µCEC) and µCE devices,\textsuperscript{124,125} and the treated PDMS microchips were employed to separate synthetic peptides\textsuperscript{124} and DNA markers.\textsuperscript{125} Unfortunately, polymer chains are immobilized on the surface through Si-O-C bonds in cerium(IV)-catalyzed polymerization, which can be easily hydrolyzed in aqueous buffer solutions, leading to grafted polymers that are not stable for long periods of time.

Atom-transfer radical polymerization (ATRP) is a transition metal-catalyzed free radical polymerization method. It was discovered by Sawamoto et al. and Matyjaszewski et al. independently.\textsuperscript{126,127} In a typical ATRP reaction, a free radical, $R\cdot$, is generated from an organic halide, $R-X$, via a one-electron oxidation catalyzed by a transition metal complex, $M_t^n-Y/\text{ligand}$ (where $Y$ may be another ligand or counter-ion), and the halogen atom, $X$, is abstracted from $R-X$ by the $M_t^n-Y/\text{ligand}$ to form an oxidized metal complex, $X-M_t^{n+1}-Y/\text{ligand}$ (1.9). Meanwhile, the radical adds to a monomer, $M$, to generate a new radical, $R-M\cdot$, and the new radical captures $X$ from $X-M_t^{n+1}-Y/\text{ligand}$ to form a new organic halide, $R-M-X$ (1.10).

\begin{align}
R-X + M_t^n-Y/\text{ligand} & \rightleftharpoons R\cdot + X-M_t^{n+1}-Y/\text{ligand} \tag{1.9} \\
R\cdot + M & \rightarrow R-M\cdot \xrightarrow{X-M_t^{n+1}-Y/\text{ligand}} R-M-X + M_t^n-Y/\text{ligand} \tag{1.10}
\end{align}

R-M-X can also form a radical and react with another monomer, $M$, to produce R-M$_2$-X according to mechanisms (1.9) and (1.10). Therefore, as this reaction proceeds, a polymer, R-M$_n$-X, will be obtained as the halogen atom is transferred to the end of the
polymer chain. Termination of polymer chain propagation by self reaction of radicals is limited in ATRP because the radicals react more rapidly with the oxidized metal complexes to form organic halides, which helps to reduce the stationary concentration of radicals and preserves the reactivity of the polymers. In ATRP, one polymer chain forms per molecule of organic halide and the growth of polymer chains is uniform. Furthermore, the resultant polymers keep their reactivity since halide atoms are always present at the end of the polymer chains. These unique advantages have been exploited to synthesize a large number of functional polymers or to graft polymer brushes on both inorganic and polymer surfaces.\textsuperscript{128, 129}

Application of ATRP in separation science has been reported. Huang et al.\textsuperscript{130} grafted polyacrylamide (PAAm) on the surface of a silica capillary using ATRP for use in capillary zone electrophoresis of proteins. Using a very similar procedure, Leinweber et al.\textsuperscript{131} grafted poly(2-hydroxyethyl methacrylate) on a silica capillary and achieved high-efficiency electrophoretic separations of both acidic and basic proteins with this capillary. Miller et al.\textsuperscript{132} further derivatized the grafted PHEMA layer with ethylenediamine and used the amine-covered capillary column to perform open tubular CEC separations of phenols and anilines. Recently, ATRP was applied to graft PAAm on a PDMS surface, and utilized this surface treatment technique in the fabrication of a PDMS $\mu$CE chip.\textsuperscript{116} The process was started by oxidizing the PDMS microchannel surface using UV/O$_3$ exposure. An ATRP initiator, 1-trichlorosilyl-2-($p$-chloromethylphenyl)ethane was then anchored on the oxidized PDMS surface via silanization. Under the protection of an argon atmosphere, an oxygen-free aqueous reaction solution containing acrylamide, CuCl, CuCl$_2$, and tris(2-dimethylaminoethyl) amine was introduced into the PDMS
microchannel and the ATRP reaction was allowed to proceed for 10 h (Figure 1.8). It was found that non-specific adsorption of proteins on the PAAm-grafted PDMS channel was reduced. Electrophoretic separation of TRITC-labeled lysozyme and cytochrome c was demonstrated using the PAAm-grafted microchip.

Chemical vapor deposition (CVD) is a widely used technique in the integrated circuit (IC) industry by which inorganic or organic thin films can be formed on silicon or glass substrates. Lahann et al.\textsuperscript{133,134} utilized CVD to deposit a layer of reactive polymer onto the surface of a PDMS microfluidic bioanalyzer. As shown in Figure 1.9, the monomer \textit{p}-xylylene carboxylic acid pentafluorophenolester-co-\textit{p}-xylylene (PPX-PPF) was sublimed and pyrolyzed to produce free radicals. When the free radicals were deposited onto a PDMS substrate positioned on a low temperature sample holder, polymerization occurred and a thin film of poly(PPX-PPF) formed on the PDMS surface.
Figure 1.9. CVD polymerization of PPX-PPF.

The deposited poly(PPX-PPF) thin film was homogeneous, stable and resistant to dimethylformamide, chloroform, acetone, ethanol, and aqueous solutions. Since carboxylic acid pentafluorophenol esters were present on the film surface, further derivatization could be readily performed.

Modification of rigid polymer surfaces. Research on permanent surface modification techniques suitable for rigid polymeric microfluidic devices used in protein assays has not received as much attention as for PDMS microchips. This may be due to the more challenging microfabrication procedures required for rigid polymeric materials. To date, most reported permanent surface modification techniques for rigid polymeric materials are concerned with surface activation.

To activate an inert PMMA surface (Figure 1.10), Henry et al. \(^{135}\) mixed ethylenediamine and propylenediamine with \(n\)-butyl lithium to produce very reactive
intermediates, i.e., $n$-lithiodiaminoethane and $n$-lithiodiaminopropane. These intermediates were then uniformly cast on the isopropyl alcohol-cleaned PMMA surface. After a short period of time, the reaction was quenched with deionized water. The resultant PMMA substrates had a layer of amine groups which provided handles for further treatment.\textsuperscript{135,136} Besides aminolysis, the pendant ester groups of PMMA can be reduced or oxidized using chemical or physical treatments. Cheng et al.\textsuperscript{137} immersed PMMA sheets in a lithium aluminum hydride diethyl ether solution for 24 h, and attenuated total reflectance Fourier transform infrared spectra confirmed that the ester groups were reduced to hydroxyl groups. Other moieties including amine, thiol, and perfluoroalkyl can also be covalently attached to PMMA substrates by silanization of the hydroxyl layer. However, because these functionalities are anchored to the surface through Si-O-C bonds, which can be easily hydrolyzed in aqueous solution, these derivatized PMMA surfaces are not stable for long periods of time. To convert the
pendant ester groups of PMMA to carboxyl groups, a 254-nm UV lamp or a pulsed UV excimer laser (KrF, 248 nm) below the ablation threshold was employed. The resultant carboxyl moieties could be subjected to further derivatization or used to manipulate EOF in the microchannels.

Zangmeister and Tarlov used UV/ozone (UV/O₃) to produce hydroxyl functionalities on the channel surface of a PC-PMMA microdevice. MPTS was then covalently linked to the activated surface by silanization. The pendant methacryl groups provided anchoring sites for further immobilization of DNA-containing PAAm hydrogel plugs. Although not mentioned by the authors, it should be possible to utilize this permanent modification technique to create protein-inert microchannels in polymeric microdevices. However, the hydrogel is immobilized on the surface through MPTS, which is anchored to the channel surface via Si-O-C bonds. Again, since these chemical bonds are easily hydrolyzed in aqueous solution, the long-term stability of the grafted layers is questionable.

Coating an adhesive on a substrate is a simple method to alter the surface properties of the material. In the fabrication of a PMMA enzyme microreactor, Qu et al. synthesized a copolymer adhesive from butyl methacrylate (BMA) and MPTS and coated it on the walls of a PMMA microchannel. The copolymer strongly adhered to the PMMA surface and provided anchoring sites for the immobilization of trypsin encapsulated in a silica sol-gel. Since the adhesive layer contained trimethoxysilyl moieties, the silica sol-gel could form Si-O-Si bridges with these functionalities during condensation and bind to the PMMA channel surface.
Wang et al.\textsuperscript{142} reported a method by which microfabrication and surface modification of PMMA could be performed simultaneously. In this “bulk modification” method, a monomer solution containing methylmethacrylate (primary monomer), a “chain modifier” (methacrylic acid, 2-sulfoethylmethacrylate, or 2-aminoethylmethacrylate), and a photoinitiator was subjected to 365-nm UV radiation for a specified period of time. The dense prepolymer solution was then poured into a cartridge with a silicon template fixed inside. Next, the whole cartridge was exposed to UV radiation again to fully cure the prepolymer. To finish the fabrication, a blank PMMA substrate of the same composition was thermally bonded to the patterned one. It was observed that when different “modifiers” were doped into the primary monomer solution, the resulting polymeric microchannels exhibited different surface properties. The activation of PET surfaces can be realized by hydrolyzing the ester bonds in the PET chains using aqueous sodium hydroxide solution to produce both hydroxyl and carboxyl groups.\textsuperscript{143} Carboxyl moieties can also be produced by ablating the polymer in an oxygen atmosphere with a 248-nm KrF excimer laser.\textsuperscript{144} PC substrates can be sulfonated using sulfur trioxide gas at elevated temperature,\textsuperscript{145} or activated by using UV/O\textsubscript{3} \textsuperscript{140} or 254-nm UV radiation \textsuperscript{146} to produce hydroxyl and carboxyl groups, respectively. To decrease the hydrophobicity of PCOC, Gaudioso et al.\textsuperscript{147} used an oxygen plasma to treat the surface. Stachowiak et al.\textsuperscript{148} filled a channel with a solution containing BP, methyl methacrylate and ethylene diacrylate and then exposed it to UV radiation. The illumination time was controlled so that only limited polymerization occurred. After removal of the excess monomer from the channel, a grafted polymer layer containing unreacted double bonds was chemically attached to the channel surface, which could be exploited to anchor other
species. To perform isoelectric focusing (IEF) with a PCOC microchip, Li et al.\textsuperscript{149} used UV-induced grafting to coat the surface of the channel with PAAm. They first introduced a grafting solution containing AAm, BP, Pluronic F-68 surfactant, and deionized water into the channel. Two UV sources, 254-nm and 302-nm, were then positioned both above and below the microchip and used to initiate the grafting reaction. The distances between the UV lamps and the device were chosen to create a consistent grafted PAAm layer on both top and bottom channel surfaces. IEF of a protein mixture including Alexa Fluor 488 labeled conalbumin and Alexa Fluor 546-labeled $\beta$-lactoglobulin A was demonstrated using the PAAm-grafted PCOC microchip. All three isoforms of conalbumin and $\beta$-lactoglobulin A were resolved in the experiment.

1.3 Microchip Electrophoresis

1.3.1 Introduction

Electrophoresis is one of the most common separation techniques for biological molecules, in which charged species migrate along a separation length under the effect of an external electric field and are resolved according to their differences in both net charge and size. Conventional electrophoresis is usually used to separate large-size analytes including proteins and nucleic acid fragments. However, to minimize skewing and broadening of separated bands caused by Joule heat and diffusion, supporting media such as paper, cellulose acetate, agar, agarose or polyacrylamide must be used. Furthermore, it is impractical to employ this technique to separate molecules with molecular weight less than 1000 Da due to their strong diffusion and small size.\textsuperscript{150} Modern capillary electrophoresis (CE) was first introduced by Jorgenson et al.\textsuperscript{151,152} in the early 1980s and
it quickly became a very important and powerful analytical tool. In comparison to conventional electrophoresis, capillaries with small inner diameters (<100 µm) are used in CE, and electrophoresis can be carried out with or without supporting media. Because of the good heat dissipation by the capillaries, high voltages up to 120 kV can be used to establish a very strong electric field, which substantially improves the resolution of CE and shortens the analysis time. Additionally, the use of sensitive detection methods such as laser-induced fluorescence (LIF) greatly decreases the detection limits of CE. CE has many operating modes including capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MECC/MEKC), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP). The range of analytes that can be analyzed by CE covers peptides, proteins, oligonucleotides, saccharides, inorganic ions, and small organic molecules. Armed with both CE and high-performance liquid chromatography (HPLC), researchers can significantly enhance their capability to solve real-world analytical problems.

After introduction of the µTAS concept in 1990, a revolutionary trend in instrument miniaturization appeared and rapidly spread throughout the science community. Because of its simplicity and convenience, CZE was one of the first analytical techniques to be microfabricated. To date, microfabricated capillary zone electrophoresis or microchip electrophoresis (µCE) has become a very popular analytical technique, and µCE devices are often used as stand-alone analyzers or the core components in many complex µTASs. The most mature application of µCE is DNA analysis.
1.3.2 Fundamental Theory of Capillary Zone Electrophoresis

CZE is the most popular operating mode of conventional or microfabricated CE.\textsuperscript{150} Its fundamental theory was first described by Jorgenson and Lukacs.\textsuperscript{151, 152}

![Figure 1.11. Schematic diagram of a simplified CZE device.](image)

A schematic diagram of a simplified CZE device is shown in Figure 1.11, which is comprised of a capillary with a length, $L$, and two buffer reservoirs. When a constant external voltage, $V$, is applied to the reservoirs, an electric field, $E$, forms in the capillary and the charged analytes start to migrate. Given the $i$th analyte has an electric charge of $q_i$, then, the coulombic force, $F_i$, on this species is

$$F_i = q_i E = q_i \frac{V}{L}$$  \hspace{1cm} (1.11)

Meanwhile, the migrating analyte is also retarded by a friction force, $F_{fi}$, as it moves through the buffer solution, which balances the coulombic force

$$F_i = F_{fi}$$  \hspace{1cm} (1.12)

$F_{fi}$ can be obtained from Stokes’ Law, which is

$$F_{fi} = 6\pi \eta r_i v_i$$  \hspace{1cm} (1.13)

By substituting (1.11) and (1.13) into (1.12), the electrophoretic velocity of the $i$th analyte, $v_i$, is obtained
\[
    v_i = \frac{q_i}{6\pi \eta r_i} \frac{V}{L} = \mu_i E
\]

where \( \eta \) is the buffer viscosity, \( r_i \) is the hydrodynamic radius of the \( i \)th analyte, and \( \mu_i \) is the electrophoretic mobility of the \( i \)th analyte, which is defined as

\[
    \mu_i = \frac{q_i}{6\pi \eta r_i}
\]

Because different charged analytes have different \( q_i \) and \( r_i \), their electrophoretic mobilities or velocities will not be the same, which results in electrophoretic separation of the charged analytes.

It should be noticed that in a typical CZE system, an open tubular silica capillary is used and the surface silanol groups are ionized to form a negatively charged layer when contacted with most commonly used buffers. This layer strongly attracts cations from the buffer solution and gives rise to a positively charged layer, or the Stern layer. The positively charged Stern layer cannot neutralize all of the negative charge in the silanoate layer, which further attracts a second layer of cations, or the mobile layer (Guoy-Chapman layer). The two layers constitute the electrical double layer, and the boundary between the Stern layer and the mobile layer is named as the shear plane (Figure 1.12). By convention, the electrical potential at the shear plane is defined as the zeta potential, \( \zeta \). The cations in the mobile layer are not tightly bound because of their

![Figure 1.12. Schematic diagram of the electrical double layer on a silica surface.](image)
increasing distance from the capillary wall. Therefore, when an external voltage is applied across the capillary, the cations migrate toward the cathode. Since the cations are hydrated, the water molecules also move together with the ions, which results in bulk flow inside the capillary, or electroosmotic flow (EOF). The velocity of the EOF is given by the Helmholtz-Smoluchowski equation (1.6), and the EOF mobility, $\mu_{\text{EOF}}$, is

$$\mu_{\text{EOF}} = \frac{\varepsilon \zeta}{\eta} \quad (1.16)$$

where $\varepsilon$ is the permittivity of the solution, $\zeta$ represents the zeta potential, and $\eta$ denotes the viscosity of the solution. If the EOF is considered, the total or effective velocity of the $i$th charged analyte, $v_{\text{Total},i}$, is equal to the vector sum of both the electrophoretic and the EOF velocities,

$$v_{\text{Total},i} = v_i + v_{\text{EOF}} \quad (1.17)$$

and the total mobility, $\mu_{\text{Total},i}$, is the vector sum of the electrophoretic and the EOF mobilities.

$$\mu_{\text{Total},i} = \mu_i + \mu_{\text{EOF}} \quad (1.18)$$

The elution time of the $i$th charged analyte, $t_i$, is

$$t_i = \frac{L}{v_{\text{Total},i}} = \frac{L^2}{\mu_i + \mu_{\text{EOF}}} \quad (1.19)$$

If diffusion is the only factor causing band broadening in CZE, the spatial variance of the analyte band, $\sigma_i$, can be obtained according to the Einstein equation

$$\sigma_i^2 = 2D_i t_i \quad (1.20)$$
where $D_i$ is the diffusion coefficient of the $i$th analyte. The number of theoretical plates, $N_i$, can be derived from Equations 1.19 and 1.20

$$N_i = \frac{L^2}{\sigma_i^2} = \frac{L^2}{2D_i t_i} = \frac{[\mu_i' + \mu_{EOF}]}{2D_i} V$$

(1.21)

To evaluate the resolution of two charged analytes ($R_s$) in CZE, Jorgenson and Lukacs derived an equation

$$R_s = 0.177\left(\mu_1 - \mu_2\right)\left[\frac{V}{D\left(\mu_{avg} + \mu_{EOF}\right)}\right]^{\frac{1}{2}}$$

(1.22)

where $\mu_1$ and $\mu_2$ are electrophoretic mobilities of two adjacent analytes, $\mu_{avg}$ is their average mobility, and $D$ is the diffusion coefficient of the analytes.

It can be learned from Equations 1.19, 1.21, and 1.22 that when a higher external voltage is applied in CZE, the elution time of an analyte is shortened, and both the separation efficiency and the resolution are improved. Increasing the voltage can improve the performance of CZE; however, the highest voltage one can apply is limited by the Joule heat generated in the buffer-filled capillary. In addition, it can be found from Equation 1.21 that the best resolution can be achieved when the vector sum of $\mu_{avg}$ and $\mu_{EOF}$ is close to 0, which means that the EOF just counterbalances the electrophoretic migration. Under this circumstance, the elution time of the analyte will be very long according to Equation 1.18.

### 1.3.3 Injection in Microchip Electrophoresis

The total volume of a capillary or channel ranges from nanoliters to microliters. Therefore, to avoid excessive band broadening, only a minute amount of sample can be
injected into the capillary or channel during CZE. In conventional CZE, samples are injected into a capillary using an external pressure (pressure-driven injection), or by applying a voltage to the sample reservoir (electrokinetic injection) for a short period of time. Since the electrophoretic migration velocities of analytes are different, when electrokinetic injection is used, an analyte with higher mobility will be sampled to a greater extent than an analyte with lower mobility, which results in an “injection bias” phenomenon.

Electrokinetic injection is the most widely used in µCE because of its simplicity. Unlike conventional CE, electrokinetic injection in µCE is typically implemented using microfabricated manifold structures or injectors. The simplest injector is comprised of a single-T structure (Figure 1.13). To inject samples, an injection voltage is applied across reservoirs 1 and 2, which drives the analytes into the separation channel. After a short period of time, a separation voltage is applied across reservoirs 1 and 3 while reservoir 2 is floated, and a plug of the analytes is injected into the separation channel.

**Figure 1.13. Single-T injector. (A) Injection, (B) separation.**

Unfortunately, “injection bias” can be introduced if the single-T injector is used. To avoid this phenomenon, a double-T injector (Figure 1.14) should be used. During injection, a voltage is applied across reservoirs 1 and 3 while reservoirs 2 and 4 are floated. In this way, the analyte composition in the sample stream is kept the same as the original sample after a short period of time. Following sample injection, a separation
Figure 1.14. Double-T injector. (A) Injection, (B) separation.

Voltage is applied across reservoirs 2 and 4 while reservoirs 1 and 3 are floated. Consequently, an analyte plug is pushed down to the separation channel.

However, when using μCE devices with a double-T injector, sample may leak from the injection channels to the separation channel during electrophoretic separation because of convection and diffusion, which results in an increase in the signal background and reduced resolution. As shown in Figure 1.15, to minimize sample leakage, electrical potentials are applied to all four reservoirs to form streams from reservoir 2 to 3 (buffer stream), 1 to 3 (sample stream), and 4 to 3 (buffer stream),

Figure 1.15. Pinched injection. (A) Injection, (B) separation.
respectively. As a result, a well-defined sample plug appears at the intersection.

Following the formation of the sample plug, a small electrical potential is applied to reservoirs 1 and 3 to drive the sample in the injection channel toward the reservoirs, while a high voltage is applied across reservoirs 2 and 4 to move the sample plug to the separation channel. This “pinched injection” can not only avoid sample leakage, but also give reproducible separation.\textsuperscript{16}

Gated injection\textsuperscript{166} is a continuous injection method. As shown in Figure 1.16, first, sample is electrokinetically driven from reservoir 2 to 3 by applying a voltage across reservoirs 2 and 3. Meanwhile, to prevent leakage of the sample into other channels, a buffer stream from reservoir 1 to 4 is introduced by applying a voltage across reservoirs 1 and 4. Next, reservoirs 1 and 4 are momentarily floated and a small amount of the sample enters the buffer and separation channels. When the voltage is re-applied across 1 and 4, the buffer stream forms again and a sample plug is “squeezed” into the separation channel for further separation. A unique feature of gated injection is that by carefully controlling the injection time, various amounts of analytes can be introduced; this is difficult to realize in pinched injection.

![Gated injection diagram](image)

**Figure 1.16.** Gated injection. (A) Loading, (B) injection, (C) separation.
1.3.4 Common Detection Approaches in Microchip Electrophoresis

**Optical detection.** UV-absorbance detection is a standard optical detection method for both conventional CE and HPLC. However, due to the small optical length (i.e., width) of microchannels and the strong UV absorption of polymer substrates, this detection method is unsuitable for polymeric μCE devices.

Laser-induced fluorescence (LIF) detection with a typical excitation wavelength of 488 nm is the most common optical detection method for μCE devices due to its very high sensitivity. Unfortunately, most analytes such as proteins and peptides do not have fluorophores that can be excited at 488 nm. Fluorescent dyes should be coupled to the analytes prior to, or after, CE separation. However, derivatization chemistry is inconvenient and different numbers of fluorescent tags may be linked to one analyte at multiple reaction sites, which results in electropherograms that are difficult to interpret. Three amino acid residues of proteins, tyrosine, tryptophan, and phenylalanine, can be directly excited to emit native fluorescence using a 266-nm UV laser, which was exploited to detect unlabeled proteins. However, the 266-nm excitation wavelength can be easily absorbed by many commodity polymeric materials and is therefore, not suitable for polymeric μCE devices.

Besides UV LIF, two-photon excited fluorescence (TPEF) detection can be used to detect the native fluorescence of proteins, peptides or other analytes. In a typical two-photon excitation process, a fluorophore absorbs two photons sequentially and is excited to an appropriate energy state. Fluorescence is then emitted when the excited fluorophore returns to its ground state. The time interval of the absorption of two photons is within 1 fs; otherwise, the fluorophore relaxes back to the ground electronic state and no
fluorescence can be observed. Moreover, the two-photon excitation process strongly depends on high excitation intensity, which can be obtained by a tightly focused laser beam.\textsuperscript{169, 170} In comparison to conventional single-photon excited fluorescence such as UV LIF, the excitation wavelength of TPEF is in the visible range. Moreover, the fluorescence signal and background are confined to a volume smaller than 1 $\mu$m\textsuperscript{3}, and discrimination of the signal from the excitation source and the background is easier since the wavelength of the fluorescence signal is shorter.\textsuperscript{170} TPEF was first exploited to detect analytes by Sepaniak and Yeung.\textsuperscript{171} Okerberg and Shear\textsuperscript{172} used TPEF to detect electrophoretically separated unlabeled peptides in a silica capillary. TPEF detection was also applied to a silica $\mu$CE device.\textsuperscript{173} Very recently, Paul et al.\textsuperscript{174} reported a compact, inexpensive TPEF detector specially designed for microfluidic devices. Detection of unlabeled phenylalanine, tyrosine, tryptophan, and bovine serum albumin (BSA) was demonstrated. TPEF detection of proteins and peptides in polymeric microfluidic devices has not been reported yet. However, because the excitation wavelength of TPEF is in the visible range, and emission wavelengths of proteins and peptides are within 300-350 nm, this detection method can potentially be applied to this application.

**Electrochemical detection.** Optical detection requires relatively expensive and complex instrumentation to achieve high sensitivity. The experimental setup for electrochemical detection, however, is relatively simple and easily integrated into microfluidic devices. Since the probing electrodes of an electrochemical detector can be microfabricated using standard photolithography, their significantly reduced dimensions generally result in higher sensitivity and faster response time than conventional electrochemical detectors.\textsuperscript{175, 176} Electrochemical detection has been applied to both
inorganic and polymeric μCE devices, and employed to analyze DNA fragments, amino acids, peptides, proteins, carbohydrates, neurotransmitters, and explosives, etc. Common operation modes include conductometry, amperometry, and voltammetry.

**Mass spectrometry.** Mass spectrometry (MS) is a very powerful analytical tool and is widely used in academia and industry. Besides small sample consumption, fast analysis speed, and high sensitivity, MS offers the capability of elucidating the structures of compounds. Because the fluidic structures and flow rates of μCE devices are compatible with electrospray ionization (ESI) interfaces, most μCE chips are connected to mass spectrometers through ESI interfaces. The outlet of a separation channel in μCE chips can be directly used as an electrospray emitter; however, a large droplet tends to form on the planar surface around the channel exit if there is no liquid controlling mechanism, which decreases the stability of the electrospray and creates a dead volume. To detect synthesized peptides and protein digests with μCE-MS, Li et al. directly inserted a short capillary, which served as a disposable nano-electrospray emitter, in the enlarged end of the μCE separation channel (Figure 1.17A), or connected the μCE chip to a conventional sheath-flow electrospray interface with a long capillary. When an ESI interface with the configuration in Figure 1.17A was used, additional buffer was pumped at a set flow rate from a side channel to improve the stability of the electrospray, and to supply solvent for proper analyte ionization. Wachs et al. fabricated a miniaturized electrospray device driven by a make-up flow and a nebulizing gas (Figure 1.17B) and connected it to the separation channel outlet of a PCOC μCE chip through a micro liquid junction (Figure 1.17C). The electrophoretically separated analytes in the PCOC
Figure 1.17. (A) μCE chip with a capillary nano-electrospray emitter, (B) simplified schematic diagram of a miniaturized electrospray device, (C) micro liquid junction, (D) integrated liquid junction in a μCE chip, and (E) μCE chip with a tapered-end nano-electrospray emitter.

Microchip were then introduced into a mass spectrometer through the electrospray device for subsequent detection.

A glass μCE chip integrated with a liquid junction was reported by Zhang and co-workers (Figure 1.17D). In this microdevice, a liquid junction channel perpendicular to the separation channel was fabricated. The flat end of a tapered capillary was then inserted through a precisely etched channel and aligned with the exit of the separation channel. The tapered end of the capillary was enclosed in a miniaturized ESI chamber connected to the sampling orifice of a mass spectrometer, and the pressure in the chamber
was maintained at 78 kPa to produce a stable electrospray. Highly efficient separations of proteins, protein digests, and peptides with a detection limit in the attomole range were obtained using the µCE-MS setup. In the fabrication of a PDMS µCE chip, Thorslund et al. constructed a microchannel network and a tapered end in the same substrate (Figure 1.17E). Following the microfabrication, a thin layer of graphite-doped PDMS was coated onto the surface of the tapered portion, which was used as a nano-electrospray emitter. It was found that a stable electrospray could be generated only when the emitter was accurately aligned to the sampling orifice of the mass spectrometer.

1.4 Electric Field Gradient Focusing

1.4.1 Introduction

Blood is a transporter and distributor of metabolites and cellular by-products. The concentration and distribution of proteins in blood are very sensitive to cellular conditions. Diseases such as cancer can change the expression of blood proteins. In order to monitor protein variations associated with cancer in blood samples, the target proteins must be separated from numerous other proteins with vastly different molecular weights (MW), isoelectric points (pI) and concentrations. It has been estimated that there are approximately 500,000 to 1 million proteins expressed in humans and the expressed proteins are often further modified by post-translational modifications. Therefore, protein separation is an extremely difficult task because of the enormous number of proteins in biological systems and the dynamic nature of proteins.

In order to address the difficult challenge of protein analysis, applicable analytical separation tools must provide very broad quantitative dynamic range, low detection limits,
high throughput, large peak capacity, good resolving power, and reasonably short analysis time. The most popular method for large-scale separation of proteins is two-dimensional (2-D) gel electrophoresis.\textsuperscript{191-193} However, this time-consuming and labor-intensive technique has insufficient resolution and poor reproducibility. Moreover, it cannot be employed to analyze proteins with extreme pI values and hydrophobicity. The detection limits of this technique are insufficient to detect low-abundance proteins. Researchers have been developing alternative approaches such as liquid-phase isoelectric focusing coupled to nonporous reversed-phase high performance liquid chromatography,\textsuperscript{194} 2-D liquid chromatography in combination with tandem mass spectrometry,\textsuperscript{195} and packed capillary column liquid chromatography coupled to Fourier transform ion cyclotron resonance mass spectrometry.\textsuperscript{196, 197} Although some improvements have been achieved, the performances of these techniques are still far from adequate. New analytical tools are still required to resolve complex protein mixtures.

### 1.4.2 Fundamental Theory of Electric Field Gradient Focusing

Electric field gradient focusing (EFGF) is a separation method in which a gradient or combination of gradients causes each analyte to seek an equilibrium position along the separation path.\textsuperscript{198-200} It belongs to a family of equilibrium-gradient separation methods\textsuperscript{201} such as isoelectric focusing (IEF), for which the separation mechanism is based on a pH gradient along the separation channel. In EFGF, charged analytes migrate at different velocities, depending on their electrophoretic mobilities and locations in an electric field gradient. The movement of analytes is countered by an opposing liquid flow, and each
analyte finally reaches its equilibrium position where its electrophoretic velocity just balances the countering flow velocity (Figure 1.18).

![Schematic diagram of a typical EFGF process.](image)

**Figure 1.18. Schematic diagram of a typical EFGF process.**

In the theoretical treatment by Tolley et al.,\textsuperscript{198} the general transport equation was expressed as

\[ J = W(x)c(x) - D_T \frac{\partial c(x)}{\partial x} \]  \hspace{1cm} (1.23)

where \( J \) is the flux density of the analyte, \( c(x) \) is the concentration of the analyte at point \( x \), \( D_T \) is the dispersion coefficient that represents the sum of all contributions to effective diffusion, and \( W(x) \) denotes the translational velocity of the analyte at point \( x \), which can be expressed as

\[ W(x) = mP(x) + u \]  \hspace{1cm} (1.24)
$P(x)$ is the intensity of the external field at point $x$, $m$ is the velocity of the analyte induced by the external field with unit intensity, and $u$ is the velocity of the bulk flow. The gradient of the external field intensity is given as

$$q(x) = -\frac{\partial P(x)}{\partial x}$$  \hspace{1cm} (1.25)

When the analyte is focused, or in an equilibrium state

$$\sigma = \sqrt{\frac{D_T}{mq(x_0)}}$$ \hspace{1cm} (1.26)

and

$$N = \frac{L}{4\sigma} = \frac{L}{4} \sqrt{\frac{mq(x_0)}{D_T}}$$ \hspace{1cm} (1.27)

$$R_S = \frac{\Delta x}{4\sigma} = \frac{|W(x'_0)|}{\sqrt{mq(x_0)D_T}}$$ \hspace{1cm} (1.28)

where $\sigma$ is the standard deviation of the band width of the focused analyte, $N$ is the peak capacity, $L$ is the length of the separation path, $R_S$ is the resolution of two focused analytes, $x_0$ is the focusing position of the first analyte, and $x'_0$ is the focusing position of the second analyte.

Equation 1.27 indicates that for a fixed channel length, peak capacity, or the total number of resolved bands, is increased when a relatively steep electric field gradient is used. However, from Equation 1.28, one knows that the steep electric field gradient may lead to a decrease in resolution. Simulation results show that only moderate resolving power can be achieved with a linear electric field gradient, which is insufficient to meet the requirements of protein analysis. However, both peak capacity and resolution can be improved greatly if a bilinear electric field gradient is utilized in an EFGF device. As
shown in Figure 1.19, the first segment of the electric field gradient is steep so that high peak capacity can be achieved, while the second segment provides sufficient resolving power to separate analytes moving from the first segment. By tuning the counter flow or electric field gradient, analytes within a specific mobility range can be retained in the separation channel of an EFGF device, while others are pushed out by the counter flow. This feature is very useful for applications such as sample purification and diagnostic analysis.

1.4.3 Implementation of Electric Field Gradient Focusing EFGF devices with changing-cross-section area

Koegler and Ivory\textsuperscript{202,203} used a packed dialysis tube and conical poly(methyl methacrylate) cylinder to construct a preparative EFGF device (Figure 1.20A). The shape of the chamber was determined by

\[
E(x) = \frac{I}{\sigma(x)A(x)}
\]  \hspace{1cm} (1.29)
where $E(x)$ is the electric field gradient, $I$ is the total current, $\sigma(x)$ is the conductivity of the buffer solution, and $A(x)$ is the cross-sectional area of the chamber. An electric field gradient along the length of the dialysis tube would form in the conical chamber when a voltage was applied across it. Without the dialysis tube, the variation in linear velocity along the conical shaped tube would offset the change in electrophoretic velocities of the analytes in the changing electric field and, hence, eliminate the focusing effect.

Humble et al.\textsuperscript{61} fabricated a miniaturized EFGF planar device using an ionically conductive acrylic copolymer (Figure 1.20B). Unlike previous EFGF devices, the focusing channel was formed in the shaped nanoporous copolymer. When voltage was applied across the copolymer, an electric field gradient could be established inside the focusing channel. For this system, only one syringe pump was used to provide counter flow, which reduced the complexity of the EFGF system.

Figure 1.20. EFGF device with changing-cross-sectional area.
**EFGF device with conductivity gradient.** Conductivity gradient focusing (CGF) is another form of EFGF\(^{204}\) in which a dialysis membrane is used to isolate the separation channel from a parallel flowing channel. A slow-moving high conductivity buffer is introduced into the separation channel as the counterflow liquid, and a lower conductivity buffer is introduced into the adjacent parallel channel. A concentration gradient or conductivity gradient forms along the separation channel by diffusion of buffer ions through the membrane from higher to lower concentration. According to Equation 1.28, when voltage is applied along the channel with constant cross-sectional area, and the total current is kept constant, an electric field gradient will be established in the separation channel.

An analytical hollow dialysis fiber based conductivity gradient focusing device coupled to a UV absorbance detector was fabricated by Wang et al.\(^{205}\) Focusing, preconcentration, and voltage-controlled separation of proteins were demonstrated. The system was later used for concentration of bovine serum albumin, desalting of a protein sample, and purification of ferritin from a protein mixture.\(^{206}\)

**EFGF device with multi-electrode array.** In a third form of EFGF, an array of electrodes with voltages individually controlled by a computer is used to generate an electric field gradient.\(^{207}\) Again, proteins are focused in a parallel channel which is isolated from the electrode array with a dialysis membrane. One obvious advantage of this format of EFGF is that the gradient profile can be dynamically adjusted, which offers flexibility in tailoring the separation of charged analytes. Petsev et al.\(^{208}\) recently reported a miniaturized poly(dimethylsiloxane) (PDMS) EFGF device based on the multi-electrode approach. Instead of metal electrodes, two dialysis hollow fibers in
fluidic and electrical contact with the main dialysis fiber channel were used to create the electric field gradient. Counter flow was generated from an electroosmotic pump instead of a syringe pump.

**Temperature gradient focusing.** In temperature gradient focusing (TGF), an electric field gradient is produced when voltage is applied across a capillary or microchannel in which local buffer conductivity continuously changes with an applied external temperature gradient. For TGF to work, the temperature dependence of the buffer conductivity must be independent of analyte mobility, which places a stringent limit on buffer selection; however, this technique does not require a semi-permeable membrane and, therefore, small ions can be focused using this technique and device microfabrication is greatly simplified. Furthermore, a major advantage of TGF over other EFGF forms is that the concentration of the buffer ions is unaffected by the focusing since their electrophoretic velocities are temperature independent and, hence, the electric field gradient is mainly determined by the external temperature gradient.

1.5 **Dissertation Overview**

Chapters 2 to 4 describe my research on the fabrication of polymeric microfluidic devices for protein analysis. Chapter 2 describes the development of an ATRP-based permanent surface modification technique suitable for PMMA µCE chips, in which PEG brushes were grafted onto the PMMA channel walls to improve resistance to protein adhesion. High quality peptide and protein separations using PEG-grafted PMMA µCE chips are demonstrated in this chapter. Chapter 3 presents the important physical characteristics, surface modification, and microfabrication of a surface reactive acrylic
copolymers. Separation of amino acids, peptides, and proteins using μCE chips fabricated from this copolymer is shown. Chapter 4 depicts a novel in-situ semi-permeable membrane integration approach, and utilization of this technique to construct microfabricated polymeric EFGF devices. The relationship between membrane properties and behavior of the polymeric EFGF microdevices is discussed. Chapter 5 of this dissertation contains a summary of proposed future research directions.
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2 SURFACE-MODIFIED POLY(METHYL METHACRYLATE)
CAPILLARY ELECTROPHORESIS MICROCHIPS FOR PROTEIN AND
 PEPTIDE ANALYSIS *

2.1 Introduction

As reviewed in Chapter 1, to date, most reported permanent surface modification
techniques for rigid polymeric materials are not directly applicable to protein analysis. In
the present work, I describe a technique using atom-transfer radical polymerization
(ATRP) to covalently graft PEG brushes on the surface of PMMA, and apply the
permanent surface modification to the fabrication of PMMA μCE devices for high-
quality electrophoretic separations of proteins and peptides.

2.2 Experimental Section

2.2.1 Materials

2-Bromoisobutyryl bromide (98%), poly(ethylene glycol) methyl ether
methacrylate (PEGMEMA, MW~475), 2,2’-dipyridyl (99+%), copper(I) chloride (98+%),
and copper(II) bromide (99%) were purchased from Aldrich (Milwaukee, WI) and used
without further purification. Heptane (reagent grade), tetrahydrofuran (THF, reagent
grade), absolute methanol (reagent grade), pyridine (reagent grade), urea (reagent grade),
and dithiothreitol (molecular biology grade) were obtained from Fisher Scientific (Fair
Lawn, NJ). Iodoacetamide was purchased from Amersham Biosciences (Piscataway, NJ).
Ethylenediaminetetraacetic acid disodium salt dihydrate (Na₂EDTA·2H₂O, 99+%) was
obtained from Invitrogen Life Technologies (Carlsbad, CA). Fluorescein isothiocyanate

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(FITC), bovine serum albumin (BSA), trypsin, FITC-conjugated BSA (FITC-BSA), and FITC-conjugated insulin (FITC-insulin) were purchased from Sigma (St. Louis, MO). R-Phycoerythrin (R-PE) was obtained from Polysciences (Warrington, PA). Recombinant, enhanced green fluorescent protein (GFP) was purchased from Clontech (Palo Alto, CA). The 18.2 MΩ·cm deionized water used was from a Milli-Q UF Plus water purification system (Millipore, Billerica, MA), and the buffer solution used throughout the experiments was 10 mM TRIZMA® hydrochloride (Tris-HCl) at pH 8.8, which was filtered using 0.2-µm syringe filters (Pall Gelman Laboratory, Ann Arbor, MI).

2.2.2 Microchip Fabrication

Acrylite FF sheets (Cyro, West Paterson, NJ) were used as substrates for PMMA μCE chips. The fabrication protocol was adapted from Kelly and Woolley. First, an 800-nm etch mask layer of silicon dioxide was grown on a 4-in. silicon wafer (Encompass Distribution Services, Pleasanton, CA) at 1110 °C in an atmosphere of oxygen and water. Next, Shipley 812 (Shipley, Marlborough, MA) positive photoresist was spin coated on the silicon wafer at 3500 rpm for 120 s. To increase adhesion of the photoresist and drive off residual solvent, the wafer was baked at 90 °C for 2 min. The photoresist was then exposed to UV radiation for 40 s through a photomask using a PLA-501F mask aligner (Canon, Tokyo, Japan). The photomask was designed using CAD software (CleWin, WieWeb Software) and printed onto transparency film using a 3600 dpi printer. Following exposure, the photoresist was developed with 20% aqueous Microposit 351 developer (Shipley) for 30 s. After being placed in an oven for 30 min at 150 °C, which helped to harden the photoresist, the wafer was immersed in buffered hydrofluoric acid
for 10 min to remove silicon dioxide from the areas not covered by the photoresist.

Finally, the silicon wafer was etched using 40% aqueous KOH solution at 80°C for 30 min. The resulting silicon template with protruding features was used to imprint the channel pattern (Figure 2.1) into 1.5-mm-thick PMMA substrates at 120 °C. The fabrication of μCE chips was finished after surface derivatization (see below) by thermally bonding the patterned surface to a 3.0-mm-thick PMMA substrate with access holes at 95 °C. The imprinting and bonding processes were performed in an HP 5890 gas chromatography oven. The access holes were created using a C-200 CO₂ laser engraving system (Universal Laser Systems, Scottsdale, AZ). The dimensions of the channel

Figure 2.1. (A) Schematic diagram of the μCE chips used in this work (1, sample; 2, sample waste; 3, buffer; 4, buffer waste). (B) Cross-sectional dimensions of the microchannels.
features were measured using an Alpha-step 200 profilometer (KLA-Tencor, San Jose, CA).

### 2.2.3 Oxygen Plasma Activation

An oxygen plasma generated using a DEM-451 reactive ion etcher (Anelva, Tokyo, Japan) was used to oxidize the surface of PMMA before grafting. The plasma was excited at 13.56 MHz, and the cathode/sample holder was cooled with running water. Prior to plasma oxidation, the patterned PMMA substrates and cover plates were annealed at 100 °C for 1 h. Next, the substrates were rinsed with methanol and water and dried with nitrogen gas. The PMMA substrates were loaded on the cathode in the etching chamber, and the chamber pressure was reduced below 10 mTorr using a two-stage vacuum pump. Oxygen was introduced into the chamber at a flow rate of 3.10 standard cubic centimeters per minute using a mass flow controller. The plasma was started when the chamber pressure reached 100 mTorr, and the power of the plasma was maintained at 80 W using a manual impedance matching network during the oxidation process, which took ~3 min.

During the plasma oxidation, ionized oxygen or oxygen radicals attacked the PMMA backbone or side chains. As a result, oxygen-containing functionalities such as hydroxyl and carboxyl groups were generated on the PMMA surface, which provided handles for subsequent chemical reactions.
2.2.4 Immobilization of Initiator

Immobilization of a typical ATRP initiator (Figure 2.2A), 2-bromoisobutyryl bromide, was performed according to the procedure of Carlmark and Malmstrom.\(^2\)

Immediately after oxygen plasma activation, the PMMA substrate was immersed in a heptane/THF solution containing 50 mM 2-bromoisobutyryl bromide and 55 mM pyridine. After 24 h, the substrate was removed and washed thoroughly with methanol and deionized water. Nitrogen gas was then used to dry the substrate.

A

\[
\text{PMMA} + \text{CH}_7\text{H}_12/\text{THF}, \text{Pyridine} \rightarrow \text{PMMA}
\]

B

\[
\text{PMMA} + \text{CuCl, CuBr}_2: \text{H}_2\text{O} \rightarrow \text{PMMA}
\]

Figure 2.2. Reaction scheme for grafting PEGMEMA on a PMMA substrate. (A) Immobilization of 2-bromoisobutyryl bromide on an oxygen-plasma-activated PMMA surface. (B) Grafting of PEGMEMA on a PMMA surface using ATRP.

2.2.5 Grafting of Poly(ethylene glycol) on the PMMA Surface

Grafting of PEG on the PMMA surface (Figure 2.2B) was implemented using a protocol adapted from the literature.\(^3^5\) First, 40 mL of PEGMEMA and 60 mL of deionized water were added to a 250-mL round-bottom flask. The flask was then sealed with a sleeved rubber stopper and subjected to laboratory vacuum followed by 20 psi nitrogen gas for 30 min. This purging procedure was repeated three times to remove oxygen. Afterward, CuCl (0.424 g, 1.8 mmol), CuBr\(_2\) (0.287 g, 0.54 mmol), 2,2'
dipyridyl (1.74 g, 4.68 mmol), and 40 wt % PEGMEMA aqueous solution were mixed inside a glove box. To start the ATRP reaction, the resulting dark brown mixture was transferred to a crystallization dish containing the initiator-immobilized PMMA substrate under a nitrogen atmosphere. The reaction was allowed to proceed at room temperature for 24 h. To quench the reaction, the PMMA substrate was taken out of the glovebox, immersed in saturated aqueous Na₂EDTA solution to remove residual copper(II) ions, and rinsed with a copious volume of deionized water. Before chip assembly, nitrogen gas was used to dry the PMMA substrates.

2.2.6 Contact Angle Measurements

Immediately after 4 μL of deionized water was placed on the PMMA surface using a syringe, an NRL-100 goniometer (Ramé-hart, Mountain Lakes, NJ) was used to measure the contact angle. The contact angle was calculated as the mean of the left and right contact angles of the water drop.

2.2.7 Electroosmotic Flow Measurements

The electroosmotic flow (EOF) in the PMMA microchannel was measured using the current monitoring method.⁶ In a typical measurement, 1 mL of deionized water was pumped through the microchannel at a flow rate of 50 μL/min using a syringe pump (11-Plus, Harvard Apparatus, Holliston, MA). Following this rinsing step, the channel was conditioned with 1 mL of 50 mM Tris-HCl buffer at a pH of 8.8 using the syringe pump (flow rate, 50 μL/min). The reservoirs used to provide electrical contact to the channel were emptied, 10 μL of 50 mM Tris-HCl buffer was added to one reservoir, and the same
volume of 1 mM Tris-HCl buffer was introduced into the other reservoir. A PS-350 high-voltage supply unit (Stanford Research Systems, Sunnyvale, CA) was employed to provide high voltage during the measurement, and current variation was recorded using a PCI-1200 data acquisition board (National Instruments, Austin, TX) and an in-house-written LabView 6i software program (National Instruments).

2.2.8 Tryptic Digest Preparation

A digestion protocol described in the literature was followed. Briefly, before tryptic digestion, 6 M urea-denatured BSA (1 mg) was reduced and alkylated using dithiothreitol and iodoacetamide, respectively. The pretreated BSA was then digested with 20 μg of trypsin at 37 °C at pH 8.0. The tryptic digest was desalted using a Spectra/Por cellulose ester dialysis membrane (MWCO 100, Spectrum Medical Industries, Houston, TX) for 24 h, and 6 mM FITC in acetone was added to the product at a 10:1 FITC/BSA molar ratio. The derivatization reaction was allowed to proceed in the dark at room temperature for 24 h. Before CE separation, the digest was diluted 2.5-fold with 10 mM Tris-HCl buffer at a pH of 8.8.

2.2.9 Detection

The laser-induced fluorescence detection system and the setup for data acquisition have been reported elsewhere. The sampling rate for data collection was 20 Hz.

2.2.10 Chip Operation

Channels were filled with 10 mM Tris-HCl buffer from reservoir 4 (Figure 2.1)
using a syringe pump. Prior to separation, 30 μL of protein sample was introduced into reservoir 1, and a platinum electrode was inserted into each reservoir to provide electrical contact. Voltages were applied to the reservoirs using PS-300 and PS-350 high-voltage supply units (Stanford Research Systems). The two voltage supplies were connected using a home-built switching circuit board. “Pinched” injection was used to introduce the sample into the channel with an estimated injection volume of 230 pL. During injection, reservoirs 1, 3, and 4 were grounded, and reservoir 2 was maintained at +0.6 kV. During separation, reservoirs 1 and 2 were set at +0.6 kV, reservoir 3 was grounded, and reservoir 4 was set at +2.0 kV. These conditions were used for all of the μCE experiments.

2.2.11 Conventional Capillary Electrophoresis

A Crystal CE model 300 capillary electrophoresis system (UNICAM, Madison, WI) was used to analyze unlabeled insulin. An 80-cm-long fused silica capillary column (75 μm i.d. × 360 μm o.d., Polymicro Technologies, Phoenix, AZ) was installed in this instrument, and the effective length (the distance between the injection end of the capillary and the detection window) was 65 cm.

An insulin sample was prepared in 10 mM Tris-HCl at pH 8.8; the same buffer was also used as separation buffer. Hydrodynamic injection was performed at 100 mbar for 6 s to introduce 1 mg/mL insulin into the capillary column. A voltage of 20 kV was then applied to the column to start the electrophoresis. The protein was detected using UV absorbance at 214 nm.
2.3 Results and Discussion

2.3.1 Substrate Fabrication

Unlike some commodity polymers, the resistance of PMMA to many organic chemicals is low. Acids, amines, ketones, esters, cycloethers, aromatic hydrocarbons, nitriles, and halogenated hydrocarbons can dissolve PMMA. N,N-Dimethylacetamide, N,N-dimethyl formamide, and dimethyl sulfoxide also damage PMMA. Xu and colleagues employed a THF/heptane mixture (4:7 v/v) as a solvent to modify the PMMA surface. In this work, I used THF/heptane to immobilize 2-bromoisobutyryl bromide on the PMMA surface. The ratio of THF to heptane was decreased to 1:4 (v/v) to minimize any effects on PMMA. Indeed, I found that this solvent mixture at room temperature did not damage the PMMA microchannels or cause loss of polymer clarity. Moreover, the background fluorescence of the PMMA substrates did not increase after surface treatment in this solvent mixture.

Cutting and drilling are often used in the fabrication of polymeric microdevices; however, the structures can be stressed during conventional machining. I observed that after drilled PMMA substrates were treated with organic solvents, cracks appeared and extended rapidly into the stressed structure, presumably because the solvents entered and swelled the polymer matrix. To alleviate this problem, the PMMA components were annealed in an oven before surface modification. Annealing was carried out at the glass transition temperature of PMMA (100 °C) for 1 h, which relieved the stress. Figure 2.3 shows the effect of annealing. Holes with diameters of 0.2 cm were drilled through 1 cm×1 cm, 1.5-mm-thick PMMA substrates using the CO₂ laser system. Two drilled PMMA substrates, one annealed and one that was not annealed, were immersed in absolute
methanol for 20 min. Whereas cracks were found along the edge of the hole in the untreated PMMA substrate (Figure 2.3A), cracks did not appear in the annealed PMMA substrate (Figure 2.3B).

2.3.2 Contact Angle and Electroosmotic Flow Measurements

Water contact angles for 1.5- and 3.0-mm-thick PMMA substrates (Table 2.1) were obtained, since both were utilized in the fabrication of microdevices. The contact angle for PEG-grafted PMMA substrates agreed well with results obtained from PEG monolayers grafted on a silicon surface (~41°), while the data for untreated PMMA substrates were close to those measured by Henry and coworkers (~66°). The comparison between contact angles before and after surface modification indicates that the wettability and surface chemistry of PMMA were significantly changed after PEG grafting.
Table 2.1. Contact Angles for PEG-grafted and Untreated PMMA Substrates.

<table>
<thead>
<tr>
<th></th>
<th>1.5 mm-thick substrate&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>3.0 mm-thick substrate&lt;sup&gt;a,b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-grafted PMMA</td>
<td>38.6 ± 1.2</td>
<td>41.2 ± 0.8</td>
</tr>
<tr>
<td>Untreated PMMA</td>
<td>65.7 ± 2.8</td>
<td>69.3 ± 2.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each value was averaged from the contact angle of four separate water drops on the same PMMA substrate.

<sup>b</sup> 95 % CL.

The EOF mobility for an untreated PMMA microchannel was \((1.6 ± 0.2) \times 10^{-4}\) cm²·V⁻¹·s⁻¹ (%CL = 95%; average of 4 measurements), and the direction of the EOF was from the anode to the cathode. In contrast, the variation in current during EOF testing for the PEG-grafted PMMA microchannel was very small, and the current-time profile resembled a plateau over at least 400 s. Thus, under the conditions used for separation of proteins and peptides in this study, EOF was less than \(1 \times 10^{-5}\) cm²·V⁻¹·s⁻¹ in the surface-modified PMMA microchannels.

2.3.3 Electrophoresis of Proteins and Peptides

Bovine serum albumin is well known for its nonspecific adsorption on surfaces, which can negatively affect its analysis in microdevices. Thus, FITC-BSA was selected as a test compound to evaluate the effect of the ATRP-grafted PEG layer on the performance of PMMA μCE devices.

To examine the adsorption of FITC-BSA on both untreated and PEG-grafted PMMA microchannels, FITC-BSA was introduced to the μCE chips. After 30 min, the microchannels were flushed with deionized water for 2 h at a flow rate of 30 μL/min. The microchips were then placed on the microscope stage, the injection cross region was illuminated with a 488-nm laser line, and fluorescence was observed. As shown in Figure
2.4A, FITC-BSA adsorbed strongly on the surface of the untreated PMMA microchannels and bright fluorescence could be seen in all channels. In contrast, no fluorescence was observed in the PEG-grafted microchannel (Figure 2.4B), indicating significantly less protein adsorption.

Figure 2.5 compares the μCE analysis of FITC-BSA using PEG-grafted and unmodified PMMA microdevices. As shown in Figure 2.5A, three components were resolved in the electropherograms obtained from the surface-grafted device. The two small peaks (1a and 1b) are suspected to be fragments of BSA or other bovine serum proteins. I also spiked the FITC-BSA sample with FITC, and a peak corresponding to the free label (peak 2) appeared between peaks 1b and 1c in the electropherogram (Figure 2.6), confirming that peaks 1a, 1b, and 1c were not the free fluorescent label. The same protein sample was also analyzed using an untreated PMMA μCE chip (Figure 2.5B). The migration time of peak 1c (Table 2.2) was 2.6-fold greater on the untreated PMMA μCE chip than on the PEG-grafted chip. The slower migration was due to a combination.
Figure 2.5. µCE analysis of FITC-BSA using PMMA devices. (A) PEG-grafted chip, (B) untreated chip. Electropherograms were recorded for three consecutive runs. Peaks 1a, 1b, and 1c are the three main components of the BSA sample.

of interactions between FITC-BSA and the PMMA surface, and the electroosmotic flow that opposed migration of the protein in the untreated device. In addition, strong protein-surface interactions in the untreated PMMA microchannel resulted in severe band broadening, and therefore, the column efficiency or total plate number for the untreated chip was 1 order of magnitude lower compared to the PEG-grafted chip (Table 2.2).
Moreover, adsorption of the negatively charged BSA should change the local ζ-potential on the unmodified PMMA surface, which may give rise to unstable EOF, and thus, poorer reproducibility of FITC-BSA migration times. Unstable EOF may have also caused baseline drift and poor injection reproducibility in unmodified PMMA systems. However, these phenomena were not found for the PEG-grafted microchips.

<table>
<thead>
<tr>
<th>Migration time (s)</th>
<th>RSD %</th>
<th>Total plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-grafted µCE chip</td>
<td>30.9</td>
<td>0.27</td>
</tr>
<tr>
<td>Untreated µCE chip</td>
<td>80.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Data were measured/calculated for peak 1c in Figure 2.5.
* Data were calculated from 5 consecutive runs in one microchip.

FITC-insulin was also used to test the PEG-grafted microchips. Seven peaks, three major ones and four minor ones, appeared in the electropherogram (Figure 2.7). In comparison, only one peak was observed in conventional capillary electrophoresis of unlabeled insulin (electropherogram not shown). I believe that the multiple peaks may
have resulted from insulin derivatized with different numbers of FITC tags or from fluorescent labeling of peptide chains resulting from insulin decomposition.

Polyacrylamide gel electrophoresis of FITC-insulin confirms that there are three major peptide components in the sample, which correspond to peaks 1-3 in Figure 2.6. However, μCE of FITC under the same conditions shows that the migration time of FITC is very close to peak 2. I spiked the FITC-insulin sample with FITC and found that the height of peak 2 increased. Whether or not FITC is contained in peak 2 cannot be verified without using other analytical techniques.

![Electropherograms](image)

**Figure 2.7. Electrophoresis of FITC-insulin. Electropherograms were obtained from four consecutive runs. Peaks 1-3 were used in the evaluation of chip performance.**

Peaks 1 and 3 were used to evaluate chip performance (Table 2.3). As many as $5.2 \times 10^4$ plates for a 3.5-cm-long separation channel were obtained in these analyses. The high plate numbers are a direct consequence of reducing both EOF and nonspecific adsorption on the PMMA surface with the grafted PEG layer.
Table 2.3. Column Efficiency and Migration Time Reproducibility for Insulin Fragments.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Migration time (s)a</th>
<th>RSD %</th>
<th>Total platesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1</td>
<td>19.4</td>
<td>0.57</td>
<td>$5.2 \times 10^4$</td>
</tr>
<tr>
<td>Peak 2</td>
<td>24.4</td>
<td>0.92</td>
<td>$5.3 \times 10^4$</td>
</tr>
<tr>
<td>Peak 3</td>
<td>32.3</td>
<td>1.0</td>
<td>$4.2 \times 10^4$</td>
</tr>
</tbody>
</table>

a Data were calculated from 4 consecutive runs in one microchip.

A mixture of acidic proteins containing R-PE, FITC-BSA, and GFP was separated with a PEG-grafted PMMA μCE chip, and a typical result is shown in Figure 2.8. Three peaks (peak 1a and doublet 1b) belonging to FITC-BSA appear in the electropherogram. R-PE (peak 2) appears between peak 1a and doublet 1b. GFP splits into two peaks (peaks 3a and 3b) during the separation. The total plates for (1a) of FITC-BSA, (3b) of GFP, and R-PE were $3.9 \times 10^4$, $3.9 \times 10^4$, and $7.5 \times 10^3$, respectively.

![Figure 2.8. Separation of model acidic proteins. The sample contained R-PE (peak 2), FITC-BSA (peak 1a and doublet 1b), and GFP (peaks 3a and 3b).](image)

A 40-s separation of a tryptic digest of BSA was also conducted using a PEG-grafted PMMA μCE chip (Figure 2.9). The separation was reproducible with relative
standard deviations for peaks 1, 2, and 3 of 0.47, 0.32, and 0.60%, respectively (data were calculated from 4 consecutive runs in one microchip). These results demonstrate the applicability of surface-grafted μCE systems in peptide digest analysis, an important area of proteomics research.

Figure 2.9. Separation of a BSA tryptic digest.

2.4 Conclusions

Poly(ethylene glycol) was grafted to the surface of PMMA substrates using atom-transfer radical polymerization, which substantially reduced electroosmotic flow and nonspecific adsorption of proteins on the PMMA surface. As a result, fast, highly efficient, and reproducible electrophoretic separations of proteins and peptides were achieved using PEG-grafted PMMA μCE chips. I believe that this surface modification technique can not only be utilized in the fabrication of μCE chips as shown here, but can also be applied in a much broader sense to other disposable polymeric microfluidic devices for proteomics studies.
2.5 References

3 SURFACE REACTIVE ACRYLIC COPOLYMER FOR FABRICATION OF MICROFLUIDIC DEVICES *

3.1 Introduction

The difficulties encountered in modification of polymer surfaces result from two major factors. First, the surfaces of most traditional commodity polymers lack reactive sites and, thus, harsh chemical reactions or high-energy sources such as plasma and UV radiation must be used to activate the surface. Second, because of the large diversity of polymers, different modification protocols must be used for each polymeric material, which further complicates the methods. It is well known that the ease of surface modification of glass originates from its surface silanol groups. Therefore, it can be anticipated that surface treatment of polymeric microdevices would be significantly simplified if polymers with reactive functionalities on their surfaces could be used in microfabrication.

Among the various chemical functional groups that can be integrated into polymers, the epoxy group is attractive because it can be readily modified using various chemical reactions to introduce other moieties. Second, it is relatively stable under ambient conditions. Therefore, introducing epoxy groups into traditional commodity plastics that have good physical properties may form attractive polymeric materials that are suitable for the fabrication of microdevices.

In this study, a surface reactive acrylic copolymer, poly(glycidylmethacrylate)-co-(methy1methacrylate) (PGMAMMA) (Figure 3.1), was synthesized as a substrate for

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microdevices. Using a fabrication protocol similar to that used for PMMA microdevices, I successfully fabricated capillary electrophoresis microchips (µCE) with this material.

![Chemical structure of PGMAMMA](image_url)

Figure 3.1. Surface structure of PGMAMMA.

### 3.2 Experimental Section

#### 3.2.1 Materials

Methylmethacrylate (MMA), hydrogen peroxide (ACS grade, 30%), and sodium dodecyl sulfate (SDS) were purchased from Columbus Chemical Industries (Columbus, WI). Glycidylmethacrylate (GMA, 97%), 2,2’-azobisisobutyronitrile (AIBN, 98%), sodium periodate (NaIO₄, 99.8+%), 2-bromoisobutyryl bromide (98%), poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, MW~475), 2,2’-dipyridyl (99+%), copper(I) chloride (98+%), and copper(II) bromide (99%) were obtained from Aldrich (Milwaukee, WI). Glacial acetic acid (reagent grade) was purchased from EM Science (Gibbstown, NJ). Benzyl alcohol (reagent grade), absolute methyl alcohol (MeOH, reagent grade), anhydrous ethylenediamine (reagent grade), sodium acetate trihydrate (reagent grade), sodium silicate solution (40-42° Bé), heptane (reagent grade), tetrahydrofuran (THF, reagent grade), and acrylamide (electrophoresis grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium phosphate tribasic, sodium
chloride, sodium carbonate, sodium bicarbonate, sodium hydroxide, hydrochloric acid (37 %), and sulfuric acid (96.4%) were purchased from Mallinckrodt Specialty Chemicals (Paris, KY). Isopropyl alcohol (IPA, USP grade) and ethyl alcohol (EtOH, USP grade) were obtained from AAPER Alcohol and Chemical (Shelbyville, KY). Fluorescein isothiocyanate (FITC) for labeling amino acids, tris[hydroxymethyl] aminomethane (TRIZMA, Tris), 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS), N-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid (TES), 2-[[N-morpholino]ethanesulfonic acid (MES), lysozyme, and FITC-conjugated casein were purchased from Sigma (St. Louis, MO). Glycine, DL-aspartic acid, DL-asparagine, DL-glutamic acid, and DL-phenylalanine were purchased from ICN Biomedicals (Aurora, OH). Ethylenediaminetetraacetic acid disodium salt dihydrate (Na$_2$EDTA·2H$_2$O, 99+%) was obtained from Invitrogen Life Technologies (Carlsbad, CA). (Heptadecafluoro-1,1,2,2-tetrahydrodecyl)-trichlorosilane was ordered from Gelest (Morrisville, PA). R-phycoerythrin (R-PE) was obtained from Polysciences (Warrington, PA). FITC for labeling lysozyme was ordered from Molecular Probes (Eugene, OR).

Deionized water (18.2 MΩ·cm) was prepared using a Milli-Q UF Plus water purification system (Millipore, Billerica, MA). Two different PMMA sheets, Acrylite OP-1 and Acrylite FF, were purchased from Cyro (West Paterson, NJ). Pre-cleaned microscope slides (5 x 70 x 1 mm) were obtained from Fisher Scientific (Pittsburgh, PA).

All chemicals were used as received without further purification. Buffers used in EOF measurements and µCE separations were filtered using 0.2 μm syringe filters before experiments (Pall Gelman Laboratory, Ann Arbor, MI).
3.2.2 Synthesis of PGMAMMA

PGMAMMA sheets were prepared in home-made glass containers using thermally-initiated free-radical polymerization. The glass containers had dimensions of $75 \times 50 \times 1.5$ mm and were constructed by fusing microscope slides with sodium silicate solution. The containers were cured at $80^\circ$C for 30 min to harden the sodium silicate before use. In a typical preparation process, a monomer solution containing MMA, GMA, and 0.1 % (w/w) of AIBN initiator, was prepared. The monomer solution was degassed with a Branson 3200 sonicator (Branson Ultrasoundics, Danbury, CT) for 10 min before being introduced into the containers. To seal the monomer-filled glass containers, a glass strip cut from a microscope slide was placed over the container opening, and sodium silicate solution was carefully applied around the edges to seal the glass strip in place. After the sodium silicate solution hardened at room temperature, the container was placed in an HP 5890 gas chromatograph oven and the temperature was held at $65^\circ$C for 24 h. After the polymerization reaction was complete, the container was immersed in a water bath overnight to let the sodium silicate dissolve, after which the hardened polymer could then be removed from the container. The resultant polymer substrate was thoroughly rinsed with 2% (w/v) SDS solution, DI water and IPA.

3.2.3 Fabrication of PGMAMMA μCE Devices

To help with the release of hot embossed PGMAMMA substrates from the silicon templates, the surfaces of the silicon templates were passivated with (heptadecafluoro-1,1,2,2-tetrahydrodecyl)trichlorosilane using procedures simplified from the work of Brzoska et al. Briefly, the templates were immersed in a freshly prepared mixture
containing 70% (v/v) sulfuric acid and 30% (v/v) aqueous solution of hydrogen peroxide, and the solution was held at 100°C for 30 min. After thoroughly rinsing with deionized water, the silicon templates were dried with nitrogen gas. An oxygen plasma generated using a DEM-451 reactive ion etcher (Anelva, Tokyo, Japan) was employed to oxidize the cleaned silicon templates. Before processing, the templates were loaded on the cathode in the etching chamber. After the chamber pressure was reduced below 10 mTorr using a two-stage vacuum pump, oxygen was introduced at a flow rate of 3.1 cm³/min. The plasma was excited at 13.56 MHz when the chamber pressure was stabilized at 100 mTorr using a mass flow controller, and the power of the plasma was maintained at 100 W using a manual impedance matching network during the 5 min oxidation process. Immediately after oxidation, the templates were immersed in 10 mM trichlorosilane/heptane solution for 24 h.

The general fabrication protocol for PGMAMMA was similar to that previously used for PMMA μCE chips.² Briefly, the surface-passivated silicon template was used to emboss the channel pattern into a PGMAMMA substrate at 90°C. The fabrication of the μCE chips was finished by thermally bonding (68°C) the patterned substrate to a blank piece of PGMAMMA substrate with access holes. The hot embossing and bonding processes were performed using an HP 5890 gas chromatograph oven. The access holes were created using a C-200 CO₂ laser engraving system (Universal Laser Systems, Scottsdale, AZ).

### 3.2.4 Surface Modification of PGMAMMA

For aminolysis, the PGMAMMA substrates were rinsed with MeOH and
deionized water sequentially. Following cleaning, the PGMAMMA substrates were immersed in a reaction solution containing 20% (w/w) ethylenediamine, 40% (w/w) IPA, and 40% (w/w) EtOH at room temperature for 24 h. The aminolyzed PGMAMMA substrates were rinsed thoroughly with MeOH and deionized water after reaction. This same procedure was used in the treatment of PGMAMMA substrates with 20% (w/w) ethylenediamine dissolved in 50 mM (pH 12) phosphate buffer.

To aminolyze a PGMAMMA microchannel, an 11-Pico-Plus syringe pump (Harvard Apparatus, Holliston, MA) was used to introduce the ethylenediamine alcohol solution into the channel at a flow rate of 0.1 µL/min for 24 h. Following rinsing with IPA, deionized water was used to flush the channel at a flow rate of 15 µL/min overnight to remove the unreacted amine.

LPAAm was photo-grafted onto the PGMAMMA surface using a modified procedure of Hu et al. Briefly, a 3 mL monomer solution consisting of 6% (w/v) acrylamide, 5 mM NaIO₄, and 1% (w/v) benzyl alcohol was prepared and sonicated for 30 min. After being cleaned with MeOH and dried with nitrogen gas, the PGMAMMA substrate was immersed in the solution and exposed to UV radiation from a Dymax 5000-EC UV curing system (Dymax, Torrington, CT) for 60 min. The LPAAm-grafted PGMAMMA substrates were rinsed with IPA and deionized water after the reaction to remove unreacted monomer and adsorbed polymer. During the photografting process, the UV lamp was positioned approximately 60 cm above the solution.

To graft LPAAm on a PGMAMMA microchannel surface, the monomer solution was filtered with a 0.2-µm syringe filter, sonicated for 30 min, and introduced into the channel using a syringe pump. The PGMAMMA chips were then placed 60 cm below the
UV lamp. The reaction was allowed to proceed for 60 min, during which the monomer solution was pumped through the microchannel at a flow rate of 0.3 µL/min. Unreacted monomer was flushed out with deionized water.

Poly(ethylene glycol) (PEG) was grafted on the PGMAMMA surface using atom transfer radical polymerization. First, the substrate was treated with an air plasma generated in a PDC-32G plasma cleaner (Harrick Plasma, Ithaca, NY) for 5 min (~10.5 W and 400 mTorr) to introduce hydroxyl groups on the PGMAMMA surface. 2-bromoisobutyryl bromide, a typical ATRP initiator, was then immobilized on the PGMAMMA surface by immersing the treated substrate in a 5:1 (v/v) heptane/THF solution containing 25 mM 2-bromoisobutyryl bromide and 27.5 mM pyridine. After 5 h, the substrate was removed from the reaction vessel, rinsed thoroughly with methanol and deionized water, and dried with nitrogen gas. Afterwards, the initiator-immobilized PGMAMMA substrate was immersed in a 30% (v/v) PEGMEMA aqueous solution containing CuCl (0.424 g, 1.8 mmol), CuBr₂ (0.287 g, 0.54 mmol), and 2,2’-dipyridyl (1.74 g, 4.68 mmol) inside a nitrogen-filled glovebox. The reaction was allowed to proceed at room temperature for 12 h. To stop the reaction, the substrate was taken out of the glovebox and immersed in a saturated aqueous Na₂EDTA to remove residual copper (II) ions. Before chip assembly, the substrate was thoroughly rinsed with deionized water and dried with nitrogen gas.

3.2.5 X-ray Photoelectron Spectroscopy (XPS)

An SSN-100 X-ray photoelectron spectrometer with a monochromatic Al Kα source and a hemispherical analyzer was used in the analysis of surface-modified
PGMAMMA substrates. Before analysis, the treated polymer substrates were thoroughly rinsed with IPA and deionized water and dried with nitrogen gas. Data acquisition and processing were performed with the latest version of ESCA NT 3.0 software.

3.2.6 Contact Angle Measurements

An NRL-100 goniometer (Ramé-hart, Mountain Lakes, NJ) was used to measure contact angles. The contact angle was calculated as the mean of the left and right contact angles of a 4-μL water drop.

3.2.7 Electroosmotic Flow Measurements

The electroosmotic flow (EOF) in a straight PGMAMMA microchannel was measured using the current monitoring method. In a typical measurement, IPA and deionized water were used to rinse the microchannel thoroughly. Following rinsing, the microchannel was filled with 30 mM testing buffer. Before measurement, a reservoir at one end of the microchannel was emptied and replaced with 15 mM testing buffer. The total amount of solution in each reservoir was carefully maintained the same. Platinum electrodes were then placed in both reservoirs and a PS-350 high voltage supply unit (Stanford Research Systems, Sunnyvale, CA) was employed to provide high voltage during the measurement. The variation in current was recorded using a PCI-1200 data acquisition board (National Instruments, Austin, TX) and an in-house written LabView 6i software program (National Instruments). The sampling rate for data collection was 20 Hz. The EOF of the untreated and amine-modified PGMAMMA microchannels was measured at different pH values. The test buffers included acetate (pH = 5), MES (pH =
6), TES (pH = 7), Tris (pH = 8, 9), and CAPS (pH = 10), and the ionic strengths of all 30 mM buffers were adjusted to 30 mM with NaCl.

3.2.8 DSC/TGA Analysis

The glass transition and thermal decomposition temperatures of PGMAMMA were measured using an STA 409 PC simultaneous thermal analyzer (NETZSCH Instruments, Estes Park, CO). A sample (~45 mg) of PGMAMMA was heated from 25°C to 300°C at a heating rate of 10°C/min in an air atmosphere.

3.2.9 UV/VIS Spectrometry

UV-VIS spectra of PGMAMMA were recorded using a Beckman DU 530 UV/VIS spectrophotometer (Beckman Coulter, Fullerton, CA). The percent transmittance was measured from 200 to 600 nm, and the sampling interval was 1 nm. Samples were cut from 1.5 mm thick Acrylite OP-1, Acrylite FF, and PGMAMMA sheets using a C-200 CO₂ laser engraving system (Universal Laser Systems, Scottsdale, AZ).

3.2.10 Preparation of FITC Labeled Amino Acids and Lysozyme

Prior to labeling, 2 mM stock solutions of glycine, aspartic acid, asparagine, glutamic acid, and phenylalanine, and 10 mM of an acetone solution of FITC with 5% (v/v) pyridine were prepared. Stock solutions of 20 µL amino acid, 200 µL FITC, and 380 µL of 10 mM carbonate buffer at pH 9.2 were then thoroughly mixed and the reaction was allowed to proceed in the dark for 24 h. The amino acid mixture used in
μCE separation experiments was prepared by mixing 3 µL of each FITC labeled amino acid solution and diluting to 12 mL with the carbonate buffer.

To label lysozyme, 600 µL of 1 mg/mL lysozyme in 10 mM carbonate (pH = 9.2) was thoroughly mixed with 40 µL of 6 mM FITC in DMSO at room temperature and the solution was placed in the dark for 2 days. The model protein mixture used in μCE separation experiments contained 19 µg/mL FITC-labeled lysozyme, 38 µg/mL R-PE, and 23 µg/mL FITC-labeled casein. In single protein electrophoresis experiments, the sample contained 56 µg/mL FITC-labeled lysozyme.

### 3.2.11 Separations Using μCE Chips

The laser induced fluorescence detection system and the setup for data acquisition have been reported previously. The sampling rate for data collection was 100 Hz.

![Voltage schemes for μCE experiments. (A) Injection, (B) separation.](image)

Voltages were applied to the reservoirs using PS-300 and PS-350 high-voltage supply units (Stanford Research Systems). The two voltage supplies were connected using a home-built switching circuit board. As shown in Figure 3.2, during injection, reservoirs 1,
3, and 4 were grounded, and reservoir 2 was maintained at +0.6 kV for amino acid analysis and +0.8 kV for protein/peptide analysis. During separation, reservoirs 1 and 2 were set at +0.6 kV for amino acid analysis and +0.8 kV for protein/peptide analysis, reservoir 3 was grounded, and reservoir 4 was set at +2.0 kV for amino acid analysis and +3.0 kV for protein/peptide analysis.

3.3 Results and Discussion

3.3.1 Thermal Properties and Optical Transparency of PGMAMMA

PGMAMMA has long been a model polymer in fundamental polymer science and research.\textsuperscript{7-14} The monomer reactivity ratios for MMA and GMA were found to be \(0.80 \pm 0.015\) (\(95\%\) CL) and \(0.70 \pm 0.015\) (\(95\%\) CL) respectively,\textsuperscript{9} which suggests that MMA and GMA form a copolymer in which the two different units distribute randomly in the polymer chain, producing epoxy functionalities on the surface of PGMAMMA substrates which can be utilized for surface modification.

To the best of my knowledge, the application of PGMAMMA in separation science has been limited to the preparation of micron-sized particles which were used in affinity chromatography.\textsuperscript{15} One monomer used to prepare PGMAMMA was MMA, which suggests that this polymer should possess some of the properties of PMMA, a commonly used polymeric material in microfabrication. Therefore, I reasoned that PGMAMMA could also be used for microfluidic devices, and the fabrication protocol for PMMA microdevices should be transferable to this polymer. To verify its suitability as a substrate for microfabrication, I investigated the thermal properties and optical transparency of this polymer.
From DSC, the glass transition temperature of PGMAMMA is 82°C, which is approximately 20°C lower than PMMA. Thus, the imprinting and bonding temperatures for PGMAMMA microdevices should be lower than for PMMA. The TGA results indicated that PGMAMMA begins to decompose in air at 260°C. Since imprinting and bonding can be performed at temperatures lower than 100°C, this polymeric material should be well suited for microfabrication.

Since GMA is a component of PGMAMMA, some of its physical properties must be different from PMMA. I found that the adhesion between silicon and PGMAMMA was higher than PMMA, and thus, releasing of PGMAMMA substrates from silicon templates was more difficult. To circumvent this problem, the silicon templates were passivated with (heptadecafluoro-1,1,2,2-tetrahydrodecyl)trichlorosilane to decrease the adhesion. Moreover, several drops of MeOH were used to help release the PGMAMMA substrate. These two steps proved to be effective, and at least 100 PGMAMMA microchips could be fabricated from a treated silicon template. In contrast, only 2 or 3 microdevices could be made from an untreated template, because it was easily broken during the releasing step.

The UV/visible transmission curves of PGMAMMA, Acrylite OP-1, and Acrylite FF are shown in Figure 3.3. From 400 to 600 nm, the transmittance of PGMAMMA is close to 90%, which is comparable to both OP-1 and FF. The optical transparency of PGMAMMA in the wavelength range of 330 to 400 nm is higher than FF and almost the same as OP-1. The differences in the three polymers are most obvious in the range of 280 to 330 nm, where the transmittance increases following the order of FF, PGMAMMA, and OP-1. Below 280 nm, the transmittance of PGMAMMA is lower than the other two
polymers. The transmittance curves indicate that sensitive optical detection such as visible wavelength laser-induced fluorescence is suitable for PGMAMMA microdevices. Furthermore, PGMAMMA can transmit light down to 280 nm, which implies that PGMAMMA microdevices can also be used in applications that require UV irradiation such as in-situ fabrication or photografting.

3.3.2 Solvent Compatibility of PGMAMMA

PGMAMMA plates were cut in pieces (1 cm x 1 cm) and immersed in 16 different solvents for 72 h to examine the solvent compatibility of the material. Acrylite FF substrates of the same dimensions were used as control samples. The results are summarized in Table 3.1. No obvious physical changes were seen for both PGMAMMA and Acrylite FF in water and acidic or basic aqueous solutions, and some hydrocarbon solvents. Without annealing, cracks appeared in the edges of the Acrylite FF substrates when treated with alcohols, whereas cracks were not found in alcohol-treated PGMAMMA. PGMAMMA swelled in acetonitrile, tetrahydrofuran, acetone, ethyl acetate and ethylene chloride; in comparison, these organic solvents dissolved Acrylite
FF substrates completely. It is noteworthy to mention that neat ethylenediamine greatly swelled both PGMAMMA and Acrylite FF; however, since primary amines can attack the epoxy rings in PGMAMMA, this polymer was further broken down into granules by ethylenediamine.

### Table 3.1. Solvent Compatibility of PGMAMMA.*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>PGMAMMA</th>
<th>Acrylite FF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Hydrochloric acid, 1 M</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Sulfuric acid, 2 M</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Sodium hydroxide, 1 M</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Hexane</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Toluene</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Ethylene chloride</td>
<td>sw</td>
<td>s</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>sw</td>
<td>s</td>
</tr>
<tr>
<td>Acetone</td>
<td>sw</td>
<td>s</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Ethanol</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Methanol</td>
<td>i</td>
<td>i</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>sw</td>
<td>s</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>sw</td>
<td>s</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>sw</td>
<td>sw</td>
</tr>
</tbody>
</table>

* i = insoluble; s = soluble; sw = swells.

### 3.3.3 Surface Modification of PGMAMMA

The ring opening of an epoxy group with an amine is a well known reaction utilized to cure epoxy resins. As an epoxy-containing acrylic polymer, PGMAMMA should be easy to modify using amines. To investigate this, I used ethylenediamine solutions to modify the surface of PGMAMMA (Figure 3.4), and the elemental composition was analyzed using XPS. The XPS spectra for both amine-modified and untreated PGMAMMA are shown in Figure 3.5.
As listed in Table 3.2, the percentage of nitrogen measured on the amine-modified PGMAMMA surface increases with an increase in GMA/MMA molar ratio, which indirectly indicates that the concentration of epoxy functionalities on the surface also increases. In the aminolysis of PGMAMMA, two solvents, IPA/EtOH and phosphate buffer, were used. From the data in Table 3.2, it can be seen that at the same GMA/MMA ratio, the percentage of nitrogen is higher for PGMAMMA substrates treated with ethylenediamine alcohol solution. The reason could be that in the ring-opening reaction, hydrogen bonds are formed between the epoxy functionalities and hydrogen donors such as water and alcohol, which weakens the C-O bond of the epoxy functionality and eases bond cleavage under attack of an amine molecule. Although both water and alcohols
have the ability to act as hydrogen donors in the reaction, alcohols can swell the PGMAMMA matrix and make the polymer chain more flexible, which could increase the reaction efficiency.

<table>
<thead>
<tr>
<th>GMA/MMA Molar Ratio</th>
<th>IPA/EtOH Solution b</th>
<th>N% a</th>
<th>pH 12 Phosphate Buffer b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 10</td>
<td>2.63 ± 0.17</td>
<td>1.74 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>1 : 8</td>
<td>2.74 ± 0.21</td>
<td>2.18 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>1 : 5</td>
<td>3.11 ± 0.15</td>
<td>2.48 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>1 : 3</td>
<td>3.86 ± 0.17</td>
<td>2.79 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>1 : 2</td>
<td>4.84 ± 0.12</td>
<td>3.39 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>1 : 1</td>
<td>5.95 ± 0.07</td>
<td>4.34 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>3 : 1</td>
<td>6.52 ± 0.37</td>
<td>6.20 ± 0.38</td>
<td></td>
</tr>
</tbody>
</table>

a %CL = 95 %

b Ethylenediamine concentration was 20 % (w/w).

According to the XPS data, PGMAMMA with a GMA/MMA molar ratio of 1 had reasonably high reaction efficiency. In addition, it was found that in the microfabrication process, PGMAMMA substrates with higher GMA/MMA molar ratios were more brittle, and the bonding strengths of the substrates were unsatisfactory. Although PGMAMMA substrates could be prepared from different GMA/MMA molar ratios, I chose a molar ratio of 1 for the remainder of the work reported in this paper.

Photografting is a common surface modification method for many polymers. Recently, Hu et al. successfully used this method to graft hydrophilic polymers on the surface of PDMS microdevices. I followed this protocol to graft LPAAm on PMMA sheets, however, no change on the surface was observed. Surprisingly, when the same LPAAm photografting procedures were applied to PGMAMMA substrates, I found that the hydrophobic surface of PGMAMMA became hydrophilic. Contact angle
measurement showed that the contact angle of untreated PGMAMMA (i.e., 72.1 ± 1.4°, %CL = 95%, average of 3 measurements) decreased to 16.7 ± 0.4° (%CL = 95%, average of 3 measurements) after photografting. XPS gave a nitrogen coverage of ~17% after surface modification, which is close to the theoretical value for LPAAm of 20%; the coverage was ~0.8% for the original PGMAMMA substrate. Both contact angle and XPS data indicate that LPAAm was grafted onto the PGMAMMA surface using this one-step photografting protocol.

It was suggested in the literature that direct ultraviolet radiation could provide enough energy to excite the strained epoxy ring, leading to cleavage of the ring.19 Therefore, I believe that through this UV-assisted ring-opening mechanism, radicals are generated that induce polymerization of acrylamide on the PGMAMMA surface.

To test the stability of LPAAm grafted on the PGMAMMA surface, a small substrate of LPAAm-grafted PGMAMMA (1.0 cm x 0.5 cm) was thoroughly rinsed with IPA and deionized water. Following rinsing, the polymer was immersed in a glass bottle containing 100 mL of deionized water, and the container was placed in a heated shaking water bath/incubator (VWR International, West Chester, PA). The oscillation speed was set at 40 strokes/min and the temperature was held at 50°C for 24 h. After this treatment, the contact angle of the PGMAMMA surface was measured to be 17.1 ± 0.9° (%CL = 95%, average of 3 measurements) and the nitrogen percent coverage was ~15%. These measurements indicate that LPAAm was not simply adsorbed on the surface of the PGMAMMA substrate.
3.3.4 Electroosmotic Flow

Relatively strong cathodic EOF was observed in the untreated PGMAMMA microchannel. The magnitude of the EOF mobility changed from \((3.7 \pm 0.2) \times 10^{-4}\) to \((0.82 \pm 0.02) \times 10^{-4}\) cm\(^2\)·V\(^{-1}\)·s\(^{-1}\) (%CL = 95\%, average of 4 measurements) when the pH decreased from 10 to 5 (Figure 3.6). In comparison, microchannels fabricated from PMMA had a pH independent cathodic EOF, and the reported EOF mobility varied from \(2.2 \times 10^{-4}\) cm\(^2\)·V\(^{-1}\)·s\(^{-1}\) to \(2.6 \times 10^{-4}\) cm\(^2\)·V\(^{-1}\)·s\(^{-1}\) within a pH range of 3 to 11.\(^{21}\) Interestingly, the direction of EOF in the PGMAMMA microchannel could be easily manipulated by ethylenediamine treatment. As shown in Figure 3.6, the EOF in an amine-modified PGMAMMA microchannel was anodic below pH 6.4, and the EOF mobility reached \((-0.8 \pm 0.2) \times 10^{-4}\) cm\(^2\)·V\(^{-1}\)·s\(^{-1}\) (%CL = 95\%, average of 4 measurements, one microchip) at pH 5. Above pH 6.4, the EOF changes from anodic to cathodic, and the variation in EOF mobility is similar to the untreated PGMAMMA microchannel. At the same pH, the magnitude of EOF mobility for an amine-treated PGMAMMA channel is lower than that

![EOF mobility variation versus pH for untreated and amine-modified PGMAMMA microchannels.](image)

Figure 3.6. EOF mobility variation versus pH for untreated and amine-modified PGMAMMA microchannels.
for an untreated channel. These results indicate that amine functionalities were immobilized on the channel surface; however, unreacted epoxy groups still existed, which could cause a cathodic EOF when the pH is higher than 6.4.

3.3.5 Separation of FITC-Labeled Amino Acids

I attempted to use unmodified PGMAMMA µCE chips to separate FITC-labeled amino acids, however, I observed that the tagged samples strongly adsorbed to the channel surface. Moreover, a cathodic EOF was generated to oppose the movement of the analytes when high voltage was applied. Obviously, a surface covered with epoxy groups is not suitable for separation of these compounds.

The epoxy groups were utilized to photograft LPAAm onto the PGMAMMA microchannel surface, and it was observed that the running buffer, 10 mM carbonate buffer at a pH of 9.2, rapidly entered the microchannel when added to one reservoir. For comparison, the buffer stayed in the reservoir in an untreated PGMAMMA µCE device.

Figure 3.7. µCE of FITC-labeled amino acids. Peak identifications: (1) aspartic acid, (2) glutamic acid, (3) glycine, (4) asparagine, (5) phenylalanine, and (6) FITC.
unless pressure or vacuum was used. The EOF in an LPAAm-grafted PGMAMMA microchannel was measured under the conditions used for the separation of amino acids. In the current-monitoring experiment, no obvious current variation over the first 600 s was measured, which indicates that the EOF was significantly reduced to less than $5 \times 10^{-6}$ cm$^2$·V$^{-1}$·s$^{-1}$ in the LPAAm-grafted PGMAMMA microchannel.

Separation of five FITC-labeled amino acids, including aspartic acid, glutamic acid, glycine, asparagine, and phenylalanine, was achieved using LPAAm-grafted PGMAMMA µCE chips. A typical result is shown in Figure 3.7, and the performance characteristics of the chip are listed in Table 3.3. The total plates measured for all five FITC-labeled amino acids were over $3.5 \times 10^4$ for a 3.5 cm long separation channel, and as many as $4.6 \times 10^4$ plates were obtained for aspartic acid, which is a direct consequence of reducing both EOF and the adsorption sites on the PGMAMMA surface.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Migration time (s)$^a$</th>
<th>RSD %</th>
<th>Asymmetry factor</th>
<th>Total plates$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.2</td>
<td>0.85</td>
<td>1.09</td>
<td>$4.6 \times 10^4$</td>
</tr>
<tr>
<td>2</td>
<td>15.5</td>
<td>0.86</td>
<td>0.84</td>
<td>$4.2 \times 10^4$</td>
</tr>
<tr>
<td>3</td>
<td>17.8</td>
<td>0.96</td>
<td>1.00</td>
<td>$3.9 \times 10^4$</td>
</tr>
<tr>
<td>4</td>
<td>18.6</td>
<td>0.94</td>
<td>1.04</td>
<td>$4.0 \times 10^4$</td>
</tr>
<tr>
<td>5</td>
<td>19.0</td>
<td>0.95</td>
<td>1.08</td>
<td>$3.8 \times 10^4$</td>
</tr>
</tbody>
</table>

$^a$ Data were calculated from 4 consecutive runs in one microchip.

3.3.6 Separation of Proteins and Peptides

As I attempted to separate proteins and peptides using LPAAm-grafted PGMAMMA µCE chips, I found that the LPAAm layer did not prevent nonspecific adsorption of FITC-labeled proteins and peptides, which could be caused by inadequate
coverage of the substrate surface by the LPAAm chains. To perform protein and peptide separation with the microchips, a different surface modification technique, atom transfer radical polymerization,\(^2\) was employed to graft PEG on the PGMAMMA surface. A separation of a protein mixture containing FITC-labeled lysozyme, R-phycoerythrin, and FITC-labeled casein was tested using the PEG-grafted PGMAMMA \(\mu\)CE chips. As shown in Figure 3.8, all major components of the proteins were separated in 15 s, and the

**Figure 3.8. \(\mu\)CE of model proteins. Peak identifications: (1) FITC-labeled lysozyme, (2) R-PE, (3) FITC-labeled casein, and (4) FITC.**

**Figure 3.9. \(\mu\)CE of FITC-labeled lysozyme decomposition products. Peak 1 is the major component of FITC-labeled lysozyme and peak 2 is FITC.**
peak corresponding to FITC-labeled lysozyme gave a theoretical plate number of $4.6 \times 10^4$. During the single protein electrophoresis experiment, it was observed that FITC-labeled lysozyme, which was prepared following the procedures described in Section 3.2.10, showed multiple peaks (Figure 3.9). This may have resulted from fluorescent labeling of peptide chains from decomposed lysozyme, or lysozyme derivatized with different numbers of FITC.

3.4 Conclusions

An acrylic-based thermoplastic, PGMAMMA, was synthesized using free-radical polymerization. The thermal and optical properties of this polymer showed that it was suitable for the fabrication of polymeric microdevices. Conventional microfabrication techniques used for PMMA microdevices were readily transferred to the fabrication of PGMAMMA microdevices. Manipulation of EOF in PGMAMMA microchannels was achieved by immobilizing amine functionalities on the PGMAMMA surface. Using a one-step photografting technique, LPAAm was grafted onto the PGMAMMA surface. Both EOF and nonspecific adsorption of analytes were reduced in the LPAAm-grafted microchannels, leading to highly efficient and reproducible electrophoretic separations of a mixture containing five FITC-labeled amino acids. PEG was also grafted on the PGMAMMA surface using the ATRP technique, and successful separations of proteins and peptides were demonstrated.
3.5 References


4 FABRICATION OF POLYMERIC ELECTRIC FIELD GRADIENT FOCUSING MICRODEVICE USING A NOVEL MEMBRANE INTEGRATION TECHNIQUE *

4.1 Introduction

Electric field gradient focusing (EFGF) was first implemented by Koegler and Ivory\textsuperscript{1,2} and their initial EFGF systems were relatively large. Since band dispersion is reduced as the separation channel cross-sectional area is reduced, the trend has been to develop microfabricated EFGF systems. With respect to most EFGF devices, the major challenge of microfabrication lies in membrane integration. To date, various methods have been used to integrate a semi-permeable membrane into a microfluidic device. The most straightforward methods are to sandwich a flat sheet of membrane between two planar substrates\textsuperscript{3-5} or embed a dialysis hollow fiber in the substrate during device assembly.\textsuperscript{6} Hisomoto et al.\textsuperscript{7} exploited multilayer flow and an interfacial polycondensation reaction to synthesize a polymeric membrane directly inside a glass microchannel. Khandurina et al.\textsuperscript{8} spin-coated a thin layer of silicate solution on a glass substrate and bonded it to another piece of glass containing etched channels. The coated silicate layer served as a porous membrane for an electrokinetic preconcentrator, and preconcentration of both DNA fragments and proteins was achieved.\textsuperscript{8,9} By using special optics, Song et al.\textsuperscript{10,11} focused a 355-nm laser beam into a thin sheet, which was then used to photo-synthesize a nanoporous membrane inside glass microchannels.

In this work, I developed a new membrane integration method for polymeric EFGF microdevices based on a surface reactive acrylic copolymer, poly(glycidyl...
methacrylate-co-methyl methacrylate) (PGMAMMA). This method is applicable to the fabrication of other devices that require integration of a membrane.

4.2 Experimental Section

4.2.1 Materials

Methyl methacrylate (MMA, 99%), butyl methacrylate (BMA, 99%), glycidyl methacrylate (GMA, 97%), 2,2’-azobisisobutyronitrile (AIBN, 98%), 2,2’-dimethoxy-2-phenylacetophenone (DMPA), hydroxypropyl cellulose (HPC, MW~80,000), poly(ethylene glycol) methyl ether acrylate (PEGMEA 454, MW~454), poly(ethylene glycol) diacrylate (PEGDA 575, MW~575), poly(ethylene glycol) dimethacrylate (PEGDMA 875, MW~875), isocyanatoethyl methacrylate (ICMA, 98%), and propylene glycol methyl ether acetate (PGMEA, 99%) were purchased from Aldrich (Milwaukee, WI, USA). Poly(ethylene glycol) 400 diacrylate (PEGDA 400) and poly(ethylene glycol) 550 methyl ether acrylate (PEGMEA 550) were obtained from Sartomer (Warrington, PA, USA). Acetone (reagent grade), absolute methyl alcohol (MeOH, reagent grade), anhydrous ethylenediamine (reagent grade), triethylamine (reagent grade), sodium silicate solution (40-42° Bé), heptane (reagent grade), and tetrahydrofuran (THF, reagent grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA); fluorescein isothiocyanate (FITC), tris[hydroxymethyl] aminomethane (TRIZMA, Tris), hemoglobin, and FITC-conjugated casein were purchased from Sigma (St. Louis, MO, USA); R-phycoerythrin (R-PE) was obtained from Polysciences (Warrington, PA, USA); recombinant, enhanced green fluorescent protein (GFP) was purchased from Clontech (Palo Alto, CA, USA). Deionized water (18.2 MΩ-cm) was prepared using a Milli-Q UF
Plus water purification system (Millipore, Billerica, MA, USA). Pre-cleaned microscope slides with dimension of 50 (width) × 70 (length) × 1 (thickness) mm and 25 (width) × 70 (length) × 1 (thickness) mm were obtained from Fisher Scientific (Pittsburgh, PA, USA) and Hardy Diagnostics (Santa Maria, CA, USA).

All chemicals were used as received without further purification. Tris-HCl buffer and ethylenediamine solution were filtered using 0.2 μm syringe filters before experiments (Pall, East Hills, NY, USA).

4.2.2 Synthesis of PGMAMMA

PGMAMMA sheets were prepared according to the previously reported protocol (see Chapter 3). Briefly, glass containers with dimensions of 75 × 50 × 1.5 mm were constructed by fusing microscope slides with sodium silicate solution. The containers were cured at 80°C for 30 min to harden the sodium silicate before use. In a typical preparation process, a monomer solution containing MMA, GMA, and 0.2 % (w/w) of AIBN initiator was prepared (molar ratio of MMA to GMA was 1) and degassed using a Branson 3200 sonicator (Branson Ultrasoundics, Danbury, CT, USA) for 10 min before being introduced into the containers. To seal the monomer-filled glass containers, a microscope slide with dimensions of 25 × 50 × 1.5 mm was placed over the container opening, and sodium silicate solution was carefully applied around the edges to seal the slide in place. After the sodium silicate solution hardened at room temperature, the container was placed in an HP 5890 gas chromatograph oven and the temperature was held at 65°C for 24 h. After the polymerization reaction was complete, the container was immersed in a water bath overnight to let the sodium silicate dissolve, after which the
sides of the glass container could then be removed from the hardened polymer. The resultant polymer substrate was thoroughly rinsed with deionized water and MeOH.

4.2.3 Preparation of the Poly(glycidyl methacrylate-co-butyl methacrylate) (PGMABMA) Adhesive

PGMABMA was synthesized by mixing 4 g of BMA, 4 g of GMA, 12 g of PGMEA, and 0.08 g of AIBN in a glass vial (molar ratio of GMA to BMA was 1). After sonicating for 20 min, the vial was sealed with a cap and placed in an HP 5890 gas chromatograph oven. The polymerization reaction was allowed to proceed at 65 °C for 24 h. The bonding adhesive was prepared by mixing the PGMABMA solution with GMA (doped with 1 wt% AIBN) at a volume ratio of 1.5 to 1 \( \frac{V_{\text{PGMABMA}}}{V_{\text{GMA}}} = 1.5:1 \).

4.2.4 Fabrication of the Silicon Template for the Polymeric µEFGF Device

The fabrication process is shown in Figure 4.1. In step 1, a layer of silicon dioxide (800–1000-nm thick) was grown on a 4” diameter silicon wafer (Encompass Distribution Services, Pleasanton, CA, USA) at 1110°C in an atmosphere of oxygen and water. Shipley 812 (Shipley, Marlborough, MA, USA) positive photoresist was then spin-coated on the silicon wafer at 3000 rpm for 120 s using a WS-400A-6NPP-LITE spinner (Laurell, Northwales, PA, USA). To increase adhesion of the photoresist and drive off residual solvent, the wafer was baked at 100°C for 2 min. The photoresist was then exposed to UV radiation for 40 s through the first photomask using a PLA-501F mask aligner (Canon, Tokyo, Japan), which transferred the pattern of the µEFGF device (pattern 1, Figure 4.1) to the wafer. In step 2, the patterned wafer was developed with
20% aqueous Microposit 351 developer (Shipley) for 30 s. After being placed in an oven for 25 min at 150°C, which helped to harden the photoresist, the wafer was immersed in buffered hydrofluoric acid for about 10 min to remove silicon dioxide from the areas not covered by the photoresist. In step 3, after rinsing the wafer with acetone and MeOH, a layer of silicon nitride was deposited on its surface using a plasma enhanced chemical vapor deposition (PECVD) system. During the deposition, a gas mixture containing SiH₄ and NH₃ was continuously introduced. The chamber pressure was controlled at 900 mTorr, while the plasma power and chamber temperature were set at 100 W and 250 °C, respectively. The whole process took 30 min, and the thickness of the deposited silicon

Figure 4.1. Fabrication procedure for the µEFGF template.
nitride layer was estimated to be 210 nm. In step 4, a photolithography process similar to step 1 was used to pattern the wafer. Pattern 2 (Figure 4.1) was first aligned using the Canon aligner so that its projection was precisely positioned on top of pattern 1 on the wafer. After UV exposure and development, a layer of photoresist bearing the shape of pattern 2 was retained on top of pattern 1. The silicon nitride layer not protected by the photoresist was then removed using a DEM-451 reactive ion etcher (Anelva, Tokyo, Japan). During the 7-min etching process, CF₄ and O₂ were introduced simultaneously in the chamber through two mass flow controllers at 25 and 3.1 cm³/min, respectively. The chamber pressure was stabilized at 100 mTorr and the plasma power was set at 100 W. In step 5, the wafer was cleaned with acetone and MeOH and then etched using a 40 wt% KOH aqueous solution at 80 °C for 15 min. The height of the protruded feature (weir) obtained in this step defined the thickness of the semi-permeable membrane. After removing the remaining silicon nitride layer using the reactive ion etcher (step 6), the wafer was etched again in the same hot KOH bath for 20 min to form the final template (step 7).

4.2.5 Fabrication of the µEFGF Device

First, the silicon template was used to emboss the µEFGF pattern into a PGMAMMA substrate at 93 °C. To seal the channel, a layer of PGMABMA adhesive was spin-coated on a blank piece of PGMAMMA substrate at a spin rate of 3000 rpm for 4 min, and the blank was thermally bonded at 52 °C to the substrate bearing the µEFGF pattern. The hot embossing and bonding processes were performed using an HP 5890 gas
chromatograph oven. Access holes were created using a C-200 CO₂ laser engraving system (Universal Laser Systems, Scottsdale, AZ, USA).

![Figure 4.2. Dimensions of the µEFGF device. (A) Top view: (1) low-field end, (2) high-field end. (B) Cross-sectional view of the circled area in (A): (3) separation channel, (4) membrane holder (weir), and (5) shaped channel.](image)

The structure and dimensions of the finished µEFGF device are shown in Figure 4.2. The microdevice has a separation channel, a membrane holder, and a shaped channel with changing cross-sectional area. It should be noticed that the profile of the shaped channel was designed following the method reported by Koegler and Ivory.¹

### 4.2.6 Surface Modification of the PGMAMMA

Before membrane fabrication, the PGMAMMA microchannel surface was treated with an aqueous reaction solution of ethylenediamine (10 wt%) and triethylamine (0.1 M) for 24 h (Figure 4.3). After thoroughly rinsing the microchannels with deionized water, the microdevice was dried in a vacuum desiccator for 1 h. Methacryl groups were then immobilized on the substrate surface by contacting the amine functionalized PGMAMMA microchannels with an ICMA solution (Figure 4.3) for 5 h, which consisted...
of 0.2 mL of ICMA, 8 mL of THF, and 42 mL of heptane. Finally, MeOH was used to remove the reaction residues and excess solvent from the microchannels.

![Chemical structures](image)

**Figure 4.3. Surface modification of the PGMAMMA substrate.**

### 4.2.7 Fabrication of the Membrane in the µEFGF Device

As shown in Figure 4.4, in step 1, the microchannel of the µEFGF device was filled with a pre-membrane solution. In step 2, nitrogen gas was bubbled through deionized water and introduced into the microchannel system. By carefully adjusting the nitrogen pressure between 1.5 to 2.5 psi, most of the pre-membrane solution could be removed except that retained on the membrane weir structure. In step 3, nitrogen was continually purged through the separation and shaped channels to prevent oxygen from interfering in the photo-curing reaction, during which the microdevice was placed 15 cm below a Dymax 5000-EC UV curing lamp (Dymax, Torrington, CT, USA) for 10 s to form the membrane. The in-situ synthesized semi-permeable membrane (Figure 4.5) was approximately 12.4 mm long, 48 µm wide, and 10 ~ 12 µm thick. The finished device was rinsed with deionized water for 1 h and then conditioned with 20 mM Tris-HCl.
Figure 4.4. In-situ fabrication of the semi-permeable membrane in the µEFGF device.

Figure 4.5. Disassembled µEFGF chip showing detachment of the membrane: (1) separation channel, (2) membrane holder, (3) shaped channel, and (4) detached semi-permeable membrane (surface modification was not performed in this chip).
buffer (pH 8.7) for at least 4 h using an 11-Pico-Plus syringe pump (Harvard Apparatus, Holliston, MA, USA). The flow rate was set at 1 µL/min.

### 4.2.8 Preparation of FITC-Labeled Hemoglobin

To label hemoglobin, 1 mL of 1 mg/mL hemoglobin in 20 mM Tris-HCl (pH 8.7) was thoroughly mixed with 60 µL of 6 mM FITC in acetone at room temperature, and the solution was stored in the dark for 1 day. To remove unconjugated label, 3 mL of Tris-HCl (pH 8.7) was added to 100 µL of the FITC labeled hemoglobin solution and placed in the upper chamber of a Microsep 3K Omega centrifuge filtering tube (Pall, East Hills, NY, USA) with a semi-permeable membrane with a molecular weight cutoff of 3000 Da. The Microsep membrane filtering tube was centrifuged at 4000 rpm at 4 °C for 2 h, which forced buffer and FITC into the lower chamber. After centrifugation, the FITC-hemoglobin solution in the upper chamber (approximately 100 µL) was collected and diluted to 1 mL with 20 mM Tris-HCl (pH 8.7) buffer before use.

### 4.2.9 Operation of the µEFGF Device

During injection (Figure 4.6A), a voltage was applied to the µEFGF device through two platinum electrodes in contact with sample reservoir 1 and buffer reservoir 3 using a PS-350 high-voltage supply (Stanford Research Systems, Sunnyvale, CA, USA), which electrokinetically introduced the charged proteins into the separation channel. During focusing (Figure 4.6B), voltage was applied to buffer reservoirs 2 and 3 to focus the injected proteins. An 11-Pico-Plus syringe pump (Harvard Apparatus) was used in the
focusing mode to provide a hydrodynamic counter flow. The operation modes could be easily switched during the experiments using a home-made switching circuit board.

4.2.10 Optical System

A TE 2000-U inverted microscope (Nikon, Tokyo, Japan) was used in imaging and detection. The laser light was generated from an air-cooled 35-LAP-321-120 Ar-ion laser source (Melles Griot, Carlsbad, CA, USA), and introduced into the microscope through an optical path consisting of an excitation filter (D488/10, Chroma, Brattleboro, VT, USA), a home-made periscope, a home-made quartz diffuser which was used to homogenize images, and a scattered-light-gathering lens. Fluorescence was collected through a 10×, 0.30 N.A. objective (Nikon) and passed through a Z488LP long-pass filter set (Chroma). All images were recorded with a COOLPIX 5400 digital camera (Nikon).
To detect focused single proteins, the diffuser and the scattered-light-gathering lens were removed. The filtered laser beam was then expanded using a 50-25-10×-425-675 beam expander (Special Optics, Wharton, NJ, USA) and then passed into the inverted microscope using the periscope. The laser beam was finally focused into a beam spot with the 10× objective. After the laser-induced fluorescence signals were passed through the long-pass filter set, photons were detected by a Hamamatsu H6780-01 photomultiplier tube (Bridgewater, NJ, USA). The detector signal was amplified using a Keithley 428 amplifier (Cleveland, OH, USA) and then recorded using a WaveSurfer 454 500 MHz oscilloscope (LeCroy, Chestnut Ridge, NY, USA). To detect focused proteins, the separation channel was scanned through a fixed detection volume using a motorized microscope stage, in which a servo motor (Kaydon Switch & Instrument, Waterbury, CT, USA) and a home-built micro-stepping controller were installed.

4.3 Results and Discussion

4.3.1 Fabrication of PGMAMMA µEFGF Device

Bonding of the cover over the imprinted channel structure was a problem encountered during the fabrication of the PGMAMMA µEFGF device. As shown in Figure 4.2A, the µEFGF chip has a changing cross-section channel, a membrane holder (weir), and a separation channel. Some regions of the changing cross-section channel have a very low aspect ratio. When a blank PGMAMMA substrate was bonded to a substrate with microchannels using a procedure that was successful for preparing µCE devices, the cover plate sagged until it touched the top of the membrane holder, which eliminated the space between the cover plate and membrane holder that was prepared for
fabrication of the semi-permeable membrane. To avoid deformation of the microchannel, a lower temperature adhesive bonding technique was used. Following the method of Meng et al.,\textsuperscript{13} we synthesized a PGMABMA-containing adhesive and spin-coated it on the PGMAMMA cover plate before bonding. The temperature used in adhesive bonding of the PGMAMMA microdevices was 15 °C lower than without the adhesive layer, which was enough to eliminate microchannel deformation. Since epoxy groups were also present in PGMABMA, the resulting adhesive surface had similar chemical properties to PGMAMMA.

4.3.2 Formation of Membranes in PGMAMMA Substrates

The advantage of using PGMAMMA over poly(methyl methacrylate) (PMMA) for fabrication of μEFGF devices is that the abundant epoxy functionalities present at the polymer surface can be exploited for further chemical treatment. PMMA has appropriate mechanical, thermal, and optical properties; however, surface modification of PMMA is quite difficult because it lacks reactive surface functionalities.

To bond the semi-permeable membrane in the PGMAMMA device, I first converted the epoxylated surface into amine functionalities with ethylenediamine and, then, reacted this surface with ICMA to produce methacryl functionalities (Figure 4.3). I also tried grafting vinyl groups onto the PGMAMMA substrate surface via reaction of allylamine with the epoxy groups; however, adherence of the semi-permeable membrane to the vinyl surface was inferior to the methacryl surface. In a comparison experiment, I photo-polymerized several drops of PEG acrylate/PEG methacrylate or acrylamide membrane prepolymer solutions on both vinyl and methacryl functionalized surfaces.
After exposing the surfaces to 20 mM Tris-HCl buffer (pH 8.7) for 5 h, the membranes were subjected to a 45-psi compressed air jet. All test membranes attached to the vinyl surface were completely detached by the air stream, whereas no physical change was observed for the membranes attached to the methacryl surface. Following this air-blowing test, the methacryl-grafted PGMAMMA substrate was again immersed in the Tris-HCl buffer and kept for over 2 months; subsequent testing showed that the membrane was still firmly attached.

Two reasons can explain the difference in adherence properties between the vinyl and the methacryl surfaces. First, vinyl groups are less active than methacryl groups in free-radical polymerization; therefore, surface methacryl groups more easily react with free-radicals formed in the pre-membrane solution which bond the polymer to the surface. Second, ethylenediamine was used in the immobilization of methacryl groups, which provided a longer spacer group. The longer spacer could help to reduce steric hindrance and increase the flexibility of end groups, hence, promoting the reactivity of the methacryl groups.

4.3.3 Focusing and Separation of Proteins with µEFGF Devices

To demonstrate EFGF of single proteins, R-PE and GFP were individually diluted in 20 mM Tris-HCl buffer, loaded into the sample reservoir, and continuously introduced into the µEFGF device. The current was kept constant at 10 µA during sample introduction. After 3 min, the voltage was switched to the focusing mode and the injected protein was focused into a narrow band (Figures 4.7A and B). A voltage of 2000 V and a counter flow rate of 5 nL/min was maintained throughout the focusing process. However,
when a mixture of the two proteins was introduced into the µEFGF device using the same conditions, they were not separated, but were stacked together (Figure 4.7C), even though they could be focused at different points in the channel individually. A higher conductivity membrane (membrane 2, Table 4.1) was required to separate the proteins. After sampling for 3 min at a current of 10 µA, R-PE and GFP could be completely separated with a focusing voltage of 800 V and a counter flow rate of 10 nL/min (Figure 4.7D).

Figure 4.7. Microscopic photographs of the µEFGF channel showing: (A) focusing of R-PE, (B) focusing of GFP, (C) stacking of R-PE and GFP, (D) separation of R-PE and GFP. The buffer used was 20 mM Tris-HCl (pH 8.7).
Table 4.1. Semi-Permeable Membranes Used in µEFGF Devices.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Membrane Ingredients</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PEGDA 575, 80 wt%; 50 mM Tris-HCl (pH 8.7), 20 wt%; DMPA, 1 wt% of PEGDA 575</td>
<td>Protein focusing and stacking</td>
</tr>
<tr>
<td>2</td>
<td>PEGMEA 454, 25 wt%; PEGDA 575, 40 wt%; 50 mM Tris-HCl (pH 8.7), 35 wt%; DMPA, 1 wt% of PEGMEA 454 + PEGDA 575</td>
<td>Protein separation and preconcentration factor measurement</td>
</tr>
<tr>
<td>3</td>
<td>PEGMEA 550, 25 wt%; PEGDA 400, 26.7 wt%; PEGDMA 875, 13.3 wt%; 100 mM Tris-HCl (pH 8.7), 35 wt%; DMPA, 1 wt% of PEGMEA 550 + PEGDA 400 + PEGDMA 875</td>
<td>Selective retention of proteins</td>
</tr>
</tbody>
</table>

To determine the preconcentration ability of the µEFGF device for proteins, a calibration curve was first constructed using GFP solutions with concentrations ranging from 1 µg/mL to 100 µg/mL (Figure 4.8). To measure the fluorescence intensity of GFP at each concentration level, the separation channel was completely filled with the GFP solution of known concentration. Next, the laser beam was focused at three different positions along the channel, and the corresponding fluorescence intensities were recorded. After 60 min, the voltage was switched to the focusing mode, and a 5 nL/min counter flow was employed to focus the protein. The laser beam was then focused on the GFP band and its fluorescence intensity was recorded using the PMT. From the calibration curve, the concentration of the focused GFP was found to be 83 µg/mL, corresponding to a preconcentration factor of $4 \times 10^3$. 
Selective retention/elution of specific analytes is one of the attractive features of the EFGF technique, which would be useful in areas such as clinical diagnostics and protein preparation/purification. This was demonstrated by Wang et al.\textsuperscript{14} and Lin et al.\textsuperscript{15} using a dialysis hollow fiber-based EFGF system. In their work, the counter flow rate was kept constant while the voltage was decreased stepwise to elute proteins with different mobilities. In this work, I used a µEFGF device with membrane 3 (Table 4.1) to demonstrate selective retention/elution by varying the counter flow rate. After injection of a mixture containing GFP, FITC-casein, and FITC-hemoglobin (injection mode, 10 µA current), the proteins were focused using a constant current of 12 µA. By increasing the counter flow rate from 33 to 36 nL/min, GFP was gradually separated from FITC-casein, pushed toward the high-field end by the counter flow, and driven out of the
separation channel (Figure 4.9). By increasing the counter flow rate even further, FITC-casein and FITC-hemoglobin were eventually moved out of the channel.

Figure 4.9. Fluorescence scan of an EFGF channel showing separation and selective retention/elution of proteins. The counter flow rate was (A) 33 nL/min, (B) 34 nL/min, (C) 35 nL/min, and (D) 36 nL/min. Peak identifications: (1) GFP, (2) FITC-casein, and (3) FITC-hemoglobin. The current was maintained at 12 µA, the photomultiplier tube was operated at 0.33 V, the rise time and gain of the Keithley amplifier was 10 ms and 10^7 V/A, respectively, and the sampling frequency for data collection were 100 Hz. The scan was started from the high-field end of the channel and the counter flow was driven from the low-field end to the high-field end. The buffer used was 20 mM Tris-HCl (pH 8.7) doped with 0.1 % HPC (MW~80,000).
4.3.4 Impact of Membrane Properties on Device Performance

In the theoretical treatment by Tolley et al.,\textsuperscript{16} the general transport equation was expressed as

\[ J = W(x)c(x) - D_T \frac{\partial c(x)}{\partial x} \] \hspace{1cm} (4.1)

where \( J \) is the flux density of the analyte, \( c(x) \) is the concentration of the analyte at point \( x \), \( D_T \) is the dispersion coefficient that represents the sum of all contributions to effective diffusion, and \( W(x) \) denotes the translational velocity of the analyte at point \( x \), which can be expressed as

\[ W(x) = mP(x) + u \] \hspace{1cm} (4.2)

\( P(x) \) is the intensity of the external field at point \( x \), \( m \) is the velocity of the analyte induced by the external field with unit intensity, and \( u \) is the velocity of the bulk flow. The gradient of the external field intensity is given as

\[ q(x) = -\frac{\partial P(x)}{\partial x} \] \hspace{1cm} (4.3)

When the analyte is focused, or in an equilibrium state

\[ \sigma = \frac{D_T}{\sqrt{mq(x_0)}} \] \hspace{1cm} (4.4)

and

\[ R_S = \frac{\Delta x}{4\sigma} = \frac{|W(x'_0)|}{\sqrt{mq(x_0)D_T}} \] \hspace{1cm} (4.5)

where \( \sigma \) is the standard deviation of the band width of the focused analyte, \( R_S \) is the resolution of two focused analytes, \( x_0 \) is the focusing position of the first analyte, and \( x'_0 \) is the focusing position of the second analyte.
Equations 4.4 and 4.5 indicate that both the band width of a focused analyte and the resolution of two analytes are inversely related to the square root of the external field gradient. A steeper field gradient will give a sharper peak, whereas a shallower gradient will result in better resolution of the analytes. Therefore, any change that alters the shape of the field gradient will affect the focusing of analytes.

In this work, three different membranes, for which the ingredients are summarized in Table 4.1, were synthesized. The main difference in the three membranes is conductivity, which is primarily a function of the buffer content and monomer composition of the membrane. To measure the conductivity of the membrane, the corresponding monomer solution was filled in a PMMA channel with dimensions of 30 (length) \times 5 (width) \times 0.3 (thickness) mm and photo-polymerized in situ. A 20 mM Tris-HCl buffer (pH 8.7) was then added to the reservoirs at both ends of the channel. A voltage was applied to the channel through two platinum electrodes in contact with the reservoirs and the resulted current was recorded. The conductivity of the membrane was estimated using Equation 4.6

\[ C = \frac{LI}{AU} \]  

(4.6)

where \( C \) is the conductivity, \( L \) is the length of the channel, \( I \) is the current, \( A \) is the cross-sectional area of the channel, and \( U \) is the applied voltage. It should be mentioned that the conductivity of the 20 mM Tris-HCl buffer (pH 8.7) was measured using the same method, except that the membrane was replaced with the buffer. The conductivities of the 20 mM Tris buffer, membrane 3, membrane 2, and membrane 1 were \( 6.0 \times 10^{-2} \), \( 6.6 \times 10^{-3} \), \( 2.3 \times 10^{-3} \), and \( 2.5 \times 10^{-5} \) S/m, respectively. Since membrane 1 had the lowest buffer content and highest concentration of cross-linker, its conductivity was the lowest among
the three. Although membranes 2 and 3 had the same buffer volume, the concentration of the buffer used in membrane 3 was higher than that in membrane 2. Moreover, more bulky monomer and cross-linker were used in membrane 3, which resulted in a polymer network with larger pore size and, thus, better ion transport. Therefore, the conductivity of membrane 3 was higher than that of membrane 2. Before protein focusing and separation experiments, the weir membranes were conditioned using 20 mM Tris buffer for 4 h. It is reasonable to presume that the buffer concentration in the membranes was close to that of the conditioning buffer.

To investigate the conductivity variation of the membranes after conditioning, we used 20 mM Tris buffer in the preparation of the three membranes listed in Table 1 and, then, measured their conductivities. The conductivities were $6.0 \times 10^{-2}$, $1.4 \times 10^{-3}$, $1.3 \times 10^{-3}$, and $1.3 \times 10^{-5}$ S/m for the 20 mM Tris buffer, membrane 3, membrane 2, and membrane 1, respectively. The conductivities of all three membranes decreased after conditioning. The conductivity of membrane 3 was close to membrane 2, while the conductivity of membrane 1 was still two orders of magnitude lower than those of membranes 2 and 3.

Furthermore, in this work, five PEG acrylates/PEG methacrylates were used to synthesize the membranes. Aqueous solutions of PEGDA 575 and PEGMEA 454 (for membranes 1 and 2) had pH values between 4 and 5, while the pH values of PEGDMA 875, PEGMEA 550, and PEGDA 400 (for membrane 3) aqueous solutions were between 6 and 7, which indicate that all the PEG monomers I used had acidic impurities (possibly acrylic or methacrylic acid), and membranes 1 and 2 had higher concentrations of carboxylic functionalities than membrane 3. Therefore, it is reasonable to presume that
the electric field gradient profiles in the separation channels of these µEFGF devices are different.

To investigate the validity of this presumption, I ran a simple FEMLAB simulation (details not presented here), where ion-transport properties of the membranes were not considered, and the total current passing through the microdevice was fixed. According to the simulation, when the conductivity of the membrane was $10^3 \sim 10^4$ times lower than the buffer, which was the case for membrane 1, the profile of the electric field gradient established in the separation channel was different from that formed in the shaped channel, and the average intensity of the electric field gradient in the separation channel was very low. As the conductivity of the membrane increased, the electric field gradient in the separation channel gradually conformed to that formed in the shaped channel. When the membrane conductivity was only 10 times lower than the buffer, the electric field gradient in the separation channel agreed quite well with the one formed in the shaped channel.

Other than the conductivity of the membrane, ion depletion at the high-field end of the µEFGF device may also contribute to the alteration of the electric field gradient in the separation channel. Ion depletion was observed in all of the µEFGF devices used in this work. During sample introduction, a continuous protein band was formed from the sample reservoir to the low-field end of the separation channel. When focusing, a dark zone appeared in the long fluorescent protein band near the high-field end of the separation channel. The dark zone continued to expand and its front end was observed to push the protein toward the low-field end of the separation channel. At the same time, the counter flow forced the protein against the movement of the protein. Finally, an intense,
narrow protein band formed in the separation channel. In addition to the formation of the
dark zone, when I shifted from the focusing mode back to the sample introduction mode
to inject more proteins into the channel, an abrupt drop in current (if voltage was fixed)
or increase in voltage (if current was fixed) was observed. After a period of time (~10 s),
the current or voltage drifted back to the steady state levels. I believe that this is
additional evidence for the formation of an ion-depletion region.

The formation of an ion-depletion region in my μEFGF device could be explained
by ion transport at the high-field end of the channel. Basically, when voltage was applied
to the shaped channel in the focusing mode, a stronger electric field, and thus, a higher
current density formed at the high-field end. If the counter flow was not high enough to
provide enough buffer ions, the electromigration of the buffer ions (across the semi-
permeable membrane and toward the low-field end) in this region would quickly decrease
the local ion concentration. Moreover, because the monomers I used had acrylic or
methacrylic acid impurities, carboxylic groups present on the surface of the membrane
would be ionized when subjected to a basic buffer such as Tris-HCl used in this work,
and negatively charged groups would form. In this way, the membrane became a cation-
selective or cation-exchange membrane. Since the semi-permeable membranes I used in
the μEFGF devices were cation-selective in nature, when an electric field was established,
cations in the separation channel migrated across the membrane into the shaped channel
at the high-field end (cathode end). Anions in the shaped channel could not easily enter
the separation channel, while those in the separation channel were pulled toward the low-
field end (anode end) by the electrical force. To maintain electro-neutrality in the
separation channel, an equal number of cations had to co-migrate upstream with the
anions. Therefore, the cation-selective membrane accelerated the formation of an ion-depletion region at the high-field end.

Recently, two research groups fabricated different types of protein preconcentrators whose preconcentration mechanisms were based on ion-depletion phenomena. Wang et al. 17 fabricated a glass microdevice that could achieve preconcentration factors as high as $10^6$-$10^8$ for proteins and peptides. In their experiments, they also observed the appearance of a dark zone in a continuous protein band, which was ascribed to formation of an ion-depletion region in the microchannel. Astorga-Wells and Swerdlow 18 used a Nafion cation-selective membrane tubing to fabricate a protein preconcentrator, and they found that an ion-depletion region appeared at the cathode junction, which greatly increased the local electrical resistance and, thus, decreased the current. No separation was demonstrated by either group. This is not surprising because in both devices only a very steep electric field gradient could be created at the boundary of the ion-depletion region. According to equations 4.4 and 4.5, a steep electric field gradient could result in a sharp peak, but the resolution of analytes would be poor.

In my µEFGF devices, however, various electric field gradients could be generated using different semi-permeable membranes. Combining the effects of both membrane conductivity and ion depletion, I speculate that the real electric field gradient in the focusing area of the separation channel had a profile close to those shown in Figure 4.10. When a voltage was applied to a µEFGF device with a low-conductivity membrane (membrane 1), the electric field gradient established in the separation channel was too shallow to provide enough force for proteins to resist the counter flow. An ion-depletion
Figure 4.10. Hypothesized electric field profiles in the focusing areas of the separation channels of µEFGF devices integrated with (A) low-conductivity membrane and (B) high-conductivity membrane.

region formed and expanded slowly toward the low-field end until it reached an area where ion-transport equilibrium could be re-established (Figure 4.10A). Since a very steep electric field gradient formed at the boundary of the ion-depletion region, proteins were tightly stacked at that point. In this situation, the µEFGF device behaved like preconcentrators with a nanofilter or Nafion membrane junction.\textsuperscript{17,18} If a µEFGF device with a high-conductivity membrane (membrane 2 or 3) was used, the electric field gradient formed in the separation channel had both shallow and steep sections (Figure 4.10B). The electric field gradient in the shallow section was similar to the electric field gradient generated by the shaped channel, while a steep gradient was formed in the ion-depletion region. By carefully controlling the counter flow rate, proteins would be moved to the shallow electric field gradient and separated (Figure 4.7D). µEFGF devices with membrane 2 or 3 were used in protein separation; however, because membrane 2 had a higher concentration of carboxylic acid functionalities than 3, formation of an ion-depletion region was faster in the µEFGF device with membrane 2. Additionally, the ion-
depletion region expanded further toward the low-field end of the separation channel, which compressed the shallow region of the electric field gradient.Interestingly, in the selective elution of GFP (Figure 4.9), in which a µEFGF device with membrane 3 was used, FITC-hemoglobin could be positioned in the shallow electric field gradient, while FITC-casein moved close to the interface of the shallow and steep electric field gradients. GFP, the least mobile protein among the three, was positioned in the steep electric field gradient and its peak was sharp compared to the others.

4.4 Conclusions

A novel fabrication method was used to integrate a PEG acrylate/ PEG methacrylate semi-permeable membrane into a PGMAMMA µEFGF device. Using various microdevices, I successfully demonstrated single protein focusing/preconcentration, protein separation, and selective elution/retention of specific protein(s). I also found that membrane properties such as conductivity and ion transport could alter the electric field gradient formed in the separation channel, which greatly affected the performance of the µEFGF device.
4.5 References


5 FUTURE DIRECTIONS

5.1 Surface Modification

ATRP proved to be an effective permanent surface modification method to graft PEG brushes on PMMA microchannel surfaces (Chapter 2). In the fabrication of PMMA µCE chips, I grafted PEG on the PMMA substrate surface before thermally bonding the substrates to form enclosed microchannels. However, the bonded PEG-grafted PMMA µCE chips did not last long, and delamination occurred spontaneously during the experiments, which may be ascribed to the weak interactions between the grafted PEG brushes. One method to solve this problem is to reverse the fabrication sequence, i.e., fabricate the PMMA microchannels before surface modification. This alternation will affect the procedures and conditions of surface modification, which require further investigation. Grafting of other protein-resistant materials using ATRP should also be considered in future research. Additionally, it is necessary to continue developing new effective and efficient surface modification techniques suitable for polymeric microfluidic protein analyzers using known chemical reactions (e.g., living radical polymerization) or chemical/physical processes (e.g., chemical vapor deposition), especially those approaches that can be used for mass production of polymeric microdevices.

5.2 Improvement of PGMAMMA and Development of New Polymeric Materials

In Chapter 3, I describe an acrylic copolymer, PGMAMMA, that can be employed to fabricate microfluidic devices. A unique feature of this material is that it has epoxy groups on the surface, which provide chemical handles for further treatment.
However, I noticed that the mechanical strength and heat resistance of PGMAMMA are not very satisfactory. To improve its physical properties, therefore, additives should be doped in the PGMAMMA polymer. Another problem I found in PGMAMMA synthesis is that the silanol groups on the glass containers could react with the epoxy groups of PGMAMMA, which made the removal of polymer sheets from the forms very difficult. In future research, different materials such as stainless steel or surface-passivated glass should be used in the construction of the forms.

Surface modification has become an indispensable step in the microfabrication of polymeric microfluidic devices. However, this process, especially permanent surface modification, is still inconvenient for most researchers. Unfortunately, microfluidic devices fabricated from currently available commodity polymeric materials and surface reactive polymers such as PGMAMMA must be subjected to surface treatment before they can be used in protein-related applications. Therefore, it would be very valuable to synthesize new polymeric materials exhibiting both good physical properties and protein adhesion resistance, which could be directly used to fabricate microfluidic protein analyzers without surface modification.

5.3 Multi-Electrode EFGF Microdevices

The EFGF microchip I report in this dissertation uses a changing-cross-section channel to generate an electric field gradient in the separation channel. One shortcoming of this format of EFGF device is that the electric field gradient cannot be easily modified once the device is fabricated. To form an electric field gradient with multiple linear or nonlinear sections, a complex changing-cross-section channel is required; otherwise,
multiple EFGF devices must be linked in serial. Fortunately, multi-electrode EFGF offers an elegant solution to this problem.

To date, the development of multi-electrode EFGF devices has been reported by Huang et al.,
Myers et al., and Petsev et al. These reports indicate that multi-electrode EFGF devices offer great flexibility in protein separations. However, these EFGF devices are relatively complex in structure and difficult to fabricate. Therefore, it is necessary to simplify the fabrication of multi-electrode EFGF devices. Recently, Humble et al. used an ionically conductive acrylic copolymer to fabricate a miniaturized changing-cross-section EFGF device (Figure 1.20B), and this fabrication process can be readily adapted to fabricate multi-electrode EFGF devices. As shown in Figure 5.1, to fabricate a multi-electrode EFGF device, in step 1, a metal wire is threaded into two sections of capillary, which is treated with MPTS outside and coated with poly(vinyl alcohol) inside. The wire-capillary assembly is then sandwiched between a PDMS mold and a MPTS-treated glass slide. In step 2, PEG hydrogel monomer solution is introduced into the PDMS channels and UV-cured to form PEG hydrogel electrodes, which are covalently attached to the MPTS-treated glass slide. In step 3, PDMS posts, which are used to fabricate reservoirs, are positioned right next to the electrodes. In step 4, PEG acrylate or PEG methacrylate monomer is cast on top of the PEG hydrogel electrodes and UV-cured. To finish the fabrication, the PDMS posts and the metal wire are carefully removed from the microdevice (step 5). In comparison to the previous reports, the fabrication methods proposed here is simpler, which may facilitate the investigation of device performance and applications of multi-electrode EFGF to proteomics research.
Figure 5.1. Fabrication of multi-electrode EFGF device.
5.4 References


