DNA SEPARATION AND SEQUENCING BY ELECTRIC FIELD-FLOW FRACTIONATION (EFFF) IN A MICROCHANNEL

By

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Master of Science

DNA SEPARATION AND SEQUENCING BY ELECTRIC FIELD-FLOW FRACTIONATION (EFFF) IN A MICROCHANNEL

By

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August, 2003

Chair: Anuj Chauhan
Major Department: Chemical Engineering

DNA separation is a core activity in biology, especially in mapping and sequencing DNA molecules. Separation of DNA molecules of different chain lengths by electrophoresis is difficult because the ratio of the total charge to viscous resistance is independent of the chain length. We modeled using a lateral electric field in a microchannel to separate DNA fragments in different sizes based on Taylor dispersion theory. With this method, we can perform the separation in free solution, which avoids the difficulty of loading gel in commonly used capillary gel electrophoresis (GE) or complicated fabrications in an artificial sieving matrix.

During the research, we found that the lateral electric field will build a concentration profile in the lateral direction, which has a different distribution for different DNA molecules. Theoretically, the product of Pe and $U_Y$ determines the shape of the profile. The variant profiles combined with the parabolic velocity profile along the
lateral direction engender different mean velocities of pulse. Based on this fact, we acquired length-dependent separation of DNA molecules.

Furthermore, EFFF can also effectively separate DNA strands differing in size by a single base pair, and thus can be used to sequence DNA. The separation step in sequencing 500 base pair long DNA molecules can be done in 1.7 hours by a 0.72-meter long, 10-micron thick channel. However, since this result is from theoretical calculations, we need further experiments to test it.
CHAPTER 1
BACKGROUND

Deoxyribonucleic Acid (DNA)

Nucleic acids are important kind of biological molecules that exist in every living being. These molecules were discovered in 1868 by a young Swiss scientist when he refined an organic substance with a very large phosphorous content from a bandage. But the biological functions of nucleic acids were not recognized until 1944, when scientist Avery did the famous pneumobacillus transform experiment. This experiment successfully proved that nucleic acids but not proteins are the genetic substances in a living organic body. The next milestone in the field of molecular biology occurred in 1953 when Watson and Crick discovered the double helix structure of DNA molecules. Then in the 1970s, recombination technology was invented, leading to the foundation of the new field of Gene technology. Since then, researchers have tried to modify genes in organisms for a variety of applications.

Most living cells contain two kinds of nucleic acids—deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Most cells have both of them with DNA existing in nucleolus and RNA existing in the cytoplasm. However viruses usually contain either only DNA or RNA.

What Forms DNA and RNA Molecules

DNA and RNA are polymers known as polynucleotide, in which the monomer units are nucleotides. Each nucleotide comprises three parts—pentose, base and phosphate group [1]. In DNA or RNA, each nucleotide contains only one phosphate
group, but some cellular free nucleotides (such as ADP and ATP, which are important species in the energy transfer and storage cycle) may contain more than one.


The carbon atoms on the bases (purine and pyrimidine rings) are numbered as 1, 2, 3, 4, 5 and thus to avoid confusion the carbon atoms on the pentose are numbered 1', 2', 3', 4', and 5' (Figure 1-2). Two kinds of pentose exist in nucleic acid—2'-deoxyribose and ribose. The difference between the two is that the deoxyribose lacks a hydroxyl group at the 2'-position [2]. The deoxyribose pentose is present in DNA and the ribose pentose is present in RNA. In both the 2'-deoxyribose and ribose, the hydroxyl groups on the 5'- and 3'- carbons link to the phosphate groups.

Five different kinds of bases are present in nucleic acids. They are adenine, guanine, cytosine, thymine and uracil. Figure 1-3 shows the molecular structure of these bases.
Figure 1-2. Structure of pentose with numbering

Four of these five bases are present in DNA. They are given one letter abbreviations as shorthand (A is for adenine; G is for guanine; C is for cytosine; T is for thymine). In the RNA molecules, there are also four types of bases; A, G, C also exist in RNA, but T is replaced by U(uracil).

If the phosphate groups in a nucleotide are removed, it becomes a nucleoside, which consists of one of the bases covalently attached to the 1' position of a pentose. The five different nucleosides of DNA and RNA are deoxyadenosine (dA), deoxyguanosine (dG), deoxycytosine (dC), deoxythymidine (dT) and deoxyuracil (dU), which is present only in RNA.

The bases in RNA and DNA form pairs—A-T and G-C in DNA, and A-U and G-C in RNA. The chemical structure of uracil is simpler than thymine and uracil can pair perfectly with adenine. Thus, it puzzled researchers that A pairs with T rather than U in a DNA strand. This issue was finally resolved after we understood the details of the repairing mechanisms in DNA.

As an evolution source, mutations may occur under the influence of external factors (UV radiation, exposure to chemical agents, etc.) or cellular processes (accidental deamination, replication errors, etc.). Figure 1-4 shows one of these mutation factors: the deamination of cytosine.

Cytosine is one of four bases in DNA molecules. As shown above, Cytosine can be mutated to uracil by deamination process. Since DNA does not contain uracil, this mutation can be easily detected and repaired by base excision. If DNA were made up of uracil, the cytosine to uracil mutation could hardly be corrected. This explains why DNA
chooses thymine, instead of uracil, even though the chemical structure of uracil is simpler than thymine.

Figure 1-4. Examples of deamination which involves the removal of an amino group. Accidental deamination may change the cytosine to uracil, or the methylated cytosine to thymine (Reprinted with permission from The Web Book Publications. 2003. Molecular biology web book. Available from URL: http://www.web-books.com/MoBio. Site last visited July 2003)

Structure of DNA Molecules

Primary structure

As mentioned above, DNA molecules consists of A,T,C,G [3]. They are joined by the 3’-5’ phosphate bonds into a strand (Figure 1-5).

The sequence of these four nucleotides determines the genetic information contained in the DNA molecules, and different creatures have different sequence and
length of DNA molecules. Table 1-1 shows the typical length of DNA molecules in various organisms.

![Scheme of single strand DNA](image)

**Figure 1-5. Scheme of single strand DNA**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Genome size (Mb*)</th>
<th>Gene number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis D virus</td>
<td>0.0017</td>
<td>1</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>0.0032</td>
<td>4</td>
</tr>
<tr>
<td>HIV-1</td>
<td>0.0092</td>
<td>9</td>
</tr>
<tr>
<td>Bacteriophage l</td>
<td>0.0485</td>
<td>80</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4.6392</td>
<td>4400</td>
</tr>
<tr>
<td>S. cerevisiae (yeast)</td>
<td>12.155</td>
<td>6300</td>
</tr>
<tr>
<td>C. elegans (nematode)</td>
<td>97.</td>
<td>19000</td>
</tr>
<tr>
<td>D. melanogaster (fruit fly)</td>
<td>137.</td>
<td>13600</td>
</tr>
<tr>
<td>Mus musculus (mouse)</td>
<td>3000.</td>
<td>?</td>
</tr>
<tr>
<td>Homo sapiens (human)</td>
<td>3000</td>
<td>30000**</td>
</tr>
</tbody>
</table>

* 1 Mb = 1 million base pairs (for double-stranded DNA or RNA) or 1 million bases (for single-stranded DNA or RNA).

** The total number of human genes is still quite controversial. It could be as high as 75,000 [see a paper published on July 4, 2001].

**Code and gene**

Scientists have found that each three continuous nucleotides within the DNA encode a protein and have drawn Table 1-2, which shows the correspondence between the codes and proteins.

### Table 1-2. Genetic code (mRNA)

<table>
<thead>
<tr>
<th>1st position (5' end)</th>
<th>2nd position (middle)</th>
<th>3rd position (3' end)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>U</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe F</td>
<td>Ser S</td>
<td>Tyr Y</td>
</tr>
<tr>
<td>Phe F</td>
<td>Ser S</td>
<td>Tyr Y</td>
</tr>
<tr>
<td>Leu L</td>
<td>Ser S</td>
<td>STOP</td>
</tr>
<tr>
<td>Leu L</td>
<td>Ser S</td>
<td>STOP</td>
</tr>
<tr>
<td>Leu L</td>
<td>Pro P</td>
<td>His H</td>
</tr>
<tr>
<td>Leu L</td>
<td>Pro P</td>
<td>His H</td>
</tr>
<tr>
<td>Leu L</td>
<td>Pro P</td>
<td>Gln Q</td>
</tr>
<tr>
<td>Leu L</td>
<td>Pro P</td>
<td>Gln Q</td>
</tr>
<tr>
<td>Ile I</td>
<td>Thr T</td>
<td>Asn N</td>
</tr>
<tr>
<td>Ile I</td>
<td>Thr T</td>
<td>Asn N</td>
</tr>
<tr>
<td>Met M</td>
<td>Thr T</td>
<td>Lys K</td>
</tr>
<tr>
<td>Val V</td>
<td>Ala A</td>
<td>Asp D</td>
</tr>
<tr>
<td>Val V</td>
<td>Ala A</td>
<td>Asp D</td>
</tr>
<tr>
<td>Val V</td>
<td>Ala A</td>
<td>Glu E</td>
</tr>
<tr>
<td>Val V</td>
<td>Ala A</td>
<td>Glu E</td>
</tr>
</tbody>
</table>

**C**

|                       |                       |                      |
|                       |                       |                      |

**A**

|                       |                       |                      |
|                       |                       |                      |

**G**

|                       |                       |                      |
|                       |                       |                      |

Synthesis of a peptide always starts from methionine (Met), coded by AUG. The stop codon (UAA, UAG or UGA) signals the end of a peptide.


By definition, a gene includes the entire nucleic acid sequence used for the expression of one product (peptide or RNA). Such sequence may be separated into two categories—regulatory region and transcriptional region. The regulatory region controls the activation of the gene, which could be near or far from the transcriptional region.

And the transcriptional region consists of exons and introns. After the transcription introns will be removed, whereas exons remains encoding a peptide or functional RNA.
Figure 1-6 shows the secondary structure of DNA molecule, which is made up of genes, pseudogenes and extragenic region. Pseudogenes are nonfunctional genes, which often comes from mutation of genes that happens in the duplication process. However, because duplicated genes commonly have many copies, the organism can still survive even if some of them become nonfunctional.

![Gene structure of DNA strand](http://www.web-books.com/MoBio)

A certain piece of DNA sequence often repeats several times in the total DNA of a cell. Experimentally, the number of repeated copies is classified on the basis of DNA reassociation kinetics [1]. The entire DNA is first randomly cleaved into fragments with an average size of about 1000 bp. Then, they are heated to separate the strands of each fragment. Subsequently, temperature is reduced to allow strand reassociation. If a fragment contains a sequence which is repeated many times in the total DNA, it will have greater chance to find a complementary strand and reassociate more quickly than other fragments with less repetitive sequences.
Based on the reassociation rate, DNA sequences are divided into three classes: highly repetitive, moderately repetitive and single copy.

**The 3D structure**

In a DNA molecule, the two strands intertwine with each other, forming a double helix structure. This structure was first discovered by James D. Watson and Francis Crick in 1953. In this structure, the sugar-phosphate backbones of the two DNA strands wind around the helix axis and the bases of the individual nucleotides are on the inside of the helix.

Within the DNA double helix, base A forms 2 hydrogen bonds with T on the opposite strand and G forms 3 hydrogen bonds with C.

![Figure 1-7. Example of dA-dT and dG-dC base pair as found within DNA double helix](http://www.web-books.com/MoBio). Site last visited July 2003)
The helix turns a round every 10 base pairs (Figure 1-8). Since the distance between two base pairs is 0.34 nm, the length is about 3.4 nm per turn for DNA molecule. The intertwined strands make two grooves of different widths— the major groove and the minor groove, which may bind with specific proteins.

The human DNA molecule in a diploid cell, if fully extended, would have a total length of 1.7 meters. If one unfolds all of the DNA molecules in the body, one could reach the moon for 6000 times! [1]

In a solution with higher salt concentrations or with alcohol added, the DNA structure may change from normally B form to an A form, which is still right-handed. But every 2.3 nm makes a turn with 11 base pairs in it.

Another DNA structure is called the Z form because it seems to zigzag, which is left-handed on rotation. One turn includes 4.6 nm, comprising 12 base pairs. The DNA molecule with alternating G-C sequences in alcohol or high salt solution tends to have such structure.

Stability [3,4]

In this part, we will introduce two aspects of DNA molecules. One is the melting of helix double strand DNA, the other is the degradation.

Melting

Melting is the term given to the separation of the two strands of a DNA molecule, which is also called denaturation.

Several factors make the DNA molecules relatively stable. The DNA strands in a double helix are held together by the H-bonds between the bases. These bonds (also called Watson-Crick) attach two strands together. Moreover, base pairs sit on the top of each other at a rotation of 36° and there is strong interaction between all adjacent base pairs. This interaction, called stacking interaction, stabilizes the DNA double helix. Additionally, the phosphate groups must be neutralized (by Na+ or Mg2+ ions) to allow the negatively charged phosphates to be in close proximity.

As introduced above, two hydrogen bonds exist between A and T and three exist between G and C. If a solution of DNA is heated, the hydrogen bonds will break at high temperatures, and the stacking interactions will become weak. As some researchers concluded, the most important contribution to DNA helix stability is the stacking of the bases on top of one another. Thus, in order to denature DNA, we must overcome the stacking energies that provide cohesion between adjacent base pairs. Since AT pairs have only two hydrogen bonds, they are easier to undergo severance. Together with the fact that the stacking energies are less for AT-rich regions, AT rich area tends to separate compared to GC rich area. As a result, the base composition of the DNA influences the melting temperature (Tm), at which two DNA strands separate. The greater the proportion of G-C base pairs in the DNA is, the higher the Tm is. However,
experimentally, at temperatures higher than 80°C the GC pairs will also melt, and the DNA will become single stranded which will be present in coiled and unstructured forms.

Several methods can be used to obtain the melting of double strand DNA:

- **Reduction of Salt Concentration**—as the salt concentration is reduced, the phosphate groups are no longer neutralized by Na+ or Mg2+ ions and the negative charges of phosphate group tend to force the strands apart.

- **Extreme of pH**—it alters the ionization states of the groups on the bases which provide and accept the H-bonds. Commonly, linear DNA molecules will denature and precipitate when pH is above 12.

- **Increase in Temperature**—when temperature of a DNA solution increases to a certain value, which is called the melting point (Tm), the strands separate.

**Degradation of DNA molecules**

Degradation of DNA molecules is related to the breakup of phosphoric bonds and consequently the cleavage of DNA chain. Various factors contribute to the degradation of DNA: chemical, physical and enzymatic, etc. Commonly, the effects that result in degradation are much stronger than that for melting.

- **Prolonged heat treatment** may result in DNA hydrolysis which degrades the DNA.

- **Low pH** may increase chemical modifications and hydrolysis of DNA. For example, at low pH (pH 4), maize DNA and plasmid DNA were rapidly degraded [5]. Under low pH conditions, what will happen first in the degradation of DNA is the depurination of the nucleic acid backbone. After that, hydrolysis of adjacent 3'-5'-phosphodiester linkages occurs, resulting in measurable shortening of DNA strands [6].

- **Enzymatic degradation** of DNA by nucleases may also occur on prolonged storage. Adding EDTA can inhibit the activity of DNA enzyme by chelating metal ions with valence of 2, so that the storage time can be above 5 years under -70°C.

**Separation of DNA molecules**

Microfluidics is a sub area of the microelectromechanical systems (MEMS) and is mainly concerned with moving fluids and then performing various unit operations in micron-sized channels. Microfluidics is rapidly becoming a very important area of
research due to numerous potential applications in separation and analysis. The current trend in this field is towards development of chips that can accomplish reactions, separations, and detection at a very rapid rate, such as a chip that can separate DNA fragments of different lengths and detect them.

DNA electrophoresis has become a very important separation technique in molecular biology and, in particular, in the genome project. DNA fragments are first separated by chain length and are later processed to read the sequence of the bases that form the genetic code of all living organisms. This technique is also indispensable in forensic applications for identifying a person from a tissue sample [7]. However, separation of DNA fragments of different chain lengths by electrophoresis is difficult because the velocity of the charged DNA molecules due to an axial electric field is independent of the chain length. The reason of this independency is that the mobility of a DNA molecule is approximately inversely proportional to the length while the total charge is directly proportional to the length. This difficulty is traditionally overcome by performing the electrophoresis in columns filled with gels. In these processes, because negatively charged DNA surrounded by positive counterions moves through a matrix such as an agarose gel, the mobility is no longer inversely proportion to the length. Thus, DNA chains of different lengths traverse the gel at different speeds and separate in a series of bands. In gel electrophoresis, the electric field can either be continuous or pulsed. Continuous field electrophoresis is useful for separating DNA molecules of sizes below approximately 20000 base pairs. The migration rates of DNA strands above this size is almost independent of the length of the strand except at very low voltages with which it takes an excessively long time to accomplish separation. Pulsed field gel
electrophoresis (PFGE) was developed to separate longer DNA fragments, which can not be separated by the conventional gel electrophoresis. PFGE utilizes a pulsed electric field, which changes directions continually, resulting in changes in migration directions. These changes lead to a stronger dependency of the net migration rates on the DNA chain length, even if the chains are longer than about 20000 base pairs.

Although gel electrophoresis can separate DNA fragments, there are some problems associated with its use in DNA separation. Bubbles can form in the gel during an operation, resulting in variations in DNA electrophoretic mobility [8]. Moreover, the separation by electrophoresis of DNA fragments larger than 40000 base pairs using gel is slow; which is still one of the slowest steps in the genome project. This kind of separation typically takes more than 20 hours because a low-intensity and pulsating field is used to separate DNA fragments to prevent the long fragments from being damaged by high temperatures that may result under large fields [9].

To eliminate the temperature increase during separation, researchers developed capillary electrophoresis, which has a high surface-area-to-volume ratio, providing rapid elimination of heat and allowing application of high electric fields without a substantial temperature increase [8,10,11]. The use of capillary sequencers in the genome project resulted in an eight-fold increase in the sequencing capacity and output [12]. However, preparing uniform, homogeneous, bubble free and stable gel-filled capillaries is difficult, especially for separation of DNA fragments, which commonly involves many parallel lanes running simultaneously.

Recent advances in microfabrication techniques have led to production of microfluidic devices frequently referred to as a “lab-on-a-chip” that can perform a
number of unit-operations such as reactions, separations, detection, etc., at a much higher throughput. Gel-based DNA separations are not convenient in such devices because of the difficulty in loading the gel [9]. Thus, gels have been replaced with polymeric solutions as the sieving mediums. Electrophoresis in a free medium can also separate DNA fragments but it requires precise modifications to the DNA molecules [13]. Microfabricated obstacles such as posts [14], self-assembling colloids [15], entropic barriers [16], and Brownian ratchets [17,18] have also shown to be effective at separating DNA strands.

Craighead et. al. used an entropic trapping system, which consists of alternating thick (0.65-1.6 µm) and thin (90nm) regions in a channel flow. Since larger molecules need to reach higher entropic states to enter the thinner regions, they spend less time in the channel and exit the channel earlier than smaller ones. In this method, the number of traps is one of the most important factors that controls the separation effect. In their experiment, they did not achieve good separation for DNA molecules (24.5, 48.5, 73.0, 97.0kbp) until the number of traps reached 3700 and the total separation time reached 40 mins [19]. Turner et. al. fabricated artificial arrays of posts in a microchannel by lithography. The diameter of the posts and the interval between them were both 100nm—small enough to provide a strong sieving effect. They tested separation of 7.2 kbp and 43 kpb DNA strands and obtained a ratio of 2 between the mean velocities of these two kinds of DNA strands [20]. Baron et al. used un-crosslinked polymer solution which provides sparse sieving and thus has low resistance to DNA molecules. By this method, they reduced the operation time to about 20mins. But the separation for DNA with large molecule weight is still not satisfactory [21]. Viovy et al. used magnetic fields to drive
superparamagnetic particles to form a post matrix with the interparticle distance to be about 5.7 \( \mu m \). They successfully separated large DNA molecules (15, 33.5, and 48.5kbp) in only 10-15mins. Furthermore, when the magnetic fields are released, the viscosity of the fluid in the pipe becomes low. Consequently, this method avoids the difficulty of loading gel that exists in gel-capillary electrophoresis. Bader et al. [17,18] created a spatially periodic anisotropic potential energy field to trap the molecules at the potential energy minima. As a result of pulsating application of an electric field, the molecules that diffuse outside a trap when the field is released are attracted to the next trap. In this method, the smaller molecules with large diffusivity have larger migration speeds, and the larger molecules have lower speeds. This difference in speed leads to separation.

However, the optimal DNA separation technique should accomplish separation without any sieving medium and should be amenable to online modification to accomplish separation for a wide range of DNA sizes. Our proposed strategy utilizes lateral electric fields and no sieving medium, and the amplitude of the field can be adjusted to separate different DNA compositions. Essentially, this method is called electric field-flow fractionation (EFFF) [22-24], which is a derivation of field-flow fractionation (FFF). Giddings first proposed FFF in 1966 [25]. The basic idea is to use a field in the direction perpendicular to flow and form a concentration profile on the cross section [26]. When charged DNA molecules flow through channels in the presence of lateral fields, i.e., fields perpendicular to the flow direction, they experience an attractive force towards the wall of the opposite polarity. In the absence of any field, each DNA molecule has an equal probability of accessing different streamlines in a time scale larger than \( h^2/D \), where \( h \) is the height of the channel, and \( D \) is the molecular diffusivity.
However, due to the electric field, the molecules on average access streamlines closer to the wall, which results in a mean axial velocity smaller than the mean fluid velocity. We shall show later that the enhancement in concentration near the wall is greater for the more slowly diffusing molecules, and thus their mean velocity is reduced more than the mean velocity of the faster diffusing molecules. If a slug of DNA molecules is introduced into a channel with lateral electric fields, the difference in mean velocities leads to separation of the molecules into bands, and the bands of smaller molecules travel faster.

FFF has also been used in size based particle separation using gravity or centrifugal acceleration as the lateral force [27-29]. New trends in FFF are thermal field-flow fractionation (TFFF) [30-32] and its application in bioseparation [33,34].

In this paper, we analyze the Taylor dispersion of charged molecules such as DNA in a microchannel with pressure driven flow under lateral fields by using regular perturbation techniques. Based on our investigation, we propose a new scheme for separating DNA molecules in channels by application of lateral electric fields without using any gel or polymeric solution as sieving mediums. Brenner used the method of moments to obtain the Taylor dispersion coefficient for shear flow in a channel accompanied by a lateral flow [35]. In our proposed technique we have Poiseuille flow in a channel along with a lateral flow driven by an electric field. We obtain the dispersion coefficient by using a regular perturbation scheme. In this paper we restrict our analysis to a 2D channel because the qualitative behavior of the DNA separation is expected to be the same in 3D even though quantitatively the results may differ.
Fluidic Properties of DNA Molecules

Since we are studying the separation of DNA in free solutions, it is important to understand the behavior of DNA molecules in free solutions, particularly the mobility and diffusivity.

Mobility of DNA molecules in free solution

Most researchers define the velocity of DNA molecules under unit electric field intensity as its mobility. Consequently, the unit of mobility is m²/(s*volt).

After the invention of capillary electrophoresis (CE), it is possible to measure the mobility of DNA molecules in free solution accurately. However, one needs to ensure that the capillary walls are coated to eliminate the electroosmosis flow (EOF) of the solvent so that the data obtained is accurate.

CE experiments have shown that the mobility of small DNA strands increases with size but levels off beyond a critical size. However, there are discrepancies on the critical size beyond which the DNA mobility is independent of size. In two separate studies Stellwagen et al. determined the critical size to be 400bp and 170bp [36,37].

From the trend of changing of mobility with DNA size, researchers concluded that small, relatively rigid DNA molecules experience greater friction with the solvent. Most researchers believe that “electrolyte friction” contributes to this phenomenon. This friction is an additional source of friction induced in the bulk solvent by the migrating polyions—the DNA molecules. As we know, the counterions in the solvent will build a double layer around the DNA molecules. When the DNA molecules are in static state, the double layer will reach an equilibrium state. However, when these DNA molecules migrate, the counterions will change the distribution around the DNA molecules. Before a new equilibrium state is established, which needs some time to achieve, the counterion
cloud will create a fluctuating force on the DNA molecules. Researchers believe that this is the origin of the dependence of the mobility of small DNA molecules on the size.

**Molecular diffusivity in free solution**

The molecular diffusivity is another important parameter that affects the separation efficiency.

Several methods can be used to measure the molecular diffusivity of DNA molecules in free solutions: capillary electrophoresis (CE), NMR, Dynamic light scattering. Based on Stellwagen’s research [38], these three methods give comparable results. Here, we introduce the most commonly used method—the stop-flow method in capillary electrophoresis. In this method, the voltage is turned off after the analytes have migrated nearly half way along the capillary channel. Then, these analytes are left there for a certain period time, within which band broadening occurs without the intervention of electric field. After that, the electric field is turned on again until all analytes go through the whole channel and are detected by the detector located at the end of the capillary. By using Equation 1-1 (\( \sigma \) is the band variance)

\[
\sigma^2(t) = \sigma^2_e + 2D_0(M)t
\]

where \( t \) is the time during which the field is turned off, and by repeating the experiment for different times, we can obtain the molecular diffusivity \( D_0 \) (the slope of the curve).

By further analysis, several researchers got an accordant result about the relationship between the molecular diffusivity and the size of DNA molecules.

Stellwagen, Sorlie and Pecora, et al. got the scaling law \( D \sim 1/M^{(0.68 \pm 0.03)} \). For long flexible polyers, a classic theory, called Flory’s theory, is successfully used to describe the asymptotic behavior \( D \sim 1/M^{3/5} \).
To find a model to calculate the molecular diffusivity of DNA molecules in free solution, Axel E. Nkodo, et al, tried several models [39]. And they found that their diffusion data agree well with the Zimm theory, which is used for a nonfree draining polymer. Therefore, they concluded that one can use Zimm equation to predict the diffusion coefficient of DNA molecules in free solutions fairly accurately with a good model for the hydrodynamic radius $R_H(M)$. And they recommend the equations for $R_H(M)$. For short rod-like fragments, one can use Equation 1-2

$$D \sim \frac{kT}{3\pi \eta L/(\ln(L/d) + \gamma)}$$

(1-2)

Here, $\eta$ is the viscosity of fluid, $L$ is the length of DNA molecule; $d$ is the diameter of the DNA molecule. When the molecular size is medium compared to their persistence length, the Kratky-Porod equation provides an excellent model for $R_H(M)$. As for very long molecules, Flory’s scaling law applies.

Additionally, some researchers did some experiment to test the effect of the intensity of electric field on the molecular diffusivity of DNA molecules in free solutions. The result is that the electric field does not change the diffusivity much within applicable conditions. This result is good since we can use high voltage to acquire high velocity of DNA molecules in the free solutions without considering much about the changing of diffusion.
CHAPTER 2
THEORY

Derivation of Equations About EFFF Using Perturbation Analysis

Figure 2-1 shows the geometry of the 2D channel along with the electrodes for applying the lateral electric field. L and h are the channel’s length and height respectively, and the channel is infinitely wide in the third direction. The approximate values of L and h are about 2 cm and 5 microns respectively. Thus, continuum is still valid for flow in the channel. As we know, large Knudsen number (the ratio of mean free path to the dimension of the channel) invalidates Navier-Stokes equations. Commonly, when knudsen number is larger than 0.01, the traditional continuum based equation becomes inaccurate. The mean free path of particles in gas of about 1 atm is around 70nm. Consequently, the height should be larger than 7 microns so that the Navier-Stokes equation applies. But for particles in liquid, the mean free path is much smaller than 70nm. As a result, even when the channel height is 1 µm, our analysis still stands.

Figure 2-1. Schematic of the 2D channel
Consider diffusion of a solute in a 2D pressure driven flow in the channel. The convection-diffusion equation for the solute is

\[
\frac{\partial c}{\partial t} + u \frac{\partial c}{\partial x} + u_y \frac{\partial c}{\partial y} = D_\parallel \frac{\partial^2 c}{\partial x^2} + D_\perp \frac{\partial^2 c}{\partial y^2}
\]  

(2-1)

where \( c \) is the solute concentration, \( u \) is the fluid velocity in the axial (x) direction, and \( D_\parallel, D_\perp \) are the diffusion coefficients in the direction parallel and perpendicular to flow, respectively. \( u_y \) is the velocity of the molecules in the lateral direction due to the

electric field and can be estimated by the Smoluchowski equation, \( u_y = \frac{\varepsilon_r \varepsilon_0 \zeta}{\mu} E \), where \( \varepsilon_r, \varepsilon_0 \) and \( \mu \) are the fluid’s dielectric constant and viscosity, respectively, \( \varepsilon_0 \) is the permittivity of vacuum, and \( \zeta \) is the zeta potential. In the limit of large double layer thickness which occurs when the salt concentration is low, the electrophoretic velocity can equivalently be expressed as, \( u_y = -D \frac{Z e}{kT} \frac{\partial \Phi}{\partial y} \), where \( Z \) is the effective charge on the polion, \( \Phi \) is the electric potential, \( k \) is the Boltzmann constant and \( T \) is the temperature. Since DNA is a stable molecule, we do not propose to use any salts in our separations and thus we use the above expression to estimate the electrophoretic velocity. The flow and the lateral electric field are expected to stretch the DNA molecule in the axial direction. Therefore, the strands will have different diffusivities in the x and y directions. Generally, the diffusion coefficient of a cylindrical molecule with a large aspect ratio like a DNA strand in the direction parallel to flow is about twice the diffusion coefficient perpendicular to flow, i.e., \( D_\parallel \approx 2 D_\perp \) [40]. Although the extent of stretching and consequently the
Diffusivity varies across the cross-section due to the difference in shear rate, for simplicity we treat the DNA strands as stretched cylinders at every lateral position. The mobility of the negatively charged DNA molecules will be reduced by the positive counter-ions surrounding the DNA. This electroviscous effect is small for spherical molecules, which are similar to cylindrical molecules in this respect [41], so, we will neglect it in our analysis. We also neglect the shear-induced diffusion and the presence of other charged species such as salts in the solution in the model developed below.

Outside the nm-thin double layer the fluid is electroneutral, and the velocity of charged molecules due to the electric fields in the y direction is constant. Thus, Equation 2-1 becomes

$$\frac{\partial c}{\partial t} + u \frac{\partial c}{\partial x} + u_y \frac{\partial c}{\partial y} = D(R \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2})$$

(2-2)

where $R = D_1/D_{\perp} \approx 2$, and we denote $D_\perp$ as $D$.

The boundary conditions for solving Equation 2-2 are

$$-D \frac{\partial c}{\partial y} + u_y c = 0 \text{ at } y = 0, h.$$  

(2-3)

The boundary conditions (Equation 2-3) are strictly valid only at the wall and not at the outer edge of the double layer, which is the boundary of the domain in which our differential equation is valid. Still, since the double layer is very thin (~ nm), and the time scale for attaining steady state inside the double layer is very short, we neglect the total flux of the DNA molecules from the bulk to the double layer.
From the momentum equation, we get

\[ 0 = -\frac{\partial p}{\partial x} + \mu \frac{\partial^2 u}{\partial y^2} - (\sum Z_i e \xi) \frac{\partial \Phi}{\partial x} \]  

\[ (2-4) \]

Due to electroneutrality in the bulk, the velocity profile remains unaffected by the lateral electric field. Thus, the fluid velocity profile in the axial direction is parabolic, i.e.,

\[ u = 6 <u> \left( \frac{y}{h} - \left( \frac{y}{h} \right)^2 \right) \]  

\[ (2-5) \]

where the mean velocity in the channel is

\[ <u> = \frac{1}{3\mu} \left( -\frac{\partial p}{\partial x} \right) h^2 \]  

\[ (2-6) \]

Here again we neglect the change in the axial velocity across the double layer because it is very thin.

Our model shows that the electric field will affect the mean velocity of the molecules only if the electric field driven velocity in the lateral direction is comparable to the mean velocity, i.e.,

\[ u^e_y = -\frac{DZe \frac{\partial \Phi}{\partial y}}{k_B T} \sim <u> \]  

\[ (2-7) \]

The approximate values of \( D, h \) and \(<u>\) are \( 10^{-9} \text{ m}^2/\text{s}, 10 \mu\text{m} \) and \( 1\text{ mm/s} \), respectively. Using these values in Equation 2-7 and assuming \( Z \sim 1 \), which is a very conservative assumption, gives \( \Delta \Phi \sim \frac{<u> k_B T h}{DZe} = 0.1 \text{V} \). In addition, there will be a potential drop of about a volt in each of the double layers at the wall. We note that we are neglecting adsorption of molecules at the channel walls and the streaming potential that may result because of the charge variation in the double layer. However, streaming
potential alters the mean velocity of all the molecules by the same amount and does not affect the dispersivity.

Our aim is to determine the Taylor dispersion of a pulse of solute introduced into the channel at $t = 0$. In a reference frame moving with a velocity $\overline{u}^*$, the mean velocity of the pulse (comprising pure kind of DNA molecules), Equation 2-2 becomes

$$\frac{\partial c}{\partial t} + (u - \overline{u}^*) \frac{\partial c}{\partial x} + u_y \frac{\partial c}{\partial y} = D \left( \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right)$$

(2-8)

Since we are interested in long-term dispersion, the appropriate time scale is $L/\langle u \rangle$ where $L$ is the total channel length, and $\langle u \rangle$ is the mean fluid velocity. In this time, a pulse will spread to a width of about $l \sim \sqrt{DL/\langle u \rangle}$, which is the appropriate length scale in the $x$ direction. These scales ensure that the convective time scale is comparable to the diffusive time scale in the axial direction. The scaling gives

$$\frac{l^2}{D} = \frac{L}{\langle u \rangle} \Rightarrow \frac{L}{h} = \frac{\langle u \rangle h}{D} \left( \frac{l}{h} \right)^2 \equiv \frac{\langle u \rangle h \; 1}{D \epsilon^2}$$

(2-9)

where $\epsilon \equiv \frac{h}{l} \sim \sqrt{\frac{h}{L}} \ll 1$, since $Pe = \frac{\langle u \rangle h}{D} \sim 1$.

We use the following de-dimensionlization:

$$T = \frac{t}{L/\langle u \rangle}, \quad U = u/\langle u \rangle, \quad \overline{U}^* = \overline{u}^*/\langle u \rangle,$$

$$U_y = u_y/\langle u \rangle, \; C = c/c_0, \; X = x/l, \; Y = y/h$$

(2-10)

where $L$ is the length of the channel; $l$ is the width of the pulse as it exits the channel; $h$ is the height; $\langle u \rangle$ is the average velocity of the flow; and $Pe$ is the Peclet number based on
D = D_. In dimensionless form, Equation 2-8 and the boundary conditions (Equation 2-3) become

\[
\frac{\partial C}{\partial T} + \frac{Pe}{\varepsilon}(U - \bar{U}^*) \frac{\partial C}{\partial X} + \frac{Pe}{\varepsilon^2} \frac{\partial C}{\partial Y} = R \frac{\partial^2 C}{\partial X^2} + \frac{1}{\varepsilon^2} \frac{\partial^2 C}{\partial Y^2} \tag{2-11}
\]

\[-\frac{\partial C}{\partial Y} + PeU_\gamma C = 0 \text{ at } Y = 0, 1. \tag{2-12}\]

We assume a regular expansion for C in \( \varepsilon \),

\[
C = C_0 + C_1 \varepsilon + C_2 \varepsilon^2 + \ldots \quad \tag{2-13}
\]

Substituting the regular expansion for C into Equation 2-11 gives the following sets of equations and boundary conditions to different orders in \( \varepsilon \).

(1/\( \varepsilon^2 \)):

\[
PeU_\gamma \frac{\partial C_0}{\partial Y} = \frac{\partial^2 C_0}{\partial Y^2}; \quad \frac{\partial C_0}{\partial Y} - PeU_\gamma C_0 = 0 \text{ at } Y = 0, 1
\]

\[\Rightarrow \quad C_0 = A(X,T) \exp(PeU_\gamma Y) \tag{2-14}\]

(1/\( \varepsilon \)):

\[
Pe(U - \bar{U}^*) \frac{\partial C_0}{\partial X} + PeU_\gamma \frac{\partial C_1}{\partial Y} = \frac{\partial^2 C_1}{\partial Y^2}; \quad \frac{\partial C_1}{\partial Y} - PeU_\gamma C_1 = 0 \tag{2-15}\]

Substituting \( C_0 \) from Equation 2-14 gives

\[
Pe(U - \bar{U}^*) \frac{\partial A}{\partial X} \exp(PeU_\gamma Y) + PeU_\gamma \frac{\partial C_1}{\partial Y} = \frac{\partial^2 C_1}{\partial Y^2} \tag{2-16}\]

Integrating Equation 2-16 in Y from 0 to 1 gives

\[\int_0^1 U \exp(PeU_\gamma Y) dY = \bar{U}^* \int_0^1 \exp(PeU_\gamma Y) dY \tag{2-17}\]

Equation 2-17 gives the average velocity of pulse.
\[
\bar{U}^* = \frac{6 + 6\exp(\alpha) + 12 - 12\exp(\alpha)}{\alpha(\alpha^2)} \frac{\exp(\alpha) - 1}{(\exp(\alpha) - 1)}
\]  
\hspace{1cm} (2-18)

where

\[
\alpha = \text{Pe}U^e_y
\]  
\hspace{1cm} (2-19)

In Equation 2-16 we assume

\[
C_1 = \frac{\partial^2}{\partial X} G(Y)
\]  
\hspace{1cm} (2-20)

This gives

\[
\text{Pe}(U - \bar{U}^*) \exp(\text{Pe}U^e_y Y) + \text{Pe}U^e_y \frac{\partial G}{\partial Y} = \frac{\partial^2 G}{\partial Y^2}
\]  
\hspace{1cm} (2-21)

Solving Equation 2-21 with boundary conditions gives

\[
G = \text{Pe} \left( \frac{12(e^{-\alpha Y} + \alpha Y)}{\alpha^3 (e^{-\alpha Y} - 1)} + \frac{3Y^2}{\alpha} + \frac{6Y^2}{\alpha^2} - \frac{2Y^3}{\alpha} + \text{const} \right) e^{\alpha Y}
\]  
\hspace{1cm} (2-22)

and the constraint \( \int_0^1 GdY = 0 \) determines the const in Equation 2-22. However, this const does not affect the mean velocity and the dispersion coefficient.

\( \varepsilon^0 \):

\[
\frac{\partial C_0}{\partial T} + \text{Pe}(U - \bar{U}^*) \frac{\partial C_1}{\partial X} + \text{Pe}U^e_y \frac{\partial C_2}{\partial Y} = R \frac{\partial^2 C_0}{\partial X^2} + \frac{\partial^2 C_2}{\partial Y^2} ; \quad \frac{\partial C_2}{\partial Y} - \text{Pe}U^e_y C_2 = 0 \text{ at } Y = 0, 1
\]  
\hspace{1cm} (2-23)

Integrating Equation 2-23, using the boundary conditions and using Equation 2-24

\[
< C_0 > = \int_0^1 C_0 dY = \frac{A}{\text{Pe}U^e_y} [\exp(\text{Pe}U^e_y) - 1]
\]  
\hspace{1cm} (2-24)
gives

\[
\frac{\partial <C_o>}{\partial T} = \frac{\partial^2 <C_o>}{\partial X^2} [R - Pe^2 \frac{U_y^c}{e^{PeUR}} - 1] \int_0^1 (U - U^*)G(Y) dY
\]  

(2-25)

Thus, the dimensionless dispersion coefficient \(D^*\) is

\[
D^* = [R - Pe^2 \frac{U_y^c}{e^{PeUR}} - 1] \int_0^1 (U - U^*)G(Y) dY
\]  

(2-26)

Substituting \(G\) from Equation 2-22 into Equation 2-26 and integrating gives

\[
D^* = R - Pe^2 (720e^a\alpha + 504e^a\alpha^2 - 24e^a\alpha^4 - 144e^a\alpha^3 - 6048e^{2a} - 720e^{2a}a + 24e^{2a}a^2
\]

\[-144e^{2a}\alpha^3 + 72e^{3a}\alpha^2 - 720e^{3a}\alpha + 6048e^a + 2016e^{3a} - 2016 - 720a - 72a^2)/((e^a - 1)^3 \alpha^3)
\]  

(2-27)

**Comparison with Brenner’s Theory**

Howard Brenner used the method of moments to calculate \(\overline{U}^*\) and \(D^*\) in FFF with a shear flow between two infinite plates. To test our method, we solve the same problem with the perturbation method.

According to Brenner, the equation for mean velocity of pulse is

\[
\overline{u}^* = \frac{G'h\gamma(2, \alpha)}{\alpha h(1, \alpha)} \quad \text{and} \quad \overline{U}^* = \frac{\gamma(2, \alpha)}{\alpha h(1, \alpha)}
\]  

(2-28)

where, \(G'\) is the parameter for \(v>G'h\) (\(v(h)\) is the velocity profile for shear flow),

\(\alpha=PeU_y^c\), and

\[
\gamma(n + 1, \alpha) = \int_0^2 \xi^n \exp(-\xi) d\xi
\]  

(2-29)

To obtain the effective diffusivity, one needs to solve Equation 2-30,

\[
\frac{d}{dY} [\exp(-\alpha Y) \frac{dB}{dY}] = \exp(-\alpha Y)(\overline{U}^* - U) \quad \text{(B.C.,} \frac{dB}{dY} = 0 @ Y = 0,1) \]

(2-30)
The result for $D^*$ is:

$$D^* = R + Pe^2 k_\nu(\alpha)$$  \hspace{1cm} (2-31)

$$k_\nu(\alpha) = \frac{\alpha}{1 - e^{-\alpha}} \int_0^1 \hat{B} \exp(-\alpha Y)(U - \bar{U}^*) dY$$  \hspace{1cm} (2-32)

$$\hat{B} = \frac{Y^2}{\alpha} - \frac{2(\alpha^2 - \alpha Y)}{\alpha^2 (e^\alpha - 1)} + \text{const tan t}$$  \hspace{1cm} (2-33)

After considerable simplification

$$k_\nu(\alpha) = \frac{4}{\alpha} \frac{\gamma(2,\alpha)}{\gamma(1,\alpha)} \left[ 2 \frac{\gamma(2,\alpha)}{\gamma(1,\alpha)} + \frac{3 \gamma(3,\alpha)}{\gamma(2,\alpha)} \right]$$  \hspace{1cm} (2-34)

Comparing Equation 2-32 with our result Equation 2-26, we get

$$G(Y) = -Pe \cdot \hat{B} \cdot \exp(-\alpha Y) \Rightarrow \hat{B} = -\frac{G(Y)}{Pe} \exp(\alpha Y)$$  \hspace{1cm} (2-35)

substituting it into Equation 2-30 gives

$$\frac{d}{dY} e^{-\alpha Y} \cdot \left( \frac{\partial}{\partial Y} \frac{e^{\alpha Y}}{Pe} + \frac{G}{Pe} \cdot \alpha \cdot e^{\alpha Y} \right) = e^{-\alpha Y} (U - \bar{U}^*)$$  \hspace{1cm} (2-36)

$$\Rightarrow$$

$$-\alpha \cdot e^{-\alpha Y} \left( \frac{\partial}{\partial Y} \frac{e^{\alpha Y}}{Pe} + \frac{G}{Pe} \cdot \alpha \cdot e^{\alpha Y} \right) + e^{-\alpha Y} \left( \frac{\partial^2 G}{\partial^2 Y} \frac{e^{\alpha Y}}{Pe} + 2 \frac{\partial}{\partial Y} \frac{\partial}{\partial Y} \frac{e^{\alpha Y}}{Pe} + \frac{2}{\partial Y} \frac{G}{Pe} \cdot \alpha \cdot e^{\alpha Y} + \frac{G}{Pe} \cdot \alpha^2 \cdot e^{\alpha Y} \right) = e^{-\alpha Y} (U - \bar{U}^*)$$  \hspace{1cm} (2-37)

Simplifying it gives

$$\frac{\partial^2 G}{\partial^2 Y} + 2 \frac{\partial}{\partial Y} \frac{\partial}{\partial Y} \frac{G}{Pe} \cdot \alpha \cdot e^{\alpha Y} = (U - \bar{U}^*) Pe \cdot e^{-\alpha Y}$$  \hspace{1cm} (2-38)

Comparing Equation 2-38 with Equation 2-21, we find that they are actually the same

(since the direction of lateral velocity in Brenner’s model is opposite to that in ours, $\alpha$
should be replaced by $-\alpha$ when comparing these two results). Thus as expected, the two techniques yield the same results.
CHAPTER 3
RESULTS AND DISCUSSION

Limiting Cases

The dispersion coefficient depends on the Peclet number and \( U_y^e \). In the limit that \( U_y^e \) approaches zero, we expect \( \overline{U}^* \) and \( D^* \) to approach the respective value for a 2D pressure driven flow in a channel without electric field, which are

\[
\overline{U}^* = 1 \quad ; \quad D^* = \frac{1}{210} \text{Pe}^2 \tag{3-1}
\]

\( U_y^e = 0 \) implies \( \alpha = \text{Pe} U_y^e = 0 \). To check whether our results match Equation 3-1, we expand our results for \( D^* \) in the limit of \( \alpha \to 0 \). This gives

\[
D^* = \frac{1}{210} + \frac{1}{1800} \alpha^2 - \frac{89}{1663200} \alpha^4 + O(\alpha^5) \tag{3-2}
\]

To leading order, Equation 3-2 reduces to \( \frac{1}{210} \text{Pe}^2 \), which is the same as Equation 3-1.

Also, we expand Equation 2-27 as \( \alpha \) goes to infinity. The result is

\[
D^* = R + \text{Pe}^2 \left( \frac{72}{\alpha^4} - \frac{720}{\alpha^5} + \frac{2016}{\alpha^6} \right) \tag{3-3}
\]

Figure 3-1 shows the curves from Equation 3-3, 3-2, and 2-27. The asymptotic solutions match the numerical solution if \( \alpha < 2 \) or \( \alpha > 8 \).
Figure 3-1. Dependency of \((D^*-R)/Pe^2\) on the product of Pe and \(U_y^e\). The dotted line is the small \(\alpha\) approximation (Equation 3-2), and the dashed line is the large \(\alpha\) approximation (Equation 3-3).

Similarly the asymptotic behavior of \(U\) in the limits of small and large \(\alpha\) is

\[
U^* = 1 - \frac{1}{60} \alpha^2 + \frac{1}{2520} \alpha^4 - \frac{1}{100800} \alpha^6 + \frac{1}{3991680} \alpha^8 \quad \alpha \to 0 \quad (3-4)
\]

\[
U^* = \frac{6}{\alpha} - \frac{12}{\alpha^2} \quad \alpha \to \infty \quad (3-5)
\]

The result for \(U^*\) in the limit of \(\alpha \to 0\) also reduces to 1 to leading order in \(\alpha\). Figure 3-2 shows the comparison of these asymptotic results and the numerical results from Equation 2-18. The small \(\alpha\) and the large \(\alpha\) results match in the limit of \(\alpha<2\) and \(\alpha>40\), respectively. These asymptotic results help us in understanding the physics of the dispersion and the DNA separation, as discussed below.
Figure 3-2. Dependency of mean velocity $U^e$ on the product of Pe and $U^e_y$. The dotted line is the small $\alpha$ approximation (Equation 3-4), and the dashed line is the large $\alpha$ approximation (Equation 3-5)

**Dependence of the Mean Velocity on $U^e_y$ and Pe**

Figure 3-2 shows the dependence of the mean velocity on $U^e_y$ and Pe. The mean velocity of pulse depends only on the product of $U^e_y$ and Pe. $U^e_y$ changes the mean velocity of the pulse because the presence of an electric field leads to a higher concentration of the charged particles near the wall of the opposite polarity. The lateral concentration profile is a balance of the dimensionless electric flux, which is equal to $\text{Pe} U^e_y c$, and the dimensionless lateral diffusive flux, which is equal to $-\frac{\partial C}{\partial Y}$. Thus, an increase in either Pe or $U^e_y$ leads to an increase in the electric flux that has to be balanced by a larger diffusive flux, which leads to particle buildup in a thinner region near the wall. Since the particles near the wall access streamlines with a smaller velocity than that
in the middle, an increase in $\text{Pe} U^e_y$ reduces the mean velocity of the pulse. As discussed above, in the limit of $\text{Pe} U^e_y$ approaching zero, the mean velocity approaches the mean fluid velocity, i.e., $\overline{U^e} \to 1$.

**Dependence of $D^*$ on $U^e_y$ and Pe**

The effective dispersion coefficient $D^*$ depends separately on $U^e_y$ and $\text{Pe}$. However, $\left(D^* - R\right)/\text{Pe}^2$ depends only on $\alpha$, the product of $U^e_y$ and $\text{Pe}$ (Figure 3-1). At small $\alpha$, with an increase of $\alpha$, the particle concentration near the wall of opposite polarity ($Y = 1$ in our case) begins to increase, and at the same time the particle concentration near $Y = 0$ begins to decrease. However, a significant number of particles still exist near the center. The increase of $\alpha$ results in an average deceleration of the particles (Figure 3-1), but a significant number of particles still travel at the maximum fluid velocity. This results in an increase in $D^*$, and a consequent spread of the pulse. At larger $\alpha$, only very few particles exist near the center as most of the particles are concentrated in a thin layer near the wall, and any further increase in $\alpha$ leads to thinning of this layer. Thus, the maximum velocity of the majority of the particles goes down, resulting in a smaller spread of the pulse. Finally, as $\alpha$ approaches infinity, the mean particle velocity approaches zero, and the dispersion coefficient approaches the molecular diffusivity. Figure 3-1 shows that the maximum value of $\left(D^* - R\right)/\text{Pe}^2$ is about .007. This implies that the convective contribution to dispersion is at most .007 $\text{Pe}^2$. Thus, even at $\text{Pe} = 10$, the convective contribution is only about 35% of the diffusive contribution $R$, which is approximately equal to 2.
Separation of DNA Fragments of Different Lengths

DNA fragments of different lengths have the same $U_y^e$ because the total charge on the molecule is directly proportional to the length (the charge is from the phosphoric structure), and the diffusion coefficient is inversely proportional to the length. Thus, a pure axial field cannot separate DNA molecules in free solution. However, as shown above, in the presence of lateral fields, the mean velocity of the molecules depends on the product of $U_y^e$ and $Pe$, where

$$Pe\ U_y^e = -\frac{<u>\ h\ DZ e \ \partial \Phi}{k_t T_t}\ \frac{\partial \Phi}{\partial y} = -\frac{<u>\ he}{k_t T_t}\ Z\ \frac{\partial \Phi}{\partial y}$$  \hspace{1cm} (3-6)$$

In Equation 3-6, $<u>, T_t, \frac{\partial \Phi}{\partial y}$ and $h$ are fixed for all the DNA molecules. Thus, the product in Equation 3-6 only depends on $Z$, which is directly proportional to the length of the DNA fragments. Since charge $Z$, and consequently $U^e$ and $D^*$ are different for molecules of different sizes, a mixture of DNA fragments of different sizes separates into bands that contain same size DNA molecules, and these bands travel with their mean velocity and disperse as a Gaussian with the dispersion coefficient corresponding to their chain length. Thus, we can separate DNA strands according to their sizes by applying a lateral field instead of an axial field. Figure 3-3 shows the separation of a pulse containing two types of DNA molecules into two individual peaks as the molecules traverse the channel. At $t = 0$, a 1:1 mixture of two types of DNA molecules is introduced as a pulse at the channel entrance. For this simulation, the ratio of the diffusion coefficients of the two types of molecules is 2, and all the other physical
constants are given in the caption. Figure 3-3 shows that, as time progresses, the DNA molecules separate into two separate Gaussian distributions.

\[ u = 1 \text{ mm/s}, \quad D_1 = 1 \times 10^{-9}, \quad D_2 = 2 \times 10^{-9}, \quad U_y = 1, \quad \text{Pe}_1 = 10, \quad h = 10 \, \mu\text{m} \]

**Separation Efficiency**

Consider separation of two types of DNA molecules in a channel. We assume that when the distance between two pulse centers is larger than 3 times of the sum of their half widths, they are separated, i.e.,

\[
|\bar{u}_2 - \bar{u}_1| \geq 3(\sqrt{4D_1D_1't} + \sqrt{4D_2D_2't})
\]

where the subscripts indicate the two different DNA fragments. If the channel is of length L, the time available for separation is the time taken by the faster moving species through the channel, i.e., \( L / \max(\bar{u}^*_1, \bar{u}^*_2) \). Substituting for \( t \), and expressing all the variables in dimensionless form gives
In the discussion below, we used $L/h$ to indicate the efficiency of separation, i.e., smaller $L/h$ implies more efficient separation.

\begin{equation}
L/h \geq 36 \frac{1}{Pe_1} \max(U'_1, U'_2)[\sqrt{D'_1} + \sqrt{D'_2}]^2 \left( \frac{D_2}{D_1} \right)^2
\end{equation}

Figure 3-4. Dependency of $L/h$ on $U_y^e$ and $Pe$ for separation of DNA strands of different sizes. $U_{y1}^e = U_{y2}^e = U_y^e$, $Pe_1 = Pe$, and $Pe_1/Pe_2 = D_2/D_1 = 10$

In Figures 3-4 and 3-5, we show the dependence of $L/h$ on $Pe$ and $U_y^e$ in the case of $U_{y1}^e = U_{y2}^e$, which corresponds to DNA fragments of different lengths. Figure 3-5 is similar to Figure 3-4; the only difference is the ratio $D_2/D_1$. Figure 3-5 shows that increasing $U_y^e$, which is physically equivalent to increasing the electric field, leads to a reduction in $L/h$ required for separation. As $Pe U_y^e$ increases, the mean velocities of both kinds of molecules decrease (Figure 3-1). But the dispersion coefficients do not change
significantly because they are very close to the diffusive value of R for small $\text{Pe} (\text{Pe}<10)$.

Thus, $L/h$ is primarily determined by the ratio $\frac{U_2^*}{\text{Pe}_1} \left( \frac{1}{U_2^* - U_1^*} \right)^2$.

![Figure 3-5. Dependency of $L/h$ on $U_\gamma^e$ and $\text{Pe}$ for separation of DNA strands of different sizes. $U_{\gamma_1}^e = U_{\gamma_2}^e = U_\gamma^e$, $\text{Pe}_1 = \text{Pe}$, and $\text{Pe}_1/\text{Pe}_2 = D_2/D_1 = 2$](image)

As shown earlier, in the small $\alpha$ regime $U^* \sim 1 - \frac{1}{60} \alpha^2$, thus,

$$\frac{U_2^*}{\text{Pe}_1} \left( \frac{1}{U_2^* - U_1^*} \right)^2 \sim \frac{1}{\text{Pe}_1 (U_\gamma^e)^4 (\text{Pe}_2^2 - \text{Pe}_1^2)^2}.$$ Since the ratio $\text{Pe}_2/\text{Pe}_1$ is fixed,

$$\frac{1}{\text{Pe}_1 (U_\gamma^e)^4 (\text{Pe}_2^2 - \text{Pe}_1^2)^2} \sim \text{Pe}_1^{-5} (U_\gamma^e)^{-4}. \quad \text{Thus, an increase in either } \text{Pe} \text{ or } U_\gamma^e \text{ leads to a reduction in } L/h \text{ in the regime of small } \alpha. \quad \text{The constant } \text{Pe} \text{ plots in Figure 3-4 and 3-5 show the } (U_\gamma^e)^{-4} \text{ dependency when } U_\gamma^e \text{ is small. Also, the constant } \text{Pe} \text{ curves shift down}$$
with increasing Pe, due to the Pe\(^{-5}\) dependency shown in the above scaling. On the other side, as shown earlier, in the limit of large \(\alpha\) \(\bar{U}^* \sim 6/\alpha\), thus,

\[
\frac{U_2^*}{Pe_1} \left( \frac{1}{\bar{U}_2^* - \bar{U}_1^*} \right)^2 \sim \frac{U_y^e}{Pe_1 Pe_2} \left( \frac{Pe_1 Pe_2}{Pe_2 - Pe_1} \right)^2 \sim U_y^e.
\]

This implies that in the large \(\alpha\) regime and at \(O(1)\) Pe, \(L/h\) becomes independent of Pe and begins to increase with an increase in \(U_y^e\), as shown in Figure 3-4.

Since \(L/h\) scales as \((U_y^e)^4\) in small \(\alpha\) regime, and as \(U_y^e\) in the large \(\alpha\) regime, it must have a minimum. The minimum in \(L/h\) is clearly visible in Figure 3-5. In Figure 3-4, the minimum occurs for slightly larger \(U_y^e\) than shown in the Figure 3-5. Physically, the minimum arises because at small field strength, the molecules accumulate near the wall, but a finite thickness of the region of accumulation still remains. Since the thickness of the region is different for the two types of molecules, the mean velocities of the two types of molecules differ. However, as the field strength becomes very large, both the mean velocities approach zero, and thus their difference also approaches zero.

Subsequently, the difference in the mean velocities is zero for zero field because both the mean velocities are equal to the fluid velocity, and is also zero at very large fields because both the mean velocities approach zero; this implies that a maximum in the difference between the mean velocities of the two types of molecules must exist at some intermediate field. This maximum combined with other secondary effects results in a minimum in \(L/h\) required for separation.
Figure 3-6. Dependency of $L/h$ on $U_y^e$ and Pe for separation of molecules of the same size but different charges. $U_y^e = U_{y1}^e$, $U_{y2}^e / U_{y1}^e = 10$, and $Pe = Pe_1 = Pe_2$, i.e., $D_2 = D_1$.

The effect of changing $Pe$ while keeping $U_y^e$ fixed is more difficult to understand physically. Due to the dedimensionalization of $U_y^e$, the only way to change $Pe$ while keeping $U_y^e$ fixed is to increase the fluid velocity and the field by the same factor. As a result, if we want to verify the effect of only an increase in the mean velocity $<u>$, we need to increase $Pe$ and concurrently reduce $U_y^e$. Thus, we first move to the smaller $U_y^e$ value and then follow the larger $Pe$ curve. This keeps $Pe U_y^e$ and consequently $D^*$ and $\overline{U}^*$ unchanged, and thus, $L/h \sim 1/Pe$. Physically, this inverse dependency on the mean fluid velocity arises because the dimensional mean velocity of the molecules depends linearly on $<u>$. Thus, an increase in $<u>$ results in a linear increase in the difference
between the mean velocities of the two types of molecules, i.e., $\bar{u}_1^* - \bar{u}_2^*$. The distance between the peaks at the channel exit is independent of $<u>$ because although $\bar{u}_1^* - \bar{u}_2^*$ increases linearly with $<u>$, the time spent by the molecules in the channel varies inversely with $<u>$. However, because $D^*$'s do not change with changes in only $<u>$, the spread of each of the Gaussians decreases with an increase in $<u>$ due to the reduction of time spent in the channel. Consequently, the spread of the peaks becomes smaller making it easier to separate the two types of DNA.

Figure 3-7. Dependency of $L/h$ on $U^e_y$ and Pe for separation of molecules of the same size but different charges. $U^e_y = U^e_{y1}$, $U^e_{y2} / U^e_{y1} = 2$, and Pe=Pe$_1$ = Pe$_2$, i.e., $D_2 = D_1$

In Figure 3-6 and 3-7, we explore the separation for particles having same diffusivity but different charges. By comparing them, we find that under the same Pe and
large $U_{y2}^e / U_{y1}^e$, results in better separation. This result is similar to the effect of an increase in $D_2/D_1$ shown in Figures 3-4 and 3-5. Furthermore, all the trends discussed above for the effect of $Pe$ and $U_y^e$ on $L/h$ for separation shown in Figures 3-4 and 3-5 persist in Figures 3-6 and 3-7 because the arguments presented above are valid even when the Peclet numbers are the same for the two types of molecules and their $U_y^e$ are different. Thus, an increase in $Pe$ for fixed $U_y^e$ reduces $L/h$, and an increase in $U_y^e$ for a fixed $Pe$ first reduces $L/h$ at small $\alpha$ ($\alpha=Pe U_y^e$), and then increases $L/h$ at larger $\alpha$ resulting in a minimum.

**Comparison of Lateral and Axial Electric Field**

As shown above, a lateral field can be used to separate particles in instances in which axial fields are ineffective because the ratio of the charge to the viscous resistance is the same for all the molecules. In this section, we wish to compare the effectiveness of lateral fields with axial fields in cases in which pure axial fields can result separation, i.e., in cases where the ratio of charge to viscous resistance is different for the molecules that need to be separated. As a special case, we consider two kinds of particles with equal and isotropic diffusivities, and $Z_2 = 2 Z_1$. In this case, the two types of molecules have the same Peclet number but different lateral electric velocities: $U_{y2}^e = 2 U_{y1}^e$ and $U_{x2}^e = 2 U_{x1}^e$.

Furthermore, axial electric fields simply alter the mean velocity of the molecules without affecting the dispersion coefficient of molecules, i.e., $\bar{U} = 1 + U_x^e$ and $D^* = 1 + \frac{1}{210} Pe^2$.

We adopt a same definition for the length of the channel needed for separation for the axial fields. Thus, we get
Figure 3-8 plots the L/h required for separation in the above case as a function of Pe and $U_{x1}^e$. The L/h required for separation decreases with an increase in $|U_x|$ because an increase in electric field strength increases the difference between the mean velocities of the two kinds of molecules without affecting their dispersivities. At small $U_x^e$, Equation 3-9 gives $L/h \sim (U_x^e)^{-2}$, and, at large $U_x^e$, it gives $L/h \sim (U_x^e)^{-1}$. Also, at small Pe, $L/h \sim 1/Pe$, and, at large Pe, $L/h \sim Pe$.

![Figure 3-8](image)

Figure 3-8. Dependency of $L/h$ on $U_x^e$ and Pe for an axial field for separation of molecules of the same size but different charges. $Pe = Pe_1 = Pe_2$, $U_x^e = U_{x1}^e$, and $U_{x2}^e / U_{x1}^e = 2$
To compare the axial and the lateral fields, we compute the ratio of the lateral and the axial electric fields that result in the same difference in mean velocity of two molecules with $Z_2 = 2Z_1$. For a pure axial electric field

$$\frac{\partial \Phi}{\partial x} |_{x_1} = \frac{(u_{x_1}^e - u_{x_2}^e)k_1T_1}{D(Z_2 - Z_1)} - \frac{(\bar{u}_1^* - \bar{u}_2^*)k_1T_1}{D(Z_2 - Z_1)}$$

(3-10)

For a pure lateral field

$$\frac{\partial \Phi}{\partial y} |_{y_1} = \frac{u_{y_1}^e k_1T_1}{DZ_1}$$

(3-11)

where $u_{y_1}^e$ is the electric velocity required to obtain the mean velocity difference of $\bar{u}_1^* - \bar{u}_2^*$. This relationship cannot be expressed analytically but is shown graphically in Figure 3-9. Dividing Equation 3-11 by 3-10 gives

$$\frac{\partial \Phi}{\partial y} |_{y_1} \frac{\partial \Phi}{\partial x} |_{x_1} = \frac{(Z_2 - Z_1)u_{y_1}^e}{Z_1(\bar{u}_1^* - \bar{u}_2^*)} = \frac{(Z_2 - Z_1)}{Z_1Pe} \left[ \frac{Peu_{y_1}^e}{(\bar{u}_1^* - \bar{u}_2^*)} \right] = \frac{(Z_2 - Z_1)}{Z_1Pe} \left[ \frac{PeU_{y_1}^e}{(U_1^* - U_2^*)} \right]$$

(3-12)

From Figure 3-9, the minimum value of $\frac{PeU_{y_1}^e}{(U_1^* - U_2^*)}$ is about 14. This means that if $Z_2 = 2Z_1$, in order to achieve the same velocity difference, the ratio of lateral electric field to axial field must be more than $14 \frac{1}{Pe}$. Thus, lateral fields could be more effective than axial fields above $Pe = 14$. Furthermore, at large $Pe$, $D^*$ in lateral fields is smaller than the $D^*$ in axial fields, which further adds to the effectiveness of the lateral fields. However, a lateral electric field always reduces the mean velocity of charged particles making it less than the mean velocity of fluid flow. Therefore, the maximum velocity
difference between different kinds of charged particles is less than the mean velocity of the flow. An axial electric field, on the other hand, does not have this limit. Figure 3-10 shows the dependency of \( \left( \overline{U}_2 - \overline{U}_1 \right) \) on \( U_x^e \) for the axial fields (dash-dot line) and on \( U_y^e \) for the lateral fields (solid lines; each line corresponds to a different \( Pe \)). In the region to the right of the dashed line, the axial fields are more effective. This region corresponds to \( Pe < 14 \) but only for \( \left( \overline{U}_2 - \overline{U}_1 \right) < 0.25 \). Using axial fields is the only way to achieve mean velocity differences larger than 0.25. Further research shows this critical velocity difference will increase with an increase in \( Z_2/Z_1 \) and approach 1 as the ratio approaches infinity. As a result, in certain cases lateral fields could be more effective even in instances where axial fields can also accomplish separation.

![Figure 3-9. Dependency of the difference of mean velocities of pulses of two kinds of molecules \((Z_2=2 \ Z_1, \ Pe_2/Pe_1 = 2)\) on the product of \( Pe = Pe_1=2 \) and \( U_y^e \) \(( U_y^e = U_{y1}^e \)](image-url)
Comparison with Other Promising Methods for DNA Separation

Entropic trapping has been successfully employed to separate a mixture of 24.5 kbp, 48.5 kbp, 73 kbp and 97 kbp DNA fragments in a 1.5 cm long channel in about 40 minutes[42,43]. Our simulations show that the same separation can be accomplished in a 80 µm channel by our proposed method. The operating time is about 1.5 minutes. The parameters for the two methods are listed in Table 3-1. Table 3-1 also shows the comparison of the proposed methods with the technique proposed by Doyle et al. that uses a self assembled matrix of magnetic particles as a sieving media. The authors do not report the channel length in the paper, but the time needed for separation is longer than
that for our proposed techniques. These comparisons show that our technique is promising.

In our model we have not taken into account the entrance and exit effects, and thus the length of the channel required for separation will actually be longer than the results shown in Table 3-1. In this situation, we propose that the electric field is only applied in the fully developed region and thus the mean velocity of the molecules at the entrance and the exit will be the same as the fluid velocity, and the region in between where the fields are applied will contributes to separation. There are other factors that may reduce the separation efficiency of our technique such as the extra dispersion caused by the effect of the walls in the third direction. Also, in our model the thickness of the region in which the molecules reside near the wall of the opposite polarity scales as \( \frac{D}{U_y} \). For the longest DNA molecules, the thickness of this layer is about 10 nm. Clearly, accumulation of molecules in such a thin region will be affected by the double layers surrounding the DNA molecules, and this may impact the separation efficiency. Thus we believe that our current model only serves as a guide in designing the best separation strategy. However, the results of our simulations show that EFFF in microchannel is certainly a promising technique for separating DNA fragments.

<table>
<thead>
<tr>
<th>sample (kbp)</th>
<th>method</th>
<th>Total time (min)</th>
<th>Length of channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,33.5,48.5</td>
<td>Continuous method a)</td>
<td>5</td>
<td>126.2 ( \mu )m</td>
</tr>
<tr>
<td></td>
<td>magnetic</td>
<td>15</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>24.5,48.5,73,97</td>
<td>Continuous method b)</td>
<td>1.5</td>
<td>80 ( \mu )m</td>
</tr>
<tr>
<td></td>
<td>Entropic trapping</td>
<td>40</td>
<td>1.5 cm</td>
</tr>
</tbody>
</table>

a) \( h=1 \ \mu \)m, \( U_y =0.3, <u>=0.1\text{mm/s} \)

b) \( h=1 \ \mu \)m, \( U_y =0.2, <u>=0.1\text{mm/s} \)
CHAPTER 4
DNA SEQUENCING ATTEMPT

To read the genome of an organism, chromosomes, which range in size from 50 million to 250 million bases, are broken into much shorter pieces, about 500 base pairs in length. In the Sanger process, each of the smaller pieces are primed for replication and then added to four beakers, each containing all the four bases A, T, C and G needed for replication. However, in each beaker, a fraction of one type of nucleotides is ‘defective’; the replication stops at these nucleotides. Each replication reaction then proceeds until a reaction-terminating nucleotide is incorporated into the growing strand, whereupon replication stops. Thus, in a beaker containing a ‘defective’ A, the length of the replicated fragments corresponds to location of T. The last step of the sequencing is then separation of these fragments, which differ in length by only one base pair, and this is accomplished by gel electrophoresis.

We therefore seek to determine the efficiency of our system at sequencing DNA. To sequence a fragment N base pair long, we need to separate a mixture of bases of lengths varying from 1 to N. In Figure 4-1 we plot the L/h of a channel required to separate a DNA fragment n bases long from a fragment n+1 bases long. To calculate L/h (n) we use Equation 3-8 with a slight modification; the factor 36 is replaced by 16 because a spacing of two times the sum of the half widths between two Gaussian peaks is enough to identify them as separate peaks, but we need a spacing of about three times to separate them. In Equation 3-8, we identify species 1 as the nth fragment and species 2 as
the (n+1)th fragment. Thus, $D_2 / D_1 = n / (n+1)$ and $Pe_1 = n Pe$, where $Pe$ is the Peclet number for a single nucleotide.

![Graph](image)

Figure 4-1. $L/h$ required to separate DNA fragments that differ in length by a single base pair vs. the number of base pairs in the smaller fragment. $h = 1\mu m$, $Pe = 1$, $U_y^e = 0.4$. The largest value of $L/h$ represents the size of the channel required for separation. The dashed line is calculated from the large $\alpha$ approximations.

Figure 4-1 plots $L/h$ required for separation as a function of the maximum number of the base pairs $n$ for fixed $Pe$ and $U_y^e$. For a given $Pe$ and $U_y^e$ ($h = 1\mu m$, $Pe = 1$, $U_y^e = 0.4$), $L/h$ first decreases because of an increase in $Pe_1$ and then begins to increase because the effect of an increase in $D_2/D_1$ dominates over the effect of an increase in $Pe$. Since the Peclet number will proportionally increase with the increase in the number of bases, it is easy to reach large values of the product of the Peclet number and $U_y^e$.

Usually separation of the two largest fragments, i.e., 500 and 499 base pair long
fragments, is the hardest. Therefore, to optimize the sequencing, we will first focus in the large $\alpha$ regime. Using the large $\alpha$ approximations gives $L/h \sim n^2 U_y^e$ (In this limit $D^*$ is equal to $D$). The dashed curve in Figure 4-1 is the large Pe asymptotic result. Plotting the $L/h$ vs $U_y^e$ on log-log axes gives a slope of 2, which agrees with above analysis. The time required for separation of fragments in DNA sequencing will be $L/\bar{U}'(n)$, where $\bar{U}'(n)$ is the mean velocity of the slowest moving, i.e., the longest, DNA fragment.

Large $\alpha$ approximation gives $T \sim n^3 \text{Pe} (U_y^e)^2$. Thus, one could accomplish faster separation by reducing Pe and $U_y^e$. However, these expressions are based on large $\alpha$ approximation, so Pe and $U_y^e$ cannot be made too small. In fact, if Pe $U_y^e$ is too small, the diffusivity of the DNA molecules begins to increase (Figure 3-2), resulting in long operating time and large required channel length. Specifically when both Pe and $U_y^e$ are reduced, the length needed to separate the first several base pairs increases dramatically because the difference in velocities between the first few fragments becomes small. Therefore, to get the best performance, we need a large Pe to limit the diffusion of large molecules (according to Equation 3-3). However, we cannot make Pe arbitrarily large because doing so will increase the pressure required to pump the fluid. Note that making Pe large by increasing $h$ will also increase $L$. Therefore, we essentially have an optimization problem in which we need to minimize $L$, $t$ and $P$ by manipulating $<u>$, $h$ and $U_y^e$. To guide us in this optimization we use the large $\alpha$ results, i.e.,

$$L/h \sim n^2 U_y^e \quad (3-13)$$

$$t \sim n^3 U_y^e^2 h^2 \quad (3-14)$$
Furthermore, for 2D Poiseuille flow

\[ p \sim \frac{\mu \langle u \rangle L}{h^2} \]  

(3-15)

According to Equation 3-15, we choose a relatively large h to provide large \( \langle u \rangle \) and Pe. At the same time, we reduce \( U^e_y \) to balance the effect on L and t of increasing h. After h and \( U^e_y \) are fixed, we choose a Pe that is large enough to control the diffusivity. Note that we use the large \( \alpha \) approximation only as a guide, and use the appropriate equations to finally determine L/h and t. We finally conclude that 1-500 base pairs can be separated in a 0.72-meter long, 10-micron thick channel in 1.7 hrs at Pe = 200 and \( U^e_y = 0.01 \). Figure 4-2 shows this result.

Figure 4-2. Length and Time required to separate DNA fragments that differ in length by a single base pair vs. the number of base pairs in the smaller fragment. h = 10\( \mu \)m, Pe = 200, \( U^e_y = 0.01 \), \( \langle u \rangle = 0.02 \)m/s
The length of the channel is about 0.72m, which is too long for fabrication on a chip. However, one could potentially fabricate about 36 parallel channels, each about 2 cm long, and join them at the ends to fabricate a channel with straight segments joined by curved ends. In such a channel, we need to evaluate the extra dispersion caused by the curved ends.
CHAPTER 5
CONCLUSIONS

Application of lateral fields affects the mean velocity and the dispersion coefficient of colloidal particles undergoing Poiseuille flow in a 2D channel. The dimensionless mean velocity \( \overline{U}^* \) depends on the product of the lateral velocity due to electric field

\[
U^*_y = \frac{Dze}{<u>_kT_I} \frac{\partial \Phi}{\partial y}
\]

and the Peclet number. The convective contribution to the dispersion coefficient is of the form \( \text{Pe}^2 f(\text{Pe}U^*_y) \). The mean velocity of the particles decreases monotonically with an increase in \( U^*_y \text{Pe} \), but \( (D^* - R)/\text{Pe}^2 \) has a maximum at a value of \( U^*_y \text{Pe} \sim 4 \). This maximum arises when the thickness of the region near the wall where a majority of the particles accumulate is about \( h/2 \).

Since the mean velocity of the particles under a lateral field depends on the charge \( Z \) but not on the product of the diffusion coefficient and the charge, colloidal particles such as DNA molecules that have the same ratio of charge to viscous resistance can be separated on the basis of their lengths on a chip by applying lateral electric fields. Axial fields cannot accomplish this separation unless the channel is packed with a gel. Thus, our proposed strategy of accomplishing separation by lateral fields may offer a solution to separating DNA on a chip. The length of the channel required for separation depends on the ratio of the diffusion coefficients of the two types of molecules that need to be separated and on the Pe and \( U^*_y \). Lateral fields can also be used to separate molecules that can already be effectively separated by purely axial fields, and, in certain instances
such as large Pe, the lateral fields require smaller field strengths than the axial fields. However, lateral fields are limited by the fact that the maximum difference in the mean velocity of two types of molecules that need to be separated is less than the mean velocity of the fluid. Axial fields do not suffer from this limitation.

Lateral fields can also be used for sequencing DNA on a chip. The separation step in DNA sequencing requires separating a mixture of DNA fragments that range in size from 1 to 500 base pairs and differ in length by a single base pair. Lateral fields can accomplish such a high-resolution separation in channels that are about 0.72m long. With current micro lithographic techniques, such a channel can be incorporated into a chip by fabricating about 36 parallel channels, each 2 cm long, and eventually joining them to acquire a single channel. In such a channel we will need to consider the extra dispersion caused by the curves that link two successive straight channels.

While analyzing the dispersion of molecules under lateral electric field, we assumed that the fluid away from the double layer is electroneutral, that the electric field is constant in the bulk of the fluid, and that DNA molecules are fully charged and aligned parallel to the flow. Also, we neglected the double layer at the electrodes, the interaction of the molecules with the wall, and the reduction in mobility due to the counterion cloud surrounding the charged colloidal particles. We also presented our analysis for a 2D channel, where the presence of walls has demonstrated a first order effect on the dispersion of molecules in Poiseuille flow in a channel in the absence of electric fields. In addition, the flow of current in the lateral direction will result in generation of oxygen and carbon dioxide that may affect the hydrodynamics of the flow. The DNA molecules may also be affected and possibly damaged by the high lateral fields. Thus, while our
proposed separation technique is promising, more experimental and theoretical work needs to be done to determine the effectiveness of lateral fields in accomplishing separation of DNA on a chip.
APPENDIX
NOMENCLATURES

A Variable defined in Equation 2-14

c Concentration of a kind of particle in fluid

D Molecular diffusion coefficient

D* Dimensionless effective diffusion coefficient

G Variable defined in Equation 2-20

h Height of the channel

k thermal constant

k_v Function defined in Equation 2-31

l Width of the pulse as it exits the channel

L Length of the channel

P Pressure

Pe Peclet number, <u>h/D

R Ratio of $D_1$ to $D_2$

t Time

T Dimensionless time

T_1 Temperature

u Velocity of flow at a certain position and time

u̅ Mean velocity of a pulse consisting of one kind of particle

<u> Mean velocity of flow
U  Dimensionless velocity of flow

\bar{U}^*  Dimensionless \bar{u}^*

u_x^e  Velocity of a charged particle in the x-direction due to electric field

U_x^e  Dimensionless u_x^e

u_y^e  Velocity of a charged particle in the y-direction due to a lateral electric field

U_y^e  Dimensionless u_y^e

x  Position on the x-axis

X  Dimensionless position in the x-axis

y  Position on the y-axis

Y  Dimensionless position in the y-axis

Z  Number of charges of a particle

\alpha  Variable defined in Equation 2-19

\gamma  Function defined in Equation 2-29

\Phi  Intensity of an electric field

\varepsilon  Perturbation, the ratio of h to l, or the square root of h/L

\mu  Viscosity of the fluid
LIST OF REFERENCES


38. Stellwagen NC, Gelfi C, Righetti PG. The use of gel and capillary electrophoresis to investigate some of the fundamental physical properties of DNA. Electrophoresis 2002; 23: 167-175.


BIographieshical Sketch

I was born in Apr 23, 1976 in He Zhang, a small town in Gui Zhou province, China. My father is a teacher of physics in a high school and my mother is a doctor. I established strong interest in science since childhood due to the intellectual surrounding provided by my family. My wide region of reading earned me honors in various competitions of high school. With competitive scores in the National Entrance Examination, I was admitted by the most prestigious university of China—Tsinghua University. I urged myself in my undergraduate study in Tsinghua University, took five-year courses in four years and got high scores in most courses. As a result, I graduated one year earlier than my peers, ranking top 5% in my department of 120 students and entered the graduate program of Biochemical Engineering in 1998, waived of the entrance examination. In graduate stage, I ranked 10% in my class.

During seven years in Tsinghua University, I participated in several projects. In my undergraduate diploma project, I studied the measurement of solubility of sodium sulfate in supercritical fluid, which is a part of the research of SuperCritical Water Oxidation (SCWO), a promising method for dealing with wastewater. Deeply absorbed in this wonderful supercritical world, I searched the literature extensively, discussed with professors, and did experiments carefully. Finally, I got satisfactory results, and my diploma got a high score—92/100.

In 1999, I took part in a project to undertake middle-scaled amplification of the production of PHB (poly-β-hydroxybutyrate, a kind of biodegradable plastic) with
E.Coli., which was a part of a Ninth Five-year National Key Project of China. Under my active and successful participation, we found and eliminated the scattering of nitrogen during sterilization and improved the distribution of air input. The density of bacteria reached 120g/l and the production of PHB extended to 80g/l, far beyond the original goal. The amplification succeeded and won me the honor of the first prize of outstanding performance in field practice of my department.

After the practice, I began my thesis work under the guidance of Prof. Zhongyao Shen, the Vice-Dean of School of Life Sciences and Engineering in Tsinghua University. My work focused on the coupling of fermentation and separation. In the first year, I applied the coupling of fermentation and ion exchange on the production of 2-Keto Gulonic Acid, the direct precursor of Vitamin C. However, this research was abandoned because an impossibility coming from the fermentation system. After that, my main interest was on the coupling of fermentation and membrane separation in the production of acrylamide from acrylotrile. During the process, I acquire insights on membrane, fermentation, ion exchange, and operation of analytical equipment. Finally, I got a high enzyme activity from the fermentation, which is the highest value on documents.

After I graduated from Tsinghua University in 2001, I came to the Department of Chemical Engineering, University of Florida to pursue advanced education. My research focuses on separation processes with microchannel and electric fields. And we have already got some promising results. This thesis is some of my research results. After I get master degree, I will further my work on this project, such as doing experiments to testify our theoretical results in this thesis.

Below is a list of my published papers.


4. Sun Xudong, Chen Zhi, Shi Yue, et al. Studies on Bioprocess and Bioreactors Used in Bioconversion for Acrylamide. CHEMICAL INDUSTRY AND ENGINEERING PROGRESS, 2002 Vol.21 No.5, p319