Reducing Electromagnetic Flow Enables DNA Separations in Ultrathin Channels

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REDUCING ELECTROOSMOTIC FLOW ENABLES DNA SEPARATIONS IN ULTRATHIN CHANNELS

A Thesis in
Chemistry
by
David Roger Bibeau

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Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science

August 1998
We approve the thesis of David Roger Bibeau.

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David Roger Bibeau
ABSTRACT

Research focused on the development of a procedure to reduce or eliminate electroosmotic flow (EOF) in 25 μm i.d. fused silica capillaries. An emphasis has been made on devising a procedure to coat 25 μm i.d. fused silica capillaries with a polymer that will enable DNA to migrate through the capillary, against electroosmotic flow, and continuously separate in ultrathin channels.

Chapter 2 focuses on solving the problem of analytes adsorbing on the surface of the capillaries and its effect on reproducibility and efficiency of separations. This problem is especially significant when large molecule (DNA) separations are being studied. To overcome the problem of solute-wall interaction, a procedure for modifying the surface of fused silica capillaries with acrylamide and for measuring the rate of electroosmotic flow (EOF) in capillary electrophoresis (CE) is reviewed.

Chapter 3 combines CE with ultrathin channels enabling the ability to evaluate individual and multiple plugs of double stranded DNA (dsDNA) that are detected by laser induced fluorescence (LIF). This technique combines the parallel processing capabilities of channel electrophoresis with the advantages of sample introduction with a single capillary. Ultrathin channels using 25 μm spacers between quartz plates allow for the efficient dissipation of Joule heat and decreased separation time. This technique demonstrates that the coated 25 μm i.d. capillaries can be used as a transfer device to smaller ultrathin channels.
## TABLE OF CONTENTS

LIST OF FIGURES ............................................................................................... VI

LIST OF TABLES ................................................................................................. VIII

ACKNOWLEDGMENTS ....................................................................................... IX

Chapter 1  DNA SEPARATION TECHNIQUES .................................................... 1

  Introduction ..................................................................................................... 1
  Capillary Electrophoresis .............................................................................. 4
  Capillary Surface Modification .................................................................. 7
  Ultrathin Channel Electrophoresis .............................................................. 14
  Research Goal .............................................................................................. 15
  References .................................................................................................... 17

Chapter 2  MODIFYING THE SURFACE OF FUSED SILICA CAPILLARIES .... 19

  Introduction .................................................................................................... 19
  Experimental ................................................................................................ 20
    Chemicals .................................................................................................... 20
    Capillary Coating Procedure .................................................................... 20
  Results and Discussion .............................................................................. 21
    Data Collection .......................................................................................... 21
    EOF Analysis ............................................................................................ 27
  Conclusions ................................................................................................... 37
  References .................................................................................................... 42

Chapter 3  DNA STUDIES IN ULTRATHIN CHANNELS .................................. 43

  Introduction .................................................................................................... 43
  Experimental ................................................................................................ 44
    Chemicals .................................................................................................... 44
    Ultrathin Channel Construction ............................................................... 44
    Capillary and Channel Logistics ............................................................... 48
    Laser Induced Fluorescence (LIF) Detection ............................................ 51
    Data Collection .......................................................................................... 53
  Results and Discussion .............................................................................. 54
Capillary Transfer of DNA into the Ultrathin Channel .......................... 54
Hydroxyethyl Cellulose (HEC) as a Sieving Buffer ............................ 57
Analysis of DNA Plug Width .......................................................... 57
DNA Separations with a 25-μm Transfer Capillary ........................... 69
Conclusions ...................................................................................... 78
References ....................................................................................... 79

Appendix FORMULAS ........................................................................ 80
LIST OF FIGURES

Figure 1-1: A. Chemical structure of the DNA backbone. B. Chemical structure of DNA bases

Figure 1-2: Schematic diagram of DNA base pairing

Figure 1-3: Schematic diagram of the capillary and the double layer

Figure 1-4: Chemical structure and mechanism for coating capillary surface

Figure 2-1: A. Schematic diagram for traditional CE systems with on-column detection. B. Schematic diagram of EOF in traditional CE systems

Figure 2-2: Schematic diagram of EOF in CE systems detecting DNA

Figure 2-3: Sample CE experimental results determining EOF

Figure 2-4: EOF comparison between three coated capillaries and a commercially available coated capillary

Figure 2-5: Sample CE experimental results determining DNA mobility

Figure 2-6: Electrophoretic mobility of pUC18 DNA

Figure 3-1: Schematic diagram of an ultrathin channel with spacers

Figure 3-2: A. Schematic diagram of the CE/ultrathin channel system for characterization studies. B. Schematic diagram of the CE/ultrathin channel system for separation studies

Figure 3-3: Schematic diagram of capillary tip to ultrathin channel transfer region

Figure 3-4: Chemical structure of hydroxyethyl cellulose (HEC)
Figure 3-5: A. Schematic diagram of DNA plug introduced into the ultrathin channel showing the directions of plug width and sample transfer time. B. Schematic diagram of multiple DNA plugs introduced into the ultrathin channel. C. Schematic diagram of the migration of DNA plugs over time. 61

Figure 3-6: Surface and contour plots depicting the capillary to DNA transfer region and plug width when the capillary field strength is greater than the channel field strength. 63

Figure 3-7: Surface and contour plots depicting the capillary to DNA transfer region and plug width when the capillary field strength equals the channel field strength. 65

Figure 3-8: Surface and contour plots depicting the capillary to DNA transfer region and plug width when the capillary field strength is less than the channel field strength. 67

Figure 3-9: Electropherogram of HaeIII-digested φX174 and its signal vs time response. 70

Figure 3-10: Electropherogram of HaeIII-digested pBR322 and its signal vs time response. 72

Figure 3-11: Electropherogram of HaeIII-digested pUC18 and its signal vs time response. 74

Figure 3-12: Electropherogram of multiple HaeIII-digested pUC18 DNA injections. 76
LIST OF TABLES

Table 2-1: EOF results comparing 3 days of runs for uncoated fused silica capillaries.

Table 2-2: EOF results for 3 coated fused silica capillaries and a commercially available coated capillary.
I would like to thank my thesis advisor, Dr. Andrew G. Ewing, for his guidance, encouragement and patience. Furthermore, I would also like to thank Andy for establishing a working environment that gave me the freedom and flexibility to setup my experiments, as I deemed necessary, and plenty of opportunity to learn from my mistakes.

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Chapter 1

DNA SEPARATION TECHNIQUES

Introduction

Deoxyribonucleic acid (DNA) is the genetic makeup of all organisms. It is a macromolecule made up of a large number of deoxyribonucleotides, each composed of a nitrogenous base, a pentose sugar (deoxyribose), and a phosphate group. The bases carry the genetic information, and the pentose sugar and phosphate groups form the backbone of the structure. The deoxyribose backbone is held together by phosphodiester bonds, and the bases are held together by hydrogen bonds, thus linking two complementary chains of DNA (Figure 1-1).

Our genetic differences occur due to the order in which the bases are arranged. In 1953, Watson and Crick proposed a three-dimensional model for the structure of DNA. The specificity of base pairing is the most important aspect of their double helix DNA model. The nitrogenous bases are derivatives of purine and pyrimidine. Adenine (A) must pair with thymine (T), and guanine (G) must pair with cytosine (C). Adenine cannot pair with cytosine because there would be two hydrogen atoms near one of the
Figure 1-1:  
A. Chemical structure of the DNA backbone. B. Chemical structure of DNA bases. The DNA backbone consists of pentose sugar (deoxyribose) held together by phosphodiester bonds. The DNA bases that are derivatives of purine are adenine (A) and guanine (G). The DNA bases that are derivatives of pyrimidine are thymine (T) and cytosine (C).
A. DNA Backbone Structure

Pentose Sugar
β-D-2-Deoxyribose

B. Purine

Adenine (A)

Guanine (G)

Thymine (T)

Cytosine (C)

Pyrimidine
bonding positions and no hydrogen atoms at the other. Similar interactions also apply to guanine and thymine (Figure 1-2).\textsuperscript{1,2}

DNA has a slightly negative charge due to the negative phosphate groups along the backbone, the negatively charged bases, and the hydroxide group on the opposite end of each chain. One end of the chain has a 5'-OH group and the other chain has a 3'-OH group. Neither of these hydroxide groups is linked to another nucleotide. It is these negative charges that cause DNA to interact with the inside wall of columns during separations.\textsuperscript{1,2}

Electroosmosis continuously affects the quality and reproducibility of capillary electrophoresis (CE) separations. Interactions between the inner wall of the capillary and a solute affect the amount of time the solute remains inside the capillary, thus affecting the separation efficiency and the resolution of the solute.\textsuperscript{3} A solution to this problem is to chemically modify the inner wall of the capillary to reduce and or eliminate the electroosmotic flow (EOF). Not only does DNA interact with the inside walls of columns; it migrates against EOF due to its negative charge. Polyacrylamide provides a reasonably inert surface for separations, is hydrophilic in nature, and does not interact nor attract DNA.\textsuperscript{4}

**Capillary Electrophoresis**

Electrophoresis is the separation of analytes based on their mobility in an electric field. CE is one of the most widely used techniques for the separation and analysis of
Figure 1-2: Schematic diagram of DNA base pairing. Adenine (A) must pair with thymine (T), and guanine (G) must pair with cytosine (C). Source: Villee, C. A.; Solomon, E. P.; Davis, P. W. DNA: The Secret of Life; Villee, C. A., Ed.; Saunders College Publishing: Philadelphia, 1985, pp 279-295.
small volume substances. A fused silica capillary is first filled with the separation buffer and then a sample is introduced at the inlet end. Both ends of the capillary and the electrodes from the high voltage power supply are placed into buffer reservoirs and up to 30,000 volts is applied across the capillary. As electrophoretic migration occurs, all analytes are swept towards the detector by bulk flow. The ionic species separate by their mass to charge ratio and pass the detector where the information is collected. Advantages of using CE are short analysis time, small sample requirements and high resolution. CE is at a disadvantage when large volume separations are required; it cannot rapidly pass large volumes through the separation column. CE also cannot process non-aqueous samples, and samples that adhere to columns. The use of narrow-bore capillaries allow the efficient dissipation of Joule heat generated from large applied fields because of the increase in the inner surface area to volume ratio.\textsuperscript{5,6}

**Capillary Surface Modification**

The surface of silicate capillaries contains negatively charged silanol groups (SiO\textsuperscript{−}) that attract cationic species. A double layer is formed containing ions from the analytes with the layer closest to the surface called the Inner Helmholtz Plane (IHP) and the outer, more diffuse layer the Outer Helmholtz Plane (OHP). Under an applied potential, the cations in the OHP migrate towards the cathode pulling with them the entire buffer solution. This “bulk flow” or EOF acts as a pumping mechanism to propel all molecules (cationic, neutral, and anionic) towards the detector with simultaneous separation between them. Cations move the fastest and elute first. Neutral molecules
move at the same velocity as the EOF, and elute after the cations. Anions move the slowest, therefore are the last to elute (Figure 1-3).\(^5\)

In the past, the inner surface of the capillary has been altered with both covalent and adsorptive modification. Covalent modification results in permanent changes to the inner wall surface, generally involving bonding via siloxane bonds, silane bonds, and silicone-carbon bonds. Adsorptive modification is achieved by applying a modifier that will adsorb to the inner wall surface, thus coating the capillary. These coatings can be either permanent or replaceable. Permanent coatings can not be removed from the surface or regenerated and replaceable coatings are achieved by adding additional amounts of the modifier to a rinsing buffer.\(^4\)

Hjerten in 1985 was the first to use polyacrylamide coatings on the inner wall of capillaries (0.05-0.3 mm i.d.). He covalently bonded the silane solution to the silica capillary walls and then reacted them with an acrylamide monomer to form a non-crosslinked polymer on the surface of the capillary. The bond formation was of the type \(-\text{Si-O-Si-C}\). Hjerten obtained near-zero electroosmotic flow with bonded acrylamide capillaries (Figure 1-4).\(^4,7,8\)

Kohr and Englehardt in 1991 modified Hjerten’s method of applying a polyacrylamide solution. By increasing the concentration of the monomer in the coating solution, the layer thickness of the polyacrylamide could be increased. Englehardt determined that there was no direct method to measure the effectiveness of coating
Figure 1-3: Schematic diagram of the capillary and the double layer. The double layer contains ions from the analytes with the inner static layer named the Inner Helmholtz Plane (IHP) or the stern layer and the outer, more diffuse mobile layer named the Outer Helmholtz Plane (OHP). Source: Oda, R. P.; Landers, J. P. *Handbook of Capillary Electrophoresis*; Landers, J. P., Ed.; CRC Press, Inc., 1994, p 13.
Static Layer (Stem Layer) Mobile Layer (Outer Helmholtz Plane)
Figure 1-4: Chemical structure and mechanism for coating capillary surface. A silane solution is covalently bonded to the inner wall of the fused silica capillary. Acrylamide is then reacted with it to form a non-crosslinked polymer surface on the inner wall.
Capillary Surface

\[ -\text{Si-OH} \]

Acrylamide

\[ \text{H}_2\text{C}=\text{O} \]

Silane Solution

\[ \text{H}_2\text{C}=\text{O} - \text{C} - \text{C} - \text{C} - \text{Si} - \text{O} - \text{CH}_3 \]

Mechanism

Uncoated Capillary

\[ -\text{Si-OH} \quad + \quad \text{OCH}_3 \quad \rightarrow \quad \text{Si-O-Si} - \text{CH}_3 \]

Silane

Intermediate

\[ \text{Si} - \text{O} - \text{Si}(\text{CH}_2)_3\text{O} - \text{CH}_2 \]

\[ \text{NH}, \quad 0 \]

Intermediate

Acrylamide

\[ \text{Si} - \text{O} - \text{Si}(\text{CH}_2)_3\text{O} - \text{CH}_2 \]

Coated Capillary

\[ \text{NH}, \quad 0 \]

\[ \sim \text{CH} \]

\[ \text{CH}_3 \]

\[ \text{CH}_2 \]
surfaces, therefore used the measurements of EOF as an indirect method to quantitatively study the surface modification.9-11

EOF is measured as the time it takes neutral analytes to elute from the column. Theoretically, the total or apparent mobility ($\mu_{ap}$) of a sample is calculated as the sum of the electroosmotic mobility ($\mu_{eo}$) and the electrophoretic mobility ($\mu_{ep}$) using the equation $\mu_{ap} = \mu_{eo} + \mu_{ep}$. Experimentally, $\mu_{ap}$ is calculated as the mobility of the cationic ions and the $\mu_{eo}$ is calculated as the mobility of the neutral species, with the difference between these values equaling the electrophoretic mobility, $\mu_{ep}$. These values are reported in units of cm$^2$/Vs.5,9,11,12

Other methods of permanent surface modification exist. Jorgenson and Lukacs in 1983 were able to modify the inner wall by adjusting the lengths of alkyl chains using trichlorosilane. The polymeric alkyl chains were covalently attached to the surface and crosslinked. Strong acid interactions, less than pH 2, proved detrimental to these bondings. Modifications using other hydrophilic functional groups, glycols, oxides, polyethers, carbohydrates, and poly(vinyl aclochols) have also been used. Hjerten and Kubo achieved success in bonding stability (lasting longer than four weeks) by using a carbohydrate (methylcellulose) coating,13 while Yeung has used oxides (polyethylene oxide)14 to deactivate the surface of capillaries and reduce the EOF. Surface modifications using ionic functional groups (anionic and cationic) have been used showing a reduction in the pH dependence and variability of the EOF velocity in the capillary.4
Ultrathin Channel Electrophoresis

Electrophoretic separations in millimeter structures with rectangular cross sections were first described by Tiselius in 1937. A trend toward small size has pushed electrophoresis to the micrometer scale. In 1993, Mesaros et al. developed a continuous injection and separation method that coupled a small-bore capillary to a 48 μm high rectangular quartz channel structure. In channel electrophoresis, a thin band of solute is deposited at the channel entrance. A voltage is applied across the channel, leading to the migration of the charged species away from the inlet and towards the detection region. Separation occurs because of differences in migration velocity, caused by differences in charge or differing degrees of frictional drag (in gels and sieving buffers) which opposes the electrophoretic motion.

Advantages of channel electrophoresis are the ability to separate large samples (~10 μg) and several samples simultaneously, with direct comparisons between the individual separations. Disadvantages are that separation times are long, several hours or longer, and low potential fields are used due to Joule heating. Reducing the inside gap distance between channel plates from a traditional channel (500 – 1000 μm) to that of an ultrathin channel (10 - 100 μm) leads to increased Joule heat dissipation analogous to CE. This is due to the increase in the surface area to volume ratio. Joule heating causes gel degradation at higher applied potentials, resulting in lower potential being used and an increase in the separation time. By decreasing the inner gap of the channel plates, higher separation potentials can be used, reducing the separation time.
Today, channels are useful for size based separations, including the separation of DNA fragments. Sieving mediums are used because of their reduced pore size, which impedes not only electroosmosis, but also the motion of large solute molecules (i.e. DNA). The sieving effect leads to increased frictional drag, with the smaller fragments eluting first and the larger fragments eluting last.

**Research Goal**

The goal of this research is to develop a method that will reduce or eliminate electroosmotic flow in 25 μm i.d. capillaries, enabling sized-based DNA separations in smaller ultrathin channels. The smallest commercially available zero EOF coated capillaries are 50 μm i.d. (Scientific Resources, Eatontown, NJ), and the intent of this research project is to develop a procedure to manufacture smaller i.d. coated capillaries until a commercially available means is a viable option.

In a manner similar to Mesaros et al. and Hietpas et al., the coated 25 μm i.d. capillaries will be coupled to ultrathin 25 μm i.d. channels and demonstrate that the EOF has been reduced in the capillaries, enabling DNA to quickly elute from the capillaries. If the EOF is not significantly reduced or eliminated, it could take up to several hours for DNA and other negatively charged analytes to elute from the capillary. Once the DNA has eluted from the capillary, the shape of the DNA plug and its ability to separate in the ultrathin channel will be analyzed.
Hietpas et al. found that the plug width of DNA increased due to lateral diffusion in free solution when there was an increase in the distance between the capillary tip and the channel entrance. Reducing both the internal diameter of the coated capillary and the inner gap distance in the ultrathin channel, will also decrease the gap distance between the edge of the capillary tip and the channel entrance, thus eliminating any capillary tip to channel entrance mismatch.
References


12) Kohr, J.; Engelhardt, H. *Capillary Electrophoresis with Coated Capillaries*; Guzman, N. M., Ed.; Marcel Dekker, Inc.: New York, 1993; Vol. 64, pp 357-381.


Chapter 2

MODIFYING THE SURFACE OF FUSED SILICA CAPILLARIES

Introduction

For molecular biologists to use CE as a routine separation technique for biomolecules, a better understanding of the capillary surface chemistry is needed. This is especially true when analytes adsorb to the surface of the capillary affecting the reproducibility of separations, lowering efficiency, and possibly resulting in an inability to detect analytes due to adsorption. This problem is magnified with large molecule separations, especially with DNA and proteins. To overcome difficulties associated with solute-wall interactions, covalent and adsorptive modification of the capillary wall has been employed.¹

In the experiments presented in this chapter, an uncoated 25 μm i.d fused silica capillary has been coated with an acrylamide solution to reduce electroosmotic flow inside the capillary during CE experiments. Acrylamide was chosen to coat the capillary surface because it provides a reasonably inert surface for separations, is hydrophilic in nature, and does not react with DNA.¹
Experimental

Chemicals

γ-Methacryloxypropyltrimethoxysilane, acrylamide, tris(hydroxymethyl)-
aminomethane (tris), 3-Hydroxytryamine (dopamine), and phenol were purchased from
Sigma Chemical Company (St. Louis, MO). The N,N,N′,N′-tetramethylethylene diamine
(TEMED), acetic acid, and potassium persulphate were purchased from Aldrich
Chemical Company (Milwaukee, WI). Disodium ethylenediaminetetra acetate (EDTA)
was purchased from Fisher Scientific Company (Fairlawn, NJ) and the boric acid was
purchased from EM Industries (Gibbstown, NJ). The HaeIII-digested pUC18 dsDNA
was obtained from Sigma.

Capillary Coating Procedure

The uncoated fused silica capillary (25 μm i.d., 360 μm o.d.) was purchased from
Polymicro Technologies (Phoenix, AZ). Thick-walled capillaries (360 μm o.d.) were
used instead of thinner-walled capillaries (150 μm o.d.) because of better Joule heat
dissipation, with the thickness of the walls acting as a heat sink.2 The 25 μm i.d.
capillaries were coated with a modified version of the procedure used by Hjerten et al. for
coating narrow-bore electrophoresis tubes with a mono-molecular layer of non-cross-
linked polyacrylamide.3-6 A solution of 240 μL of γ-methacryloxypropyl-
trimethoxysilane was mixed with 60 mL of distilled water, and adjusted to pH 3.5 with acetic acid. This silane solution was pushed through the capillary under pressure for 10 minutes and allowed to remain inside the capillary at room temperature overnight (at least 18 h). The capillary was then rinsed with distilled water for 10 to 15 minutes and filled with a deaerated 3% (w/v) acrylamide solution containing 1 μL TEMED (catalyst) and 1 mg potassium persulphate (initiator) per mL solution. In order to fill the capillary, the acrylamide solution was pushed through the capillary by argon gas (120 psi) for 30 min. Afterwards, the solution was pushed out of the capillary by Argon gas for at least 30 min to remove the excess acrylamide solution. The capillary was then rinsed with distilled water for 10 to 15 min, dried for at least 30 min with argon gas and then in an oven at 35 to 40°C for at least three h. All of the above solutions were filtered through a 0.22-μm filter purchased from Supelco Co. (Bellefonte, PA) prior to injecting the solutions into the capillary.

Results and Discussion

Data Collection

The CE system utilized to measure EOF is similar to the CE system used by Hayes et al. and consists of an “in-house” constructed double plexiglas box designed for personal safety.7 One box houses the high voltage electrode used for the separation potential along with the inlet buffer reservoir and/or sample reservoir. The other box
houses the voltage regulator and resistors. A Bertan high-voltage power supply Series 230 (Hicksville, NY) provides the separation potential (Figure 2-1). The system polarity is reversed during experiments with HaeIII-digested pUC18 DNA, using a negative potential to push the DNA through the EOF towards the cathode (Figure 2-2).

A Spectra 100 variable wavelength detector (Barnstead Thermolyne, Dubuque, IA) is used to measure the changes in the absorbance of the cationic and neutral markers as they migrated past the detection window. The UV detector utilizes a deuterium lamp and is set up for on-column detection similar to the procedure of Olefirowicz et al. The results have been plotted on a Linear chart recorder (Reno, NV), where the beginning of each peak is recorded as the time of the peak.

Prior to performing experiments, the capillary is filled with the separation buffer at the desired pH and the sample is introduced momentarily (1-15 s) when the inlet end of the capillary is placed in the sample reservoir. Prior to injecting the separation buffer and the sample into the capillary, both are filtered through a 0.22-μm filter to remove any particulate matter.

A 50 mM TBE buffer (pH 8.5) is used as the separation buffer, 0.01 M dopamine (DA) for my cationic marker and 0.01 M phenol as a neutral marker. For determining EOF, the UV detector wavelength is set to 200 nm, a wavelength that has been found experimentally to be good for the detection of most organic compounds in most buffers. An on-column detection window is formed by placing a drop of concentrated sulfuric acid where the window is desired and touching the drop with a hot
Figure 2-1:  A. Schematic diagram for traditional CE systems with on-column detection. B. Schematic diagram of EOF in traditional CE systems. Cations elute first, followed by neutral species that elute with EOF. Anions elute last.
A. Traditional CE Setup

![Diagram of Traditional CE Setup]

B. EOF in Traditional CE Setup

![Diagram of EOF in Traditional CE Setup]
Schematic diagram of EOF in CE systems detecting DNA. The polarity is reversed with the cathode inserted into the inlet buffer reservoir for DNA experiments. EOF flows from the outlet reservoir towards the inlet reservoir, pushing against the analytes. Anions elute first, followed by neutral species and lastly, cations.

EOF in DNA Experiments

Electroosmotic Flow
soldering iron burning off the polyimide surface covering. This method prevents
destruction of the internal coating that would be expected from the traditional method of
heating the polyimide outer coating and forms a smaller-sized detection window.\textsuperscript{9}

EOF is measured in these experiments as the time for neutral analytes to reach the
on-line detector, therefore the value of EOF is equivalent to the $\mu_{\infty}$ of the neutral marker,
phenol. The apparent solute mobility, $\mu_{\text{app}}$, is also calculated for the cationic marker
using $\mu_{\text{app}} = \frac{L_d L_t}{V t}$.\textsuperscript{9} The difference between these values equals the electrophoretic
mobility, $\mu_{\text{ep}}$.

**EOF Analysis**

The CE/UV results have been plotted on a chart recorder where the beginning of
the neutral peak, phenol, is recorded as the time of the neutral peak. This is repeated
when a cationic analyte, dopamine, is used and recorded as the time of the cationic peak
(Figure 2-3). This experiment has been repeated 10 or more times for 3 different coated
capillaries to ensure an adequate sample population. These results are compared with a
variety of other capillaries, including an uncoated 25 $\mu$m i.d. capillary, a commercially
available surface deactivated capillary, and compared to a 50 $\mu$m i.d. commercially
coated, “zero EOF” capillary from Scientific Resources (Tables 2-1 and 2-2).

The value of EOF for each capillary varies with time. The greatest difference is
between the first and second run for each capillary, with a smaller value of $\mu_{\infty}$ for the
first run and the time for the neutral marker to elute increasing significantly. To avoid
Figure 2-3: Sample CE experimental results determining EOF. Run #1 on 17 Nov 97. Conditions were: 25 μm i.d., 360 μm o.d., 20 kV applied potential, 3 s injection, 50 mM TBE buffer (pH 8.5), 200 nm detection wavelength, 0.01 AUFS, 1.0 rise time, room temperature, neutral (EOF) marker was phenol (PhOH), cationic marker was dopamine (DA). Capillary length to the detector was 56.4 cm, and total length was 66.4 cm.
Cationic Peak
39.5 min
(2370 s)

Neutral Peak
50.0 min
(3000 s)
Table 2-1: EOF results comparing 3 days of runs for uncoated fused silica capillaries. Experimental conditions were: Capillary 50 \( \mu m \) i.d., 360 \( \mu m \) o.d., 20 kV applied potential, 5 to 10 s injections, 50 mM TBE buffer (pH 8.5), 200 nm detection wavelength, 0.01 to 0.02 AUFS, 1.0 rise time, room temperature, neutral (EOF) marker was phenol (PhOH), cationic marker was dopamine (DA). Capillary length to the detector was 59.6 to 60 cm, and total length was 69.8 to 70.4 cm.
<table>
<thead>
<tr>
<th>Day</th>
<th>EOF Mean (cm$^2$/Vs)</th>
<th>Std Dev (cm$^2$/Vs)</th>
<th>N</th>
<th>Buffer</th>
<th>Markers</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3.08E-04</td>
<td>7.69E-06</td>
<td>5</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
<tr>
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<td>2.97E-04</td>
<td>1.30E-05</td>
<td>5</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
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<td>3.29E-06</td>
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<td>TBE</td>
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</tbody>
</table>
Table 2-2: EOF results for 3 coated fused silica capillaries and a commercially available coated capillary. Experimental conditions for coated capillaries were: 25 µm i.d., 360 µm o.d., 20 kV applied potential, 1 to 10 s injections, 50 mM TBE buffer (pH 8.5), 200 nm detection wavelength, 0.005 to 0.02 AUFS, 1.0 rise time, room temperature, neutral (EOF) marker was phenol (PhOH), cationic marker was dopamine (DA). Capillary length to the detector was 56.4 to 59.7 cm, and total length was 66.4 to 70.0 cm. Conditions for the commercially available coated capillary was the same as above except for: 50 µm i.d., 360 µm o.d., length to the detector was 40 cm, and total length was 50.7 cm. Regenerated capillaries were rinsed with 0.1 M HCl (optional), distilled water (if rinsed with HCl), methanol, and distilled water again for 10 to 15 min prior to refilling the coated capillary with the separation buffer.
<table>
<thead>
<tr>
<th>Capillary</th>
<th>Size</th>
<th>EOF Mean (cm²/Vs)</th>
<th>Std Dev (cm²/Vs)</th>
<th>N</th>
<th>Buffer</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 (Coated)</td>
<td>25id/360od</td>
<td>6.11E-05</td>
<td>1.07E-05</td>
<td>13</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
<tr>
<td>#1 (Regenerated)</td>
<td>25id/360od</td>
<td>5.88E-05</td>
<td>7.93E-06</td>
<td>9</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
<tr>
<td>#2 (Coated)</td>
<td>25id/360od</td>
<td>5.60E-05</td>
<td>5.77E-06</td>
<td>14</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
<tr>
<td>#2 (Regenerated)</td>
<td>25id/360od</td>
<td>6.34E-05</td>
<td>2.34E-06</td>
<td>6</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
<tr>
<td>#3 (Coated)</td>
<td>25id/360od</td>
<td>7.57E-05</td>
<td>4.66E-06</td>
<td>10</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
<tr>
<td>Commercial</td>
<td>50id/360od</td>
<td>8.59E-05</td>
<td>2.59E-06</td>
<td>4</td>
<td>TBE</td>
<td>DA &amp; PhOH</td>
</tr>
</tbody>
</table>
this discrepancy, all of the capillaries can be filled with several volumes of the separation
buffer and then allowed to run at the separation voltage for at least the time of one
separation to allow the capillaries to equilibrate.\(^9\)

After several separations, the rate of EOF increases in all of the capillaries. This
is due to adsorption of the analytes on the capillary wall, changing the surface
characteristics of the capillary, zeta potential. This fluctuation in EOF is seen throughout
a single run and during successive runs.\(^{11}\) A temporary solution to increase the
serviceability of the coated capillaries is to regenerate the capillaries by rinsing with 0.1
M HCl (optional), distilled water (if rinsed with HCl), methanol, and distilled water again
for 10 to 15 min prior to refilling the coated capillary with the separation buffer. This
removes most of the analytes that adhered to the surface coating of the capillary without
damaging the coating itself.\(^9,^{12}\)

Figure 2-4 shows that all of the capillaries coated with this method perform better
than the commercially available 50 \(\mu\)m i.d. coated capillaries. For all of these
experiments, the separation buffer and applied potential have been held constant. The
lengths of the coated capillaries are similar in total length \((L_t)\) and length to the on-line
detector \((L_d)\), thus having similar field strengths \((V/cm)\). The values for \(L_d\) are in the
range from 56 to 60 cm and the values for \(L_t\) are 66 to 70 cm (field strength 303 – 286
V/cm). The commercially coated capillary has \(L_d\) at 40 cm and \(L_t\) at 50.7 cm (field
strength 394 V/cm), thereby having significantly higher field strength than the coated
capillaries.
Figure 2-4: EOF comparison between three coated capillaries and a commercially available coated capillary. Conditions same as Table 2-2. Chart table depicts EOF value for each iteration.
Ten additional CE/UV experimental runs have been conducted by reversing the polarity in the setup and injecting HaeIII-digested pUC18 DNA (Figure 2-5). The DNA eluted from the capillary in 9 min, proving that EOF is significantly reduced. The mean electrophoretic mobility of DNA is 3.48x10^-4 cm²/Vs, which is close to the theoretical value of 3.4x10^-4 cm²/Vs. The value for EOF is the difference between the experimental and theoretical mobilities of DNA, averaging 7.8x10^-6 cm²/Vs (Figure 2-6).

**Conclusions**

An advantage to this capillary coating procedure is that it is a fast and simple procedure that takes less than 30 h to prepare a coated capillary, compared to 2 or more days in time when using other more difficult methods like Englehardt’s or Novotony’s.13,14

A known weakness to this capillary coating procedure is that the Si-O-Si-C bonds created by the methoxy groups reacting with the silanol groups on the capillary surface are prone to nucleophilic cleavage at high pH (above 11) due to siloxane bond hydrolysis and instability of the amide bond after several weeks.1,4,12 This has not been found to be a significant problem at slightly alkaline pH ranges with DNA and use of a TBE buffer. Coated capillaries have been successfully used in DNA experiments 4 months after preparation. Thus, the experimental coatings prepared and discussed here appear to be at least stable for that period of time.
Figure 2-5: Sample CE experimental results determining DNA mobility. Run #7 on 18 Nov 97. Conditions were: Capillary 25 μm i.d., 360 μm o.d., -20 kV applied potential, 15 s injections, 50 mM TBE buffer (pH 8.5), 260 nm detection wavelength, 0.005 AUFS, 0.1 rise time, room temperature. Capillary length to the detector was 56.4 cm, and total length was 66.4 cm.
Injection

DNA
9 min
(540 s)

39
Figure 2-6: Electrophoretic mobility of pUC18 DNA. Experimental conditions were: Capillary 25 μm i.d., 360 μm o.d., -20 kV applied potential, 15 s injections, 50 mM TBE buffer (pH 8.5), 260 nm detection wavelength, 0.005 AUFS, 0.1 rise time, room temperature. Capillary length to the detector was 56.4 cm, and total length was 66.4 cm.
References


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10) Karger, B. L.; Foret, F. *Capillary Electrophoresis: Introduction and Assessment*; Guzman, N. M., Ed.; Marcel Dekker, Inc.: New York, 1993; Vol. 64.


Chapter 3

DNA STUDIES IN ULTRATHIN CHANNELS

Introduction

A coated capillary is coupled to an ultrathin channel utilizing a sieving buffer to enhance sized-based DNA separation efficiency. This method combines the parallel processing capabilities of channels with the advantages of sample introduction obtained with a single coated capillary. This procedure allows for rapid sequential or simultaneous separations to be carried out in ultrathin channels with efficient heat dissipation.

Molecular biologists have used channel electrophoresis for sized-based separations for decades. It has proven to be a useful technique for separations of DNA fragments in a variety of means, including pathogen detection, restriction mapping, and sizing of large DNA fragments. Increases in speed and resolution are possible due to the increased heat dissipation that results when the surface area to volume ratio of the channel is increased by use of thinner channels.\(^1\)

In this chapter, samples of HaeIII-digested dsDNA fragments are introduced into the ultrathin channel from a coated 25 µm i.d. capillary. The capillary is translated along the channel entrance, depositing DNA fragments, which are then separated into different lanes.
Experimental

Chemicals

Tris(hydroxymethyl)-aminomethane (tris) was purchased from Sigma Chemical Company (St. Louis, MO) and the hydrofluoric acid (HF) was purchased from Aldrich Chemical Company (Milwaukee, WI). Disodium ethylenediaminetetra acetate (EDTA) was purchased from Fisher Scientific Company (Fairlawn, NJ) and the boric acid was purchased from EM Industries (Gibbstown, NJ). Hydroxyethyl cellulose (HEC), M.W. 140,000 was purchased from Polysciences, Inc. (Warrington, PA).

The HaeIII-digested pUC18, pBR322, and ϕX174 were obtained from Sigma. Ethidium bromide intercalating dye was obtained from Molecular Probes Inc. (Eugene, OR) and was used only in the HEC sieving buffer at a concentration of 0.400 mg/400 mL.

Ultrathin Channel Construction

The ultrathin channel construction procedure was similar to that used by Mesaros et al. in which narrow channels were coupled to small-bore capillaries.\(^2,3\) The channels were constructed of two pieces of polished quartz (20 cm long x 5 cm wide x 0.25 in. thick) purchased from Technical Glass Products, Inc. (Mentor, OH). Both quartz plates had one end (5 mm) beveled to an angle of 45\(^\circ\), facilitating easier alignment of the capillary to the channel entrance. Two spacers (0.001 in. thick), amber plastic shim
stock, purchased from Small Parts, Inc. (Miami Lakes, FL) were trimmed to 0.5 cm and placed along the outside edge of the bottom quartz plate. When the top plate was placed on top of the bottom quartz plate, a rectangular opening was formed between the two plates, with an internal gap of ~25 μm (Figure 3-1).

Prior to filling the plates with sieving buffer, the plates were rinsed with water and/or methanol and then rinsed with distilled water and dried. Special care was taken to ensure that no methanol residue remained on the quartz plates, and that the plates were wiped clean without streaks and fingerprints.

A 400 mL solution of 1% (m/v) HEC (M.W. 140,000) was prepared from 10 mM tris-borate-EDTA (TBE) buffer and distilled water. The intercalating dye, ethedium bromide, was added to the 1% HEC solution in the amount of 0.4 mg per 400 mL. Special care was rendered when adding the HEC. The HEC must be slowly poured into the TBE to reduce the amount of clumps forming in the solution. Excess clumps were removed by placing the beaker containing the HEC solution in an ultrasonic cleaner, (Cole-Parmer Instrument Company, Chicago, IL) until most of the clumps were removed. This solution was then poured onto the bottom quartz plate, in between the spacers. The top quartz plate was then carefully placed on top of the bottom plate, causing the excess HEC solution to spill out of the channel and leaving no air bubbles in between the quartz plates. Immediately after pouring, the two quartz plates were held together with six ¾ in. Acco binders along the edges. The edges were then sealed with 5-min epoxy, Devcon Corp. (Danvers, MA). The epoxy was allowed to cure until it was dry to touch (approx. 1 h), prior to removing the Acco binders. During this time, the beveled entrance edge and
Figure 3-1: Schematic diagram of an ultrathin channel with spacers.
Quartz Plates

Channel Spacers

5 cm

20 cm

25 μm

5 cm

20 cm
the exit edge of the channel was continuously kept moist with the HEC sieving buffer solution.

After curing, the ultrathin channel was suspended across two quartz buffer reservoirs. High-vacuum grease (Dow Corning, Midland, MI) was used to adhere the channel to both buffer reservoirs. A platinum electrode was epoxied into both reservoirs and a Bertan High Voltage Power Supply Series 230 (Hicksville, NY) was used to provide the separation voltage for the channel (Figure 3-2).

**Capillary and Channel Logistics**

The exit end of each coated capillary was etched in HF to a tip with an o.d. of 45 to 55 μm in accordance with the procedure outlined by Olefirowicz et al. The etched end was then easily positioned with a micromanipulator into the beveled channel entrance.

The coated capillary was filled with 10 mM TBE buffer, and a negative potential field (-4 to -20 kV) was applied to the inlet buffer reservoir by a Spellman High-Voltage Power Supply (Plainview, NY). The cathode was placed in the inlet buffer reservoir and the ground was placed in the buffer reservoir located at the junction between the capillary and the channel. The anode was placed in the outlet buffer reservoir of the ultrathin channel. This ensured that the potential field across the capillary would be independent of the potential field in the channel, yet electromigration would continue through the entire system.
Figure 3-2:  A. Schematic diagram of the CE/ultrathin channel system for characterization studies. B. Schematic diagram of the CE/ultrathin channel system for separation studies. Note that the detector is set up at the entrance to the channel in the characterization studies and is set up at the channel exit for the separation studies.
DNA was transported through the capillary by electromigration and deposited into the channel. Individual injections of DNA were initiated by moving the entrance end of the capillary and the cathode to the DNA vial and applying a negative potential field momentarily. Afterwards, the entrance end of the capillary and the cathode were returned to the inlet buffer container. This process was repeated for each plug, spaced by a 3 to 4 min delay for the separation buffer to act as a spacer between the consecutive DNA plugs.

To deposit several DNA plugs along the entrance edge of the channel, a Hurst Stepper Motor (Princeton, NJ) was utilized in a similar manner to Mesaros et al.2,3 The stepper motor was controlled by a Gateway 2000 386/25 computer (Souix Falls, SD) with in-house written software using the Labview programming language. The timing of the capillary movement was correlated with the calculated elution time of the DNA plugs. The capillary was moved 5 mm every 3 to 4 min allowing 5 mm spacing between plugs. As the DNA plugs entered the channel, they encountered a sieving buffer that would separate their fragments based on size.

**Laser Induced Fluorescence (LIF) Detection**

Laser induced fluorescence is a detection method that is currently used for detection of DNA in ultrathin channels. The detection zone is an area identified on the quartz plates that detects the DNA fragments as they migrate past the detection region. The LIF setup was similar to that of Sweedler et al. and others.1, 5, 6 A 6 W, high-powered argon ion laser (Coherent, Santa Clara, CA) was used in the single wavelength
mode at 514 nm. The detector was a cryogenically cooled charge-coupled device (CCD) purchased from Photometrics (Tucson, AZ). The CCD has a rectangular detection array of 1024 x 256 with each detection element 27 x 27 μm². These detection elements were read out as groups of 220 by 160 pixels for the plug width studies or as a group of 1024 by 10 pixels in the separation studies.

The argon ion laser line, 514 nm, was expanded with a Newport Optics beam expander (20x, Irvine, CA). The laser beam was directed with two Newport Optics mirrors through a Newport Optics cylindrical lens (50.8 mm x 50.8 mm, F = 150 mm) that shaped the expanded laser beam into a line (approximately 5 cm long x 500 μm width). This was focused onto the top channel plate at Brewster's angle to ensure maximum transmittance of light into the channel detection region. This line was placed at a distance of 0 to 16 cm from the channel entrance. When the line was placed at the entrance of the channel, it was possible to observe the DNA elute from the coated capillary and migrate into the channel. Consequently, as the line was moved further into the channel (i.e. 12 cm) and away from the channel entrance, it was possible to observe DNA separations.

The DNA emits fluorescence when it contacts the ethidium bromide intercalating dye in the HEC sieving buffer. This fluorescence was collected at a 90° angle as it passed through a 550 nm cutoff filter, eliminating stray light, including the reflected laser light from the HEC sieving buffer reservoir. This fluorescence was demagnified 1.1 times by a 60 mm Nikkor camera lens (Nikon f2.8) before reaching the charge coupled device (CCD) camera.
Data Collection

For analyzing the plug widths of DNA, the CCD was controlled by using a NTC Pentium 133 Mhz computer (State College, PA) and a Photometrics AT200 card (Tucson, AZ). The spectroscopic software utilized to monitor the DNA injections at the channel entrance was MAPS95 (Photometrics). The detection region of the channel was focused onto a 220 x 160 pixel array for the CCD. The 220 axis represents the width of the channel and the 160 axis represents the depth into the channel monitored by the CCD. This array was binned into 16 different tracks, each track being 220 x 10 pixels. This data was transferred into a 3-D surface plot (width, depth, intensity) using Transform 2-D (Fortner Research LLC, Sterling VA).

To analyze the widths of the DNA plugs entering the channel, a peak width program was written in-house using the Labview programming language. This peak width program was able to measure the widths of the plugs by determining its width at each track level (depth). Thus being able to measure the width sixteen times, with the largest value calculated being the plug width. Another quick method of measuring the plug width was to raise the elevation level of the Transform surface plots to a level that equaled the maximum height of the laser line. By hiding the laser line, one can quickly measure the plug width by pixel numbers above line.

For evaluating DNA separations in ultrathin channels, CCD9000 (Photometrics) spectroscopic software was utilized. The detection region of the ultrathin channel was focused onto a 1024 x 10 pixel array of the CCD. A new image was taken every second, and this 1024 x 10 pixel array was binned to a 1024 x 1 array. The CCD9000 software
allowed for 900 to 1800 consecutive images of this size to be stored in a single file. This file was then analyzed using Transform 2D (Fortner Research LLC) software to visualize the separation and identify the DNA fragmentation peaks.

To improve the visualization of the DNA separations in the channel, a signal-averaging background subtraction program was written in-house using the Labview programming language. This background subtraction program was able to reduce the high intensity laser background from the specific regions in the channel, enhancing the identification of the DNA fragmentation peaks.

Results and Discussion

Capillary Transfer of DNA into the Ultrathin Channel

Smaller coated capillaries (25 μm i.d.) are utilized in smaller ultrathin channels (25 μm i.d.) in order to reduce the distance between the edge of the capillary tip and the inside of the channel. Previously, Hietpas et al. have used a sulfonic acid coated zero EOF capillary (50 μm i.d., 362 μm o.d.), purchased from Scientific Resources, Inc. (Eatontown, NJ) in 25 μm and 57 μm channels.1,7 A capillary tip to channel entrance mismatch was encountered in 25 μm channels, with the widths of the DNA plugs increasing due to lateral dispersion in free solution prior to entering the channel. Utilizing smaller coated capillaries should help eliminate this size mismatch (Figure 3-3).
Figure 3-3: Schematic diagram of capillary tip to ultrathin channel transfer region. Gap distance between the capillary tip and the channel entrance is reduced to ~12.5 μm.
Tip is 12.5 μm Away from 25 μm Channel Entrance
Hydroxyethyl Cellulose (HEC) as a Sieving Buffer

The use of uncross linked polymer solutions enables high-resolution DNA separations through the use of their reduced pore size with DNA restriction fragments less than 23 kbp in size. This impedes not only electroosmosis, but also the motion of the DNA fragments. The mechanism of DNA separations in uncross linked HEC solutions may involve entanglement coupling between the DNA and the uncross linked HEC. This "sieving" effect leads to an increased frictional drag, with the smaller fragments eluting first and the larger fragments eluting last resulting from traveling a more tortuous path through the HEC pores (Figure 3-4).

Currently, DNA is theorized to travel through HEC under the biased reptation concept. The DNA moves in reptilian fashion by repeated stretching, slippage, relaxation and re-extension. Under the influence of a strong potential field, the DNA becomes more elongated as it moves through the pores of the HEC. Ultimately, it can achieve a rodlike shape under a very strong potential.8-10

Analysis of DNA Plug Width

To evaluate the DNA as it enters the ultrathin channel, the laser line had to be focused on the channel entrance. This region contained the capillary tip, the gap between the capillary tip and the channel entrance, and a region of the channel past the entrance.
Figure 3-4: Chemical structure of hydroxyethyl cellulose (HEC). Three HEC chain monomers are shown.
Figure 3-5A demonstrates how the plug width is measured by pixel numbers and is perpendicular to the migration path of DNA as it transverses the channel. Figure 3-5B illustrates multiple injections of DNA plugs as they migrate through the channel and figure 3-5C depicts the DNA plugs separating based on their fragmentation size.

The experimental results indicate that there is a slight variation in plug width when varying the field strength of the capillary to the field strength of the ultrathin channel. When the field strength of the capillary is greater than the field strength of the channel (Figure 3-6), the plug width is 0.209 cm wide ($\sigma = 0.01052$). This trend continues as the field strength of the capillary is lowered to equal the field strength of the channel (Figure 3-7) where the plug width is 0.201 cm wide ($\sigma = 0.00383$). When the field strength of the capillary is less than the field strength of the channel (Figure 3-8), the DNA plug width decreases to 0.191 cm wide ($\sigma = 0.00924$).

When the field strength of the capillary is greater than the field strength of the ultrathin channel, there is a large variation in the intensity (height) of the plug over the top of the laser line and depth as it crosses the laser line. The intensity of the plug is much greater and rises far above the height (intensity) of the laser line. This trend continues as the field strength of the capillary is lowered to an amount equal to or less than the field strength of the channel, the DNA plug intensity and depth decreases. At these potentials, the DNA appears to be more strongly drawn into the channel and not allowed to build up and loiter outside the channel entrance.
Figure 3-5: A. Schematic diagram of DNA plug introduced into the ultrathin channel showing the directions of plug width and sample transfer time. B. Schematic diagram of multiple DNA plugs introduced into the ultrathin channel. C. Schematic diagram of the migration of DNA plugs over time.
A.

B.

Capillary Movement
Inj #1

Capillary Movement
Inj #2

Capillary Movement
Inj #3

C.

Migration Time

Sample Introduction
Figure 3-6: Surface and contour plots depicting the capillary to DNA transfer region and plug width when the capillary field strength is greater than the channel field strength. A. Surface plot of the capillary to DNA transfer region. B. Surface plot of the capillary to DNA transfer region where the intensity plane is raised to a level that removes the laser line (background). C. Contour plot showing plug width. D. Contour plot showing plug width where the intensity plane is raised to a level that removes the laser line (background). 250 pixels equals 1 cm.
Capillary Field Greater than the Channel Field

A.  

B.  

C.  

D.
Figure 3-7: Surface and contour plots depicting the capillary to DNA transfer region and plug width when the capillary field strength equals the channel field strength. A. Surface plot of the capillary to DNA transfer region. B. Surface plot of the capillary to DNA transfer region where the intensity plane is raised to a level that removes the laser line (background). C. Contour plot showing plug width. D. Contour plot showing plug width where the intensity plane is raised to a level that removes the laser line (background). 250 pixels equals 1 cm.
Capillary Field Equal to the Channel Field

A.

B.

C.

D.
Figure 3-8: Surface and contour plots depicting the capillary to DNA transfer region and plug width when the capillary field strength is less than the channel field strength. A. Surface plot of the capillary to DNA transfer region. B. Surface plot of the capillary to DNA transfer region where the intensity plane is raised to a level that removes the laser line (background). C. Contour plot showing plug width. D. Contour plot showing plug width where the intensity plane is raised to a level that removes the laser line (background). 250 pixels equals 1 cm.
Capillary Field Less than the Channel Field

A.

B.

C.

D.
DNA Separations with a 25-μm Transfer Capillary

DNA separations have been carried out with the coated 25-μm i.d. capillary developed in this work as a transfer capillary to an ultrathin channel filled with sieving matrix. The results of these experiments indicate that several DNA samples can be simultaneously separated in the same ultrathin channel. A capillary field strength that was greater than the channel field strength has been used to increase the rate at which the DNA enters the channel and to ensure enough DNA (< 1 ng) is injected into the channel. This also makes it possible to visualize the DNA eluting from the capillary and entering the channel.

This is accomplished by temporarily reflecting the laser beam with a small compact mirror towards the channel entrance and monitoring the tip of the capillary. The DNA plug visually appears (through argon laser glasses) to be reddish in color and elongate into a rod-like structure upon entering the channel. The stepper motor is started after the DNA finishes eluting from the capillary. The capillary is moved along the entrance of the channel a predetermined distance (5 mm) prior to the next DNA plug’s elution. Figure 3-9 shows a top view and a cross section of a separation of HaeIII-digested φX174 DNA fragments. Nine of the 11 peaks expected are observed. Figures 3-10 and 3-11 show individual separations of pBR322 and pUC18 DNA fragments, respectively, in the smaller capillary to channel system. Finally, multiple plugs of HaeIII-digested pUC 18 have been separated sequentially in a 25-μm thick channel with 1% HEC (Figure 3-12).
Figure 3-9: Electropherogram of HaeIII-digested ϕX174 and its signal vs time response. Experimental conditions for the capillary were: 25 μm i.d., 360 μm o.d., 3 s injection, -15 kV applied potential, field strength, 270 V/cm, 10 mM TBE buffer, room temperature. Total length was 48.5 cm. Channel conditions were: spacing, 25 μm, length, 20 cm, width, 5 cm, 2.5 kV applied potential, field strength 125 V/cm, 1% HEC sieving buffer, length to detector 12 cm, CCD start time 13 min.
Figure 3-10: Electropherogram of HaeIII-digested pBR322 and its signal vs time response. Experimental conditions for the capillary were: 25 μm i.d., 360 μm o.d., 3 s injection, -20 kV applied potential, field strength, 350 V/cm, 10 mM TBE buffer, room temperature. Total length was 56.8 cm. Channel conditions were: spacing, 25 μm, length, 20 cm, width, 5 cm, 2.5 kV applied potential, field strength 125 V/cm, 1% HEC (140,000 MW) sieving buffer, length to detector 12 cm, CCD start time 15:10 min.
Figure 3-11: Electropherogram of HaeIII-digested pUC18 and its signal vs time response. Experimental conditions for the capillary were: 25 μm i.d., 360 μm o.d., 3 s injection, -20 kV applied potential, field strength, 350 V/cm, 10 mM TBE buffer, room temperature. Total length was 56.8 cm. Channel conditions were: spacing, 25 μm, length, 20 cm, width, 5 cm, 2.5 kV applied potential, field strength 125 V/cm, 1% HEC (140,000 MW) sieving buffer, length to detector 12 cm, CCD start time 18:15 min.
**Figure 3-12:** Electropherogram of multiple HaeIII-digested pUC18 DNA injections. Experimental conditions for the capillary were: 25 μm i.d., 360 μm o.d., 3 s injection, -20 kV applied potential, field strength, 350 V/cm, 10 mM TBE buffer, room temperature. Total length was 56.8 cm. Channel conditions were: spacing, 25 μm, length, 20 cm, width, 5 cm, 2.5 kV applied potential, field strength 125 V/cm, 1% HEC (140,000 MW) sieving buffer, length to detector 12 cm, CCD start time 18:15 min. Time between injections 4 min, distance between injections was 5 mm.
Conclusions

The transfer of DNA from the capillary to the ultrathin channel has been evaluated. A noticeable increase in DNA plug width occurs as the DNA elutes from the capillary and enters the ultrathin channel (capillary diameter is 25 μm and DNA plug width is 0.2 cm). Less diffusion is observed when the field strength of the capillary is less than the field strength of the channel, leading one to believe that the DNA is being quickly “pulled” inside the channel. When the capillary field strength is greater than the channel field strength, the DNA builds up outside the channel entrance and diffuses while it is trying to enter the pores of the HEC sieving buffer.

Multiple injections and separations of dsDNA have been evaluated. DNA base pair fragments ranging in size from 51 to 1353 bps have been separated with less than 1 ng of DNA injected. Multiple injections can be accomplished in serial fashion and allowed to continuously separate in separate lanes on the channel. Better resolution is observed as the amount of DNA injected is decreased.
References


Appendix

FORMULAS

Velocity:
\[ v = \mu_{\text{app}} E = \mu_{\text{app}} \frac{V}{L_{\text{tot}}} \]

Apparent Mobility
\[ \mu_{\text{app}} = \mu_{\text{sp}} + \mu_{\text{eo}} \]
\[ \mu_{\text{app}} = L_{\text{det}} \times L_{\text{tot}} / V \ t_{\text{peak}} \]

Amount Injected on Capillary
\[ \text{Amount} = \left[ \frac{\pi \ r^2 \ \mu_{\text{app}} \ V_{\text{inj}} \ t_{\text{inj}}}{L} \right][\text{Conc}] \]

Efficiency
\[ N = 5.54 \left( \frac{L_{\text{det}}}{W_{1/2}} \right)^2 \]
\[ N = 5.54 \left[ t_{\text{inj}} / \text{Peak Area} / \text{Peak Height} \right] \]

Joule Heating
\[ \text{Watts/Meter} = \frac{(\text{Voltage})(\text{Amperage})}{(\text{Column Length cm}) \times 1000} \]