Development of a microfluidic capture device for the manipulation and concentration of waterborne pathogens

by

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Abstract

Severe epidemics in North America due to consumption of polluted water containing pathogens such as *Cryptosporidium parvum* and *Girdia* led to the development of standard detection methods like USEPA 1623 (by US Environmental Protection Agency). USEPA 1623 method, although fairly practical, is still at its infancy. There are still questions and ambiguity about the precision and accuracy of this method for the separation and detection of pathogens.

USEPA 1623 includes a series of lengthy unreliable procedures that are highly prone to failure. The main purpose of this study is to examine the feasibility of developing alternative methods to improve pathogen recovery. The proposed method includes a microfluidic flow cell which implements physical properties of the pathogens (e.g., mass and electric charge) to manipulate, separate and concentrate them for future detection step. In general, the proposed device includes a flow path with an arbitrary shape through which water sample is flushed. The device then separates and concentrates the pathogens at the proximity of the “capture sites” where they are trapped by complementary antibody molecules.

In order to design such a device, first, continuum numerical simulation is used to determine the optimum device geometry from among the many possible configurations. In addition to the device geometry, the effect of two mechanisms, sedimentation and electrophoresis, related to the physical properties of the pathogen is numerically studied using continuum simulation to enhance the pathogen separation process. Discrete numerical simulation is implemented afterwards to achieve a clear understanding of physical phenomena occurring at the molecular level close to the capture sites. The results obtained from the numerical modeling are used to fabricate a microfluidic test cell. The test cell is then examined in terms of pathogen separation and capture performance, and the experimental results are compared with those obtained by the modeling. It is shown that the proposed capture method has a better recovery with a higher precision when used along with the sedimentation process. Electrophoresis, however, introduces new challenges to the problem which necessitates further investigation of the
method. Some of the most important challenges are discussed, and the potential remedies are suggested.
Preface

This research was conducted in the Advanced Thermo-Fluidic Laboratory and the Biological Solution Laboratory under the supervision of Dr. Mina Hoorfar and Dr. Deborah June Roberts. The results of this research have been published in several peer-reviewed journal articles and conference proceedings.


A version of Chapter 3 and Appendix A has been published in a journal article (S. Jomeh and M. Hoorfar, "Study of the effect of electric field and electroneutrality on transport of biomolecules in microreactors," Microfluid. Nanofluid., vol. 12, pp. 279-294, 2012) and a conference proceeding (S. Jomeh and M. Hoorfar, "Study of the effect of electrophoresis on transport of biomolecules in microreactors," Proceedings of ASME 2011 9th International Conference on Nanochannels, Microchannels, and Minichannels, Edmonton, AB, 2011). I conducted all the numerical simulations and wrote the manuscript.


Parts of the modeling described in Chapters 2 and 3, and the experimental results in Chapter 5 have been presented in a conference. S. Jomeh, D. J. Roberts and M. Hoorfar, “Development of

Chapter 5 is based on experimental work conducted in Biological Solution Laboratory, Advanced Thermo-fluidic Laboratory and Dr. C. Eskicioglu’s research laboratory by Sina Jomeh and Tasrif Rahman. I was responsible for designing all the experiments and running majority of the tests. Tasrif Rahman collaborated in conducting some of the experimental runs.
# Table of Contents

Abstract ........................................................................................................................................... ii

Preface ............................................................................................................................................ iv

List of Tables .................................................................................................................................... x

List of Figures ................................................................................................................................... xi

List of Symbols ................................................................................................................................. xvii

Greek symbols ..................................................................................................................................... xix

Acknowledgements ........................................................................................................................ xxi

Dedication .......................................................................................................................................... xxii

1 Introduction ...................................................................................................................................... 1

1.1 EPA 1623 method ...................................................................................................................... 2

1.2 Research objective .................................................................................................................... 4

1.3 Proposed separation/detection method ..................................................................................... 5

1.4 Review of relevant literature ..................................................................................................... 9

1.4.1 Pathogen cell adhesion ......................................................................................................... 9

1.4.2 Pathogen cell transport ......................................................................................................... 14

1.4.3 Pathogen physical separation techniques ............................................................................. 20

1.5 Research methodology ............................................................................................................. 24

2 Numerical modeling of particle transport: passive separation ................................................. 27

2.1 Theory of continuum medium transport ................................................................................ 28
3.4 Conclusions........................................................................................................................................... 74

4 Discrete numerical modeling of particle adhesion ................................................................................. 76

4.1 Theory of cell adhesion coupled with flow hydrodynamics ................................................................. 77

4.1.1 Cell adhesion....................................................................................................................................... 77

4.1.2 Fluid flow and cell deformation ......................................................................................................... 78

4.2 Method.................................................................................................................................................. 79

4.3 Computer program specifications .......................................................................................................... 83

4.4 Validation ............................................................................................................................................. 84

4.5 Results and discussion............................................................................................................................ 88

4.5.1 Cell adhesion: general patterns ......................................................................................................... 88

4.5.2 Effect of bending rigidity..................................................................................................................... 95

4.5.3 Effect of cytoplasm viscosity .............................................................................................................. 97

4.6 Adhesion design parameters for Cryptosporidium capture ................................................................. 98

4.7 Conclusions ......................................................................................................................................... 99

5 Particle transport and capture in the microfluidic capture device ......................................................... 101

5.1 Sedimentation: experimental study ...................................................................................................... 101

5.1.1 Materials and method ....................................................................................................................... 101

5.1.2 Experimental results and discussion ............................................................................................... 105

5.2 Electrophoresis: experimental study .................................................................................................... 110

5.2.1 Materials and method ....................................................................................................................... 110

5.2.2 Experimental results and discussion ............................................................................................... 115

5.2.3 Challenges and troubleshooting .................................................................................................... 125

5.3 Conclusions ......................................................................................................................................... 133

6 Conclusions and future work .................................................................................................................. 135
6.1 Conclusions......................................................................................................................... 135
6.2 Proposed future work ........................................................................................................... 138

Bibliography.................................................................................................................................. 139

Appendix A. Effect of electroneutrality assumption ................................................................. 148
List of Tables

Table 2.1. Numerical values of the parameters used for the simulations in Figs. 2.6-2.8 (taken from [11, 21]). ................................................................. 41
Table 3.1. Numerical values of the parameters used in the particle electrophoresis simulations ........................................................................ 70
Table 4.1. Dimensionless parameters of cell hydrodynamics and adhesion [26] ................................ 89
Table 5.1. Oocyst mobility measurements for different pH and conductivity values as reported by Particle Characterization Laboratories, Inc. (Novato, US) .......... 118
Table 5.2. Polystyrene microspheres mobility as reported by Particle Characterization Laboratories, Inc. (Novato, US) ........................................ 125
Table A.1. Values of the parameters used in the simulations for non-electroneutral condition .................................................................................. 161
List of Figures

Fig. 1.1. Schematics of the separation and capture mechanisms that pathogen cell undergoes in the microfluidic flow cell device: a) overall view and b) zoomed-in view near the capture element. ................................................................. 7

Fig. 1.2. Schematics of a field flow fractionation (FFF) method......................................................... 21

Fig. 2.1. (a) Rectangular microchannel with the reactive side at the bottom. The channel is 10 μm wide and 500 μm long. Part of the channel close to the inlet is shown here. Surface and contour plots of concentration are presented, (b) Comparison of the numerical results with the entrance and fully-developed analytical solutions for $Pe = 50$ and $Da \to \infty$........................................... 35

Fig. 2.2. Validation of the numerical model: (a) rectangular microchannel with the reactive plate shown in red and (b) the fit between the experimental data (green circles) obtained for 29 basis pairs of DNA strands and the numerical results (red solid line). .................................................................................. 37

Fig. 2.3. Geometries of three microreactors: (a) parallel-plate open channel (PP) in which top and bottom surfaces are reactive, (b) circular micropillars (CM) in which cylinder surfaces are reactive and (c) screen plates (SP) in which only front sides of the plates are reactive................................................................. 39

Fig. 2.4. View of the mesh in: (a) parallel-plate open channel, (b) circular micropillars and (c) screen plates. ......................................................................................................................... 43

Fig. 2.5. Streamlines in: (a) circular micropillars and (b) screen plates for $Re = 10^{-3}$. Edge effects are observed at the outlet far from the reactive obstacles. .............................................................................................................. 44

Fig. 2.6. Concentration plot for the parallel-plate channel: (a) in the bulk and (b) on the reactive surface. The small overshoots happen due to the edge effect.......................................................... 45

Fig. 2.7. Concentration plots for circular micropillar design: (a) in the bulk and (b)-(k) on the reactive surfaces for $Pe = 100$, $Da = 1000$, $KD =$
0.1 and \( \varepsilon = 0.01 \). Each reactive surface is a quarter of a circle numbered in Fig. 2.7a. \( x \) is measured on the perimeter of each quarter circle as shown in Fig. 2.7a.

Fig. 2.8. Concentration plots for screen plates: (a) in the bulk and (b)-(f) on the reactive surfaces (as numbered in Fig. 2.8a) for \( Pe = 100, Da = 1000, KD = 0.1 \) and \( \varepsilon = 0.01 \). Only front sides are reactive.

Fig. 2.9. Capture efficiency versus Peclet number for different designs with reactive length = 470 \( \mu \text{m} \), \( Da = 1000, KD = 0.1 \) and \( \varepsilon = 0.01 \).

Fig. 2.10. Capture efficiency of screen plates versus \( Da \) number for different \( Pe \) numbers (\( KD = 0.1 \) and \( \varepsilon = 0.01 \)).

Fig. 2.11. Nondimensional average surface concentration versus number of reactive units for \( Pe = 100, Da = 1000, KD = 0.1 \) and \( \varepsilon = 0.01 \). The single unit shown on the top right side of the plot includes two plates reactive on the front side.

Fig. 2.12. Capture efficiency versus length of the reactive surface for the single-sided (SS) and double-sided (DS) devices for different Peclet numbers (\( Da = 1000, KD = 0.1 \) and \( \varepsilon = 0.01 \)).

Fig. 3.1. Schematic view of two main forces applied on a particle in a gravitational field.

Fig. 3.2. Simulation results for sedimentation of solid particles inside a microchannel. \( \phi_{d, \text{inlet}} = 10^{-8} \), \( u_{in, \text{max}} = 1.25 \mu \text{m/sec} \), \( \rho_d - \rho_f = 50 \text{ kg/m}^3 \). Red colour shows the high dispersed-phase volume fraction.

Fig. 3.3. Schematic view of three main forces applied on a negatively-charged particle in both electric and gravitational field for a) a horizontal device, and b) a vertical device.

Fig. 3.4. Schematic view of different layers in an electric double layer around a charged particle and definition of zeta potential.
Fig. 3.5. Simulation results for the effect of electric field on particle separation. Maximum inlet velocity is 150 $\mu m/sec$ and the applied potential difference is 0.5 $V$. .......................................................................................................................... 71

Fig. 3.6. Concentration plots of bioparticle separation after 300 sec using a) sedimentation and b) electrophoresis (with negligible diffusion and $\Delta V = 5 mV$). Maximum inlet velocity is 1.25 $\mu m/sec$ in both cases. The results indicate that electrophoresis and sedimentation have similar effect on transporting the bioparticles................................................................. 74

Fig. 4.1. SPH particle representation of the domain including all five types of particles: 1) solid boundaries (cyan), 2) buffer (green), 3) cytoplasm (yellow), 4) membrane (red) and 5) periodic inlet and outlet particles (blue). .......................................................................................................................... 82

Fig. 4.2. Schematic of extensional and bending springs at the cell membrane....................... 83

Fig. 4.3. Snapshots of cell motion in the middle of a microchannel with $\eta_r = 1$, $a = 2 \mu m$ and $v_{max} = 1 cm/sec$ at a) $t^* = 0$, b) $t^* = 1$, c) $t^* = 2$, d) $t^* = 3$ and e) $t^* = 4$. .......................................................................................................................... 87

Fig. 4.4. Two snapshots of cell motion vertically displaced from the center of the channel at $t^* = 0$ and $t^* = 4$. Fluid SPH particles are removed from the plot. .......................................................................................................................... 88

Fig. 4.5. Snapshots of cell adhesion with $\gamma = 400 sec^{-1}, \alpha = 9, F_\sigma = 0.3, \kappa = 10^6, \beta = 2.5 \times 10^{-7}, \chi = 0.25, \eta_r = 1$ and $\theta = 2 \times 10^{-14}$ at a) $t^* = t\gamma = 0$, b) $t^* = 1$, c) $t^* = 2$, d) $t^* = 3$ and e) $t^* = 4$. X and Y are the coordinates. For simplicity only SPH membrane particles are shown. f) Snapshot of a Jurkat cell adhered to an EI monolayer under the shear rate of 257.7 sec$^{-1}$ (Reprinted with permission from [93]). ........................................ 92

Fig. 4.6. Comparison of cell adhesion at two different flow rates $\gamma = 400$ and 4000 sec$^{-1}$. Other parameters are the same as Fig. 4.5.......................................................... 93

Fig. 4.7. Comparison of cell adhesion for two different ligand densities at $t^* = 7$ for $\gamma = 4000 sec^{-1}$ and $\theta = 2 \times 10^{-13}$. All other parameters are the
same as Fig. 4.5. The cell initial position at $t^* = 0$ is also displayed in black. The small disconnection in the membrane line demonstrates the rotation of the cell while moving away from its initial position.

Fig. 4.8. Cell adhesion patterns at $t^* = 4$ for $\gamma = 400 \text{ sec}^{-1}$ with a) $\theta = 2 \times 10^{-15}$, b) $\theta = 2 \times 10^{-14}$ and c) $\theta = 2 \times 10^{-13}$. Other parameters are $\alpha = 9, F_s = 0.3, \kappa = 106, \beta = 2.5 \times 10^{-7}, \chi = 0.25$ and $\eta_r = 1$.

Fig. 4.9. Effect of increasing cytoplasm viscosity on cell adhesion ($t^* = 4$, $\gamma = 400 \text{ sec}^{-1}$). All other parameters are the same as Fig. 4.8.

Fig. 5.1. Experimental flow cell device for sedimentation test: a) a schematic, b) fabricated parts and c) the assembled device.

Fig. 5.2. Flowcharts of the two versions of the experimental setup used for sedimentation experiment.

Fig. 5.3. Concentration of Cryptosporidium oocysts in the effluent for different flow rates. Nominal inlet concentration was 500 oocysts/ml.

Fig. 5.4. Accumulative number of oocysts versus time for horizontal (diamonds) and vertical (squares) flow cell devices at the 1 ml/hr inlet flow rate. The initial plateau is observed due to the void volume of the device, and it determines the time it takes for the particles to enter the detector instrument.

Fig. 5.5. Two versions of the electrophoretic microfluidic device: a) steel-gold and b) gold-gold electrode combination.

Fig. 5.6. Red polystyrene microspheres (Bangs Laboratory Inc.) inside the fabricated microfluidic separator. The background is the surface of the steel.

Fig. 5.7. Experimental setup for electrophoresis analysis.

Fig. 5.8. Schematic view of particle accumulation on oppositely-charged reactive electrodes; a) power supply off, b) power supply on.
Fig. 5.9. Electric current versus time for the steel-gold electrode configuration for $pH \approx 8$ and $\sigma \approx 1400 \mu S/cm$. Steady state current is rather small (almost 5 $\mu A$) compared to the literature [75].

Fig. 5.10. Representative output graph of instantaneous number of particles (exiting the device) versus time obtained by flow microscopy (Brightwell Technologies Inc.). Each number is counted in a 0.1-minute time interval. Flow rate is 1 $ml/hr$.

Fig. 5.11. Average of five replicates for zero and nonzero electric potential runs. Flow rate is 1 $ml/hr$, $pH = 7.8$ and $\sigma = 1340 \mu S/cm$. The applied voltage is 1.2 $V$. The initial plateau is observed due to the void volume of the device, and it determines the time it takes for the particles to enter the detector instrument.

Fig. 5.12. Voltage drop at the EDLs close to the electrodes.

Fig. 5.13. Gold oxidation and slide electroplating at a large voltage differences (2 $V$). Back of the slide is shown.

Fig. 5.14. Electrophoretic mobility versus $pH$ and conductivity; a) 3D view, b) mobility-$pH$ view and c) mobility-conductivity view.

Fig. 5.15. Effect of $pH$ on mobility for constant conductivity (200 $\mu S/cm$).

Fig. 5.16. Effect of Tween on electrophoretic mobility of the oocysts.

Fig. A.1. Space charge density for a charged particle inside a symmetric solution.

Fig. A.2. a) Schematic of the microchip used by Kassegne et al. [31]. Anode diameter is 80 microns. Cathode ring is 40 microns wide. b) Comparison of the experimental data with numerical simulation. c) Error percentage of the two cases (zero and nonzero space charge density).

Fig. A.3. Electric potential contours for the cases of a) zero and b) nonzero space charge density.

Fig. A.4. Open channel parallel plate (PP) in which top and bottom surfaces are reactive.
Fig. A.5. Sample bulk concentration in PP a) without and b) with the electric field (zero space charge density) at $t = 60 \text{ sec.}$ ................................................................. 162

Fig. A.6. Sample adsorbed surface concentration at the top and bottom surfaces a) without and b) with the electric field at $t = 60 \text{ sec.}$ ................................................................. 163

Fig. A.7. Electric potential contours for a) zero and b) nonzero space charge density .......................................................................................................................... 164

Fig. A.8. Electrophoretic flux with a) zero and b) nonzero space charge density (arrows show the relative magnitude and direction of the electrophoretic flux) at $t = 60 \text{ sec.}$ ........................................................................................................ 166

Fig. A.9. Sample bulk concentration with the nonzero space charge density at $t = 60 \text{ sec.}$ .......................................................................................................................... 167

Fig. A.10. Sample surface concentration a) zero space charge density and b) nonzero space charge density. ........................................................................................................ 168

Fig. A.11. Capture efficiency comparison at different cross sections for zero and nonzero charge density .................................................................................................................................. 169

Fig. A.12. Surface occupation of binding sites with respect to time at the a) top surface and b) bottom surface for no electric field, zero charge density and nonzero charge density. Ligand concentration is $5 \times 10^{-8} \text{ mol/m}^2$. ......................... 170

Fig. A.13. Capture efficiency versus $\Lambda$ for zero and nonzero space charge density. .............. 171

Fig. A.14. Surface concentration for different ion concentrations in buffer solution ................. 172

Fig. A.15. Velocity field of the buffer with nonzero charge density ............................................. 173

Fig. A.16. Surface plot of the force ratio in the ranges of $0.01 < F_R/F_E < 0.1$. ............................. 175
## List of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>Particle radius ($m$)</td>
</tr>
<tr>
<td>$A_c$</td>
<td>Cross-sectional area ($m^2$)</td>
</tr>
<tr>
<td>$c$</td>
<td>Sample bulk concentration ($mol.m^{-3}$)</td>
</tr>
<tr>
<td>$c_o$</td>
<td>Inlet bulk concentration ($mol.m^{-3}$)</td>
</tr>
<tr>
<td>$c_d$</td>
<td>Dimensionless dispersed phase mass fraction</td>
</tr>
<tr>
<td>$CE$</td>
<td>Capture efficiency</td>
</tr>
<tr>
<td>$c_s$</td>
<td>Sample surface concentration ($mol.m^{-2}$)</td>
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<tr>
<td>$c_{s0}$</td>
<td>Ligand surface concentration ($mol.m^{-2}$)</td>
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<td>$c_{s,avg}$</td>
<td>Average surface concentration ($mol.m^{-2}$)</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion coefficient ($m^2.sec^{-1}$)</td>
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<td>$E$</td>
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<tr>
<td>$F_b$</td>
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<td>Bond force ($N$)</td>
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<td>$h$</td>
<td>Channel height ($m$)</td>
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<td>$k_b$</td>
<td>Boltzmann constant ($m^2.kg.s^{-2}.K^{-1}$)</td>
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<tr>
<td>$k_{on}$</td>
<td>Forward reaction rate ($m^3.mol^{-1}.sec^{-1}$)</td>
</tr>
<tr>
<td>$k_{off}$</td>
<td>Backward reaction rate ($sec^{-1}$)</td>
</tr>
<tr>
<td>$l$</td>
<td>Reactive length ($m$)</td>
</tr>
</tbody>
</table>
$l_{unstretched}$ Bond length at equilibrium (m)

$l_{stretched}$ Stretched bond/microvillus length (m)

$n$ Normal vector

$N_b$ Number of chemical bonds

$N_l$ Ligand density (molec.m$^{-2}$)

$N_r$ Receptor density (molec.m$^{-2}$)

$p$ Pressure (Pa)

$Q$ Electric charge (C)

$R$ Recovery

$t$ Time (sec)

$T$ Temperature (K)

$u$ Velocity vector (m.sec$^{-1}$)

$u_{avg}$ Average velocity (m.sec$^{-1}$)

$u_{slip}$ Relative velocity between dispersed phase and continuous phase

$V$ Electric potential (V)
Greek symbols

\( \varepsilon_0 \) Electric constant \((C.V^{-1}.m^{-1})\)

\( \varepsilon_0 \) Permittivity

\( \phi_d \) Volume fraction of the dispersed phase

\( \phi_{\text{max}} \) Maximum packing concentration

\( \gamma \) Receptor peeling factor

\( \dot{\gamma} \) Shear rate \((sec^{-1})\)

\( \kappa \) Debye length \((nm^{-1})\)

\( \Lambda \) Dimensionless electrophoretic to convective transport number

\( \eta \) Dynamic viscosity \((Pa.sec)\)

\( \mu \) Electrophoretic mobility \((m^2.V^{-1}.sec^{-1})\)

\( \rho \) Buffer density \((kg.m^{-3})\)

\( \rho_d \) Dispersed phase density \((kg.m^{-3})\)

\( \rho_f \) Continuous phase density \((kg.m^{-3})\)

\( \rho_s \) Space charge density \((C.m^{-3})\)

\( \psi \) Particle surface potential \((V)\)

\( \sigma_b \) Bond spring constant \((N.m^{-1})\)

\( \sigma_{\text{eq}} \) Equivalent bond/microvillus spring constant \((N.m^{-1})\)

\( \sigma_{\text{mv}} \) Microvillus spring constant \((N.m^{-1})\)
\( \sigma_{ts} \quad \text{Transition spring constant (N.m}^{-1}) \)

\( \xi \quad \text{Zeta potential (V)} \)
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Dedication

This doctorate dissertation is dedicated to:

my beloved hard-working father

who always wished me to be a doctor, although not in engineering!

my beloved kind-hearted mother

who always wished me to be what I wished to be,

my beloved compassionate brother and sister

who never really cared what I wished to be, but cared a great deal about me!
1 Introduction

The 1996 disease outbreak in Kelowna due to contaminated water infected more than 10,000 people [1]. The pathogen was *Cryptosporidium*, one of the major water-borne parasites frequently-observed in surface waters in North America. There have been reports of outbreaks in other parts of the world as well. The 1993 Milwaukee outbreak with more than 400,000 infections still remains one of the most severe *Cryptosporidiosis* outbreaks. Since then, tremendous efforts have been focused on the development of methods for detection/removal of this harmful pathogen. These efforts have resulted in the current EPA 1623 method developed by the United States Environmental Protection Agency (USEPA) [2]. EPA 1623 has been used over the past many years for the detection of *Cryptosporidium* oocysts and *Giardia* cysts in various water samples. Canadian public municipalities and private research laboratories essentially use the same method as there is no alternative effective sampling method which can be used.

Despite the general success of EPA method, there are still some major drawbacks. The method is complex and requires highly-skilled personnel. There are lengthy, labour-intensive steps that must be followed with extreme care. It can take from three days to a few weeks to analyze the samples in advanced laboratories, in addition to the time needed to transport the sample to those laboratories. In the meantime, the water might be distributed and consumed allowing another severe outbreak. To make it worse, these tests are very expensive, limiting the frequency of the analysis. EPA 1623 does not meet the limit of detection for *Cryptosporidium* (i.e., one oocysts in 10 litre of sample water). EPA 1623 includes a filtration step which is highly unreliable and usually non-replicable. Huge variations in the filtration recovery have been reported [3]. This adversely affects the overall efficiency of the method in the detection of the harmful oocysts.
In this research, an alternative diagnostic tool is proposed to monitor water effectively in the shortest time possible. The proposed tool consists of a flow cell device with reactive plates on which specific antibodies are mounted (immobilized). As the water sample flows through the device, pathogens bind to these antibodies and are captured for the next stage during which detection occurs. Therefore, there are two general stages in the proposed methodology: 1) separation and concentration, and 2) examination and detection. The main focus of this study is to develop the flow cell device to separate and concentrate as many pathogens as possible from the water sample. For the detection part, as the first step, direct epifluorescence microscopy is used similar to EPA 1623 method. Once the device is developed, more effective detection techniques can be incorporated to the proposed separation device. However, the design of a new detection stage is out of the scope of this thesis.

One of the benefits of the proposed design is that it can detect the pathogens in a rapid reliable manner. The portability of the proposed tool also eliminates the unnecessary time spent on sending the samples to the labs. In addition, it can be used by non-expert personnel enabling them to provide health organizations with more reliable results in much less time. The proposed design can also be used to capture one (e.g., Cryptosporidium) or different kinds of pathogens at once. For the latter, the device has to be modified to have an array of capture molecules and reactive plates. All these advantages assist municipalities to react rapidly against any probable pathogen epidemic and prevent possible outbreaks.

In this chapter, first, the conventional EPA 1623 method for the detection of Cryptosporidium and Giardia is briefly reviewed and its shortcomings are discussed. Then, the major objective of this research is presented and the proposed alternative separation method is introduced. The functional basis for pathogen capture by the new device is discussed and the relevant literature is thoroughly reviewed. At the end, the organization of the research chapters is presented.

1.1 EPA 1623 method

EPA 1623 method consists of four essential steps [2]:

In this research, an alternative diagnostic tool is proposed to monitor water effectively in the shortest time possible. The proposed tool consists of a flow cell device with reactive plates on which specific antibodies are mounted (immobilized). As the water sample flows through the device, pathogens bind to these antibodies and are captured for the next stage during which detection occurs. Therefore, there are two general stages in the proposed methodology: 1) separation and concentration, and 2) examination and detection. The main focus of this study is to develop the flow cell device to separate and concentrate as many pathogens as possible from the water sample. For the detection part, as the first step, direct epifluorescence microscopy is used similar to EPA 1623 method. Once the device is developed, more effective detection techniques can be incorporated to the proposed separation device. However, the design of a new detection stage is out of the scope of this thesis.

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1.1 EPA 1623 method

EPA 1623 method consists of four essential steps [2]:

In this research, an alternative diagnostic tool is proposed to monitor water effectively in the shortest time possible. The proposed tool consists of a flow cell device with reactive plates on which specific antibodies are mounted (immobilized). As the water sample flows through the device, pathogens bind to these antibodies and are captured for the next stage during which detection occurs. Therefore, there are two general stages in the proposed methodology: 1) separation and concentration, and 2) examination and detection. The main focus of this study is to develop the flow cell device to separate and concentrate as many pathogens as possible from the water sample. For the detection part, as the first step, direct epifluorescence microscopy is used similar to EPA 1623 method. Once the device is developed, more effective detection techniques can be incorporated to the proposed separation device. However, the design of a new detection stage is out of the scope of this thesis.

One of the benefits of the proposed design is that it can detect the pathogens in a rapid reliable manner. The portability of the proposed tool also eliminates the unnecessary time spent on sending the samples to the labs. In addition, it can be used by non-expert personnel enabling them to provide health organizations with more reliable results in much less time. The proposed design can also be used to capture one (e.g., Cryptosporidium) or different kinds of pathogens at once. For the latter, the device has to be modified to have an array of capture molecules and reactive plates. All these advantages assist municipalities to react rapidly against any probable pathogen epidemic and prevent possible outbreaks.

In this chapter, first, the conventional EPA 1623 method for the detection of Cryptosporidium and Giardia is briefly reviewed and its shortcomings are discussed. Then, the major objective of this research is presented and the proposed alternative separation method is introduced. The functional basis for pathogen capture by the new device is discussed and the relevant literature is thoroughly reviewed. At the end, the organization of the research chapters is presented.

1.1 EPA 1623 method

EPA 1623 method consists of four essential steps [2]:
1) sample collection and storage,
2) filtration and elution,
3) concentration and separation,
4) staining and examination.

After the water is collected in bulk samples, it has to be shipped to the laboratories under preserved conditions (cold environment). If care is not taken to preserve the samples during the shipment, the analysis may be adversely affected by pathogen degradation. This becomes worse if the collection is performed in remote areas far away from laboratories.

In the next step, the samples are filtered to remove the oocysts/cysts from water (this step may be done at the site of the sample collection as well). After the pathogens are on the filter membrane, they will be eluted and recovered thorough mechanistic washes using elution buffers already prepared in laboratory. Largely different values have been reported for the recovery percentage of the filtration/elution process [2] resulting in a wide range of mean recoveries and large standard deviations. This makes the filtration step both unreliable and imprecise. For instance, the acceptable range of final recovery reported by EPA for Cryptosporidium parvum is 38-100% with maximum relative standard deviation of 38 [2]. The actual mean recovery value obtained in laboratories is usually around 50-60% in most cases with the relative standard deviation of 20-30.

After the sample is eluted, it is centrifuged and concentrated (purified) in the next step through immunomagnetic separation (IMS). In IMS, first, centrifuged concentrate sample is resuspended with functionalized magnetic beads equipped with anti-pathogen antibodies. Once attached to the pathogen, the pathogen-bead complex is separated from the solution in a magnetic particle concentrator. The pathogen-bead complex is then dissociated by adding the dissociation buffer to the final solution. IMS has been shown to yield great recoveries. However, the method requires skilled personnel and advanced equipment as it involves complicated procedures.

After the oocysts/cyst sample is purified, it is stained using fluorescent materials and examined under the microscope [2]. Positive and negative controls are recorded and the final results are
obtained. This step is usually tedious and time consuming since it needs a human operator to confirm the morphological characteristics of the oocysts/cysts.

Before any EPA 1623 test is performed, the setup quality is controlled through previously-prepared spiked solutions. These solutions are spiked to a precise initial concentration using flow cytometry and then used in the EPA test. The final recovery is obtained through the following equation [2]

\[ R = \frac{N}{T} \times 100 \quad (1.1) \]

where \( N \) is the number of pathogens counted and \( T \) is the number initially spiked. Acceptable mean recovery range and precision (maximum relative standard deviation) are reported to be 38%-100% and 37, respectively. The wide range of recovery and precision demonstrate that although the method works practically, there is much room for improvement.

### 1.2 Research objective

Considering the above shortcomings, the main objective of this research is to investigate the possibility of developing more efficient and sensitive tools which can function with less complexity and need of resources. In this research, it is not intended to achieve the low limit of detection of one oocyst per litre, but to improve the reliability and efficiency of the pathogen separation process used in the EPA method.

The proposed tool must

- be portable so it eliminates the risk and the time associated with the sample shipment,
- not involve unreliable imprecise processes (such as filtration),
- not involve complex processes which require advanced instrumentation (such as IMS),
- yield comparable sampling rate with EPA method and better recoveries.
1.3 Proposed separation/detection method

In this section it is explained how each design criterion presented in the Research Objective is accounted for in the proposed separation/detection method. The first design criterion (portability) requires to consider a small-scale device in which most (if not all) the essential steps of EPA method or their equivalent processes occur in one stage. Also, since micron-size pathogens (averagely, 4-6 μm in diameter for Cryptosporidium oocysts) are dealt with, any proposed design must offer a high degree of sensitivity and controllability over the sample. The above requirements favour considering microscale devices as an attractive option to design the sampling flow cell. Microscale devices (such as microchips) are able to conduct very complex operations in small scale providing a great deal of convenience, sensitivity and robustness [4]. Since small amount of samples are used in these devices, the operations are readily controlled and monitored. In addition, thousands of these microchips can be incorporated in one single device performing thousand operations simultaneously while the device is still reasonably sized.

To meet the second criterion (reliability and precision), it is required that any process which is not reliable and reproducible is replaced with more precise methods. The most imprecise stage in the EPA method seems to be the filtration step. Therefore, developing more precise methods means replacing this step with alternative separation techniques that have better reproducibility of the recovery. Unlike filtration in which pore sizes smaller than the pathogens are used for the separation (mechanical separation), here larger paths are considered for the pathogen to flow through the microscale (microfluidic) device. These paths are still small, in the order of hundreds of microns, to maintain the small bulk size of the device. Instead of mechanical separation which may result in issues such as clogging and membrane failure, another approach must be considered to separate (trap) the pathogens inside these paths. The alternative approach should help maintain a large flow paths and a continuous flow of the water sample while it

1) separates and
2) holds the harmful pathogens until the end of separation stage.
To separate the pathogens, a physical separation technique must be undertaken as opposed to the mechanical separation used in filtration. This means that an effective technique, employing the physics of the event, must be found to manipulate the pathogens moving with the flow and separate them from the main stream. A review of the feasible physical separation techniques is presented in Section 1.4.3. Once separated, the pathogens must be held against the flow of sample and not leave the microfluidic device. Therefore, similar to IMS, the device should have “capture elements” binding pathogens.

The schematics of the proposed separation and capture mechanisms are shown in Fig. 1.1. A pathogen moves at an arbitrary distances from the capture elements. However, capture elements function only when the pathogen is in their close proximity. In order for the pathogen to be trapped, the microfluidic device must be designed in such a way that as many pathogens as possible are directed towards the capture elements. Once the pathogens are close to these elements, they should bind to capture sites and remain there for the entire sampling period. To achieve strong binding to the capture elements, they are functionalized using antibodies specific to the pathogen of interest. The outcome is a sturdy bond between the complementary molecules on the surface of the pathogen cell (receptors) and reactive capture element (ligands). Henceforth, this process is called cell adhesion which is shown to be based on a key-and-lock mechanism between the receptor and ligand molecules (see Fig. 1.1) [5]. Therefore, an efficient design of the microfluidic device is necessary to facilitate both particle transport and adhesion in fluid flow fields, where the pathogen cell is the particle and water sample is the fluid.
Fig. 1.1. Schematics of the separation and capture mechanisms that pathogen cell undergoes in the microfluidic flow cell device: a) overall view and b) zoomed-in view near the capture element.

The proposed microfluidic device automatically meets the third design criterion as it does not need the filtration setup and also the IMS step. In the EPA method, the IMS step is conducted to concentrate and separate the pathogens from the sample; whereas in the proposed method, the pathogens are already separated after the sample is flushed through the device. A buffer (similar to the one used in IMS) can be flushed through afterwards to dissociate the bonds between the pathogens and the antibodies, and collect the pathogens in the effluent. Then, the
effluent will be stained and examined using direct microscopy. So there is no need to perform lengthy and complex tasks of filtration and magnetic concentration conducted in EPA.

Since large volumes of water must be sampled, the device will need multiple paths (channels) to handle the flow. Parallel implementation of microfluidic channel (as shown in Fig. 1.1) produces enough sampling rate comparable to the EPA method as required by the forth design criterion. Since each channel is small, even hundreds of them will not be unacceptably large. The only question that remains is whether or not the proposed method is able to provide better recovery and precision than the USEPA method. In order to answer this question, a structured analytical approach was developed to study all the relevant phenomena (including transport and adhesion) and parameters (including flow rate, antibody surface density, cell receptor density, binding reaction rates, etc). There are a very large number of different configurations of fluid flow-capture element which can be designed to separate and capture the pathogens. With the aid of transport/adhesion modeling a great deal of time and effort can be saved and allocated to the physical experimental work on the proposed prototype.

In summary, instead of mechanical separation using filtration (based on the pathogen size), physical separation (based on the physics of particle transport and adhesion), is considered here. The followings are known about the final design:

- a microfluidic flow cell device is considered for the capture purpose,
- particles are smaller than the flow paths and brought to the capture elements by means of a separation technique,
- the separation technique has to be selected and optimized based on the physics of particle transport and adhesion,
- antibodies are used to capture and hold pathogens on the capture elements,
- once the pathogens are trapped, antibody-pathogen binding must be sufficiently strong to resist shear flow.

In the next section, a detailed overview of the theory and modeling for the transport and adhesion of biological cells is presented. Understanding the physics of transport/capture
facilitates the development of more efficient capture methods. Then potential physical separation techniques which can be used to enhance the rate of pathogen transport to the capture elements are reviewed.

1.4 Review of relevant literature

Cell transport and adhesion has been the subject of a prodigious amount of studies conducted both experimentally and theoretically. In the following sections, first, the theory of cell adhesion is reviewed, and the analytical and numerical approaches used to model adhesion are explained. A review of previous studies which incorporated cell adhesion models into particle transport models will be presented. Finally, selective physical techniques, used to increase the rate of cell transport to capture elements, are reviewed.

1.4.1 Pathogen cell adhesion

Cell adhesion is commonly observed in many biological and biotechnological applications [4]. A familiar example of cell adhesion is adhesion of leukocytes (which are a family of white blood cells) to endothelial cells on the blood vessel walls [6]. This adhesion is necessary to initiate a process called cascade which leads to transmigration of cells through the vessel into an infected tissue. Cell adhesion is also a crucial step in many other biological events such as cancer metastasis [7], preferential migration of cells in the embryonic development [8] and repair of the blood vessel by platelets [9]. Implementing the idea of cell adhesion happening in the response of the body immune system to the infective cells, one may use the complementary capture molecules of any specific pathogen to detect them within the sources likely to be consumed by humans. This is the principle reason to use antibodies for pathogen capture in the proposed methodology.

Considering the broad range of applications of cell adhesion, proposing a general model provides a robust tool applicable to different problems without making extra efforts to include new conditions for each problem. This means that once a model is developed to simulate cell motion and adhesion in a flow, it can be used in different conditions and various
biotechnological phenomena ranging from leukocyte response in human blood vessels to pathogen capture in water sampling devices. In this section, the theory of cell adhesion is explained in detail and the relevant studies reported in the literature are reviewed. It is worth mentioning that all the theories and models described here include only the specific adhesion of particles, not the transport in the bulk flow. Particle transport is discussed in the next section.

Tyler [10] and Bell et al. [11] proposed that the specificity of adhesions between cells is the result of chemical reactions between the molecules on their surfaces. Today, these molecules are generally called receptors and/or ligands, and adhesion is known to be based on the key-and-lock mechanism at the binding sites of the receptor and ligand molecules. The theoretical analysis of cell adhesion is generally divided into two categories: mechanical/thermodynamic equilibrium analysis and kinetic (dynamic) analysis [12].

**Mechanical/thermodynamic equilibrium analysis**

Bell et al. [11] were the first to investigate the equilibrium state of two cells adhered to each other using a thermodynamic approach. Their model took into account the effect of specific binding (between complementary molecules) and nonspecific repulsion including both electrostatic repulsion and steric stabilization [13]. Through minimization of the free energy function, they obtained a set of relationships which determined three distinct regions depending on the relative strengths of binding and repulsion. From all the three regions, adhesion was possible only in the second and the third region showing a greater strength of adhesion with respect to repulsion. Several modifications [14-16] that have been proposed by other researchers to generalize the solution are: 1) consideration of the mobility and interactions of the receptor molecules on the cell membrane, 2) consideration of the multiplicity of receptors (i.e., several types of receptors on a single cell), and 3) addition of the elasticity of the biological cells in the model. Evans [17, 18] included the effect of cell mechanics in the problem. He assumed the cell membrane behaves like an elastic continuum having an adherent zone (with “molecular cross-bridging” with a plate underneath) and a free zone. The
adherent zone was assumed to have either continuum [17] or discrete [18] molecular cross-bridging. A tension was applied in the free zone of the membrane which opposed the spread of the membrane due to binding in the adherent zone. Evans [17] solved the equations of mechanical equilibrium for the tension and contact angle at both adherent and free zones by imposing the continuity condition between the two zones. In his continuum cross-bridging assumption, the tension required to oppose the spread of membrane was equal to the tension necessary to separate the membrane from the surface. This was in contrast with experimental observations [17]. His model could explain the observed behaviour by assuming discrete cross-bridging binding on the membrane [18]. However, only a small portion of the cell membrane was considered in his model. The models of Bell et al. [11] and Evans [17, 18] were improved later by others [15, 19]. However, the equilibrium analyses have always been undermined by one simple fact: in many in vivo situations, the process of cell adhesion is inherently dynamic, and the cells do not have enough time to reach equilibrium. Realizing this, many researchers have worked towards a model that can describe the kinetics of binding in non-equilibrium conditions.

**Kinetic (dynamics) analysis**

Bell [5] assumed that the molecular binding between the two classes of receptors (on two separate cells) is governed by the kinetic equation of chemical reactions. He considered a force-dependent exponential equation for the backward reaction rate (also called dissociation rate or reverse rate) to take into account the changes occurring in bond breakage for the cells under the influence of external forces exerted from the flow (drag force). However, the cell was assumed to be at mechanical equilibrium with the drag force. Stokes drag force on a sphere was used as the external force applied on the bonds at the interface. Solving the first order kinetic equation, a simplified relation between the velocity of the flow and the number of bonds was obtained. Bell’s estimation of externally applied force was not accurate since he ignored other forces such as the lift force and also external torques exerted on the cell. He used the Stokes’ equation for the drag force which is only accurate for the spheres inside the bulk, not close to the walls. In addition, his exponential model of dissociation rate could not be used
for all types of bonds observed in vivo. Other functions of the dissociation rate were examined by other researchers. For instance, Evans [20] introduced a power-law form of the force-dependent equation (compared to the exponential function of Bell [5]) to model different behaviours ranging from brittle to flexible bonds. Realizing the inaccurate force estimation of Bell, Hammer et al. [21] presented a dynamic analysis of adhesion (between a cell and a surface) adding the receptor mobility and mechanical forces to Bell’s model. They used Goldman’s equations [22, 23] to calculate the shear force and torque exerted on the cell and assumed the mechanical equilibrium to transport these forces to the bonds at the interface. To obtain a more reasonable behaviour of the bonds under stress, Dembo et al. [24] suggested that spring-like bonds be used instead of assuming mechanical equilibrium (taking the idea from Bell et al. [11]). Their simulation resembled the model of Evans [17, 18] as they investigated a membrane adhered to a surface and clamped at one side. Unlike Evans, they included the kinetics of reaction and assumed the reaction rates were functions of the distance between the receptor and ligands and the bond spring constant. They developed solutions for the critical tension required for peeling the membrane off the surface and also the velocity of peeling. Nevertheless, like Evans [17, 18], they only considered a portion of the cell membrane and only a tension in the membrane while in real conditions the detachment force is a function of the coupled influence of both the cell and the flow.

All the previous models were obtained for a deterministic formulation of the kinetic approach. However, if the number of molecules involved in a reaction is small [25], the nature of the problem becomes statistical. Cozen-Roberts et al. [25] introduced a probabilistic approach to model the kinetics of cell adhesion and compared it with the deterministic approach. They realized that the deterministic model may underestimate the time required for attachment or overestimate the time needed for detachment. Also, the deterministic framework may overestimate the force of detachment. Using the probabilistic framework, the probability of a cell remaining adhered to a surface can be found for any external force instead of reporting crisp values of the detachment force which results in either attached or detached cells. Hammer et al. [26] used the probabilistic framework of reaction kinetics (using the Monte-Carlo method [27]) and also the distance-dependent relations for the reaction rates (from [24]). They
called their model the “Adhesive Dynamic (AD)” simulation. In their model, the cell was considered as a solid sphere covered with adhesive springs. The distribution of microvilli (arm-shape protrusions of the cell membrane mostly responsible for cell-surface contact), the distribution of receptors on microvilli tips and the reaction rates were chosen to have random probability functions. The velocity of the cell at each instant was determined using the Newton’s second law of motion with the balance of the bond force (as a spring force) on one side and the hydrodynamic force on the other side. Instead of solving the probabilistic kinetic equations, the Monte-Carlo method was implemented to calculate the probability of attachment and detachment at each time to determine whether or not the receptor was bound. If bound, the bond spring force was included in the equations of motion along with the hydrodynamic forces. If not, the cell was moved only by the hydrodynamic force. The cell was initially positioned at an appropriate distance from the wall so that it could probably form bonds. The adhesive dynamic (AD) model was very successful at reproducing different regimes of adhesion (observed in experiments) including completely unencumbered motion, rolling, transient adhesion and firm adhesion. This led the researchers [28, 29] to present state diagrams for different parameters governing the adhesion phenomenon. The AD model was further modified to involve different aspects of cell adhesion. For instance, the multiplicity of receptors was modeled by Bhatia et al. [29]. They used two classes of receptors; one responsible for initial arrest of leukocytes and the other one responsible for the firm adhesion of the cell on the surface. Using the AD model, they could validate the velocity of rolling with the data they obtained from the literature. In 2007, Caputo et al. [30] reproduced a shear threshold (i.e., a certain level of the shear force required for cell rolling) in leukocyte rolling by the AD modeling. The AD model has been proved to be a powerful method to describe the various behaviours observed in the experiment (at least qualitatively). However, the main shortcoming of this method is modeling the deformation of the cell, which has been shown to be very influential in the dynamic process of adhesion and still remains unsolved. Since cells move and deform in the flow, the hydrodynamics of the cell inside the flow is of great importance. Unlike the cell adhesion models described above, a complete modeling of cell capture involves the simultaneous determination of the position of the cell (membrane) as a
result of various external forces and the solution of the kinetic equation of binding at the interface. In the next section, different numerical approaches developed for the coupled modeling of transport, deformation and adhesion of cells are presented and discussed.

1.4.2 Pathogen cell transport

Numerical modeling greatly helps to characterize the transport and adhesion mechanisms, and can be used to find the optimal design among all the proposed ones. Like adhesion, particle transport analysis has also been the subject of a tremendous number of studies in the literature [31-35]. Different transport models have been considered to simulate the motion of either a single particle or a cloud of particles in fluid flows. Particle transport models are generally divided into two categories. In the first category, which lies within the Eulerian framework, the particles are assumed to behave like continuum medium (as if they are part of bulk flow), and particle concentration is determined to track the cloud. In the second category, cells are considered as discrete particles, and two-phase flow simulation methods are used to follow the particles (Lagrangian framework). That means each particle is treated individually. Accordingly, different methods are applied in each category to model particle adhesion. Each of the above modeling categories is described along with its corresponding adhesion mechanism, and the related literature is reviewed.

Continuum models

In this category, the general procedure is to solve the mass transport (convection-diffusion) equation along with the flow momentum transport equations and chemical reaction kinetics to obtain the concentration profile of the particles inside the flow and on the reactive capture elements. However, more simplified solutions were also used in the literature. For instance, Xia [36] investigated the adhesion of biological particles (such as bacteria and red blood cells) in an impinging jet apparatus. Different colloidal forces such as Van der Waals and electrostatic interactions and steric repulsion were used in his simulation. Considering the relatively small sizes of the bacteria, he assumed that the attractive Van der Waals forces were balanced by the
hydrodynamic drag force sensed by the particle approaching the wall. This is the main assumption of the Von Smoluchowski-Levich approximation [37]. This approximation provides an upper limit for the particle mass transport (bacteria in the case of Xia’s work [36]) in different device geometries. The Smoluchowski approximation was also used by Dickinson [38] to simulate the deposition of Brownian particles on a surface mediated by molecular binding. His approach combined both colloidal and molecular binding forces within a probabilistic analysis of the Smoluchowski equation. Although Smoluchowski-Levich approximation greatly simplifies the calculation, it is not valid over the entire range of particle sizes. As the particle size increases (e.g., red blood cells), the Smoluchowski assumption does not hold anymore and the full convection-diffusion equations need to be solved numerically [36]. Longest [39] solved the convection-diffusion equation with the reaction kinetics equation and compared the results with the experimental data of platelet deposition on a plate in stagnant flow. It was observed that the numerical results followed the experimental data over the regions far from the stagnation point. However, in the stagnation zone, the deposited concentration was obviously different. The researcher discussed that this behaviour existed due to the importance of the particle size and inertia. In other words, neglecting the hydrodynamic interaction of flow-particle was believed to be the main reason of deviation.

The convection-diffusion-reaction equations have extensively been used in the simulation of chemical reactions between different species in reactive flows [40-42]. These equations are mostly valid in the case of a dilute continuum solution of small particles which possess the same flow properties (such as velocity) as that of the liquid medium. However, as mentioned above, this model does not account for the hydrodynamics of the particle which is significant especially for larger particles (such as cells). These effects include, but are not limited to, the size and inertia of the particle, and the effect of particle interactions with each other and also with the flow (two-way coupling). The need to consider such effects led to another class of transport-adhesion models introduced in the next section.
**Dispersed-phase models**

In the second category of particle transport modeling, particles are essentially assumed to be a dispersed phase in a continuum medium (two-phase flow) [43]. This lies in the Lagrangian framework in which each particle is traced individually. Lagrangian dispersed-phase modeling itself is divided into two categories: 1) point-volume methods and 2) volume-resolved methods [43].

**Point-volume method** - In this method, the particles are represented as single mass points moving inside the flow. The particle motion is essentially governed by the second law of Newton (or the Langevin equation if the Brownian motion of the particles is significant). The simulation can be either one-way coupling (which ignores the effect of the presence of the particle on the surrounding fluid flow) or two-way coupling. The general solution procedure is summarized in two steps. In the first step, the momentum transport equation is solved to obtain the velocity profile throughout the domain. In the second step, this velocity is used to calculate the external forces exerted on the solid particle, and the position of the particle is updated at each time using Newton’s second law [43]. Maxey et al. [44] derived the corrected form of the equation of motion for a solid sphere moving in a flow. The right hand side of this equation included different forces such as buoyancy, pressure gradient, Stokes drag, added mass and Basset history. In general, the Stokes’ drag force and buoyancy force (for a large density difference between the particle and the fluid) are the most dominant forces. Added mass and Basset history forces are important only in the cases where the particle is starting to move in a stationary fluid (for example, under the influence of gravity) or in an oscillatory flow with high frequency [44]. These terms were obtained for a solid sphere translating in the bulk flow far away from the walls. Goldman [22, 23] modified the Stokes’ drag force by taking into account the effect of the wall in the quiescent fluid and Couette flow. Saffman [45] calculated the lift force that particles sense due to inertia when moving close to a wall. This force along with the rotation of the particle was not included in the equation presented in the work of Maxey et al. [44]. When the rotation velocity is different from that of fluid, Magnus force derived by Crowe et al. [46] is exerted on the particle.
So far, only the transport of the particle has been reviewed. But adhesion must also be modeled in this research in order to obtain a full understanding of particle-flow interactions. Different models have been introduced to include particle adhesion (sometimes called deposition) in this type of simulations. A qualitative parameter called volumetric residence time (VRT) was first introduced by Kunov et al. [47]. This parameter is a measure of the time particles spend at each location in the flow. Larger value of VRT means that the particles are more concentrated at a specific location. The concept was applied to the case of stenosis (narrowing of blood vessels) for platelets. It is assumed that platelets are activated at a specific level of the shear rate in the flow to adhere to the surface [47]. Kunov et al. [47] included VRT in their model and concluded that platelets reside longer in high shear and low-velocity regions as expected. This concept was modified later on by Longest [48] to the so-called near-wall residence time (NWRT) which considers only a small region near the walls where interactions were thought to be significant. It was found that the NWRT approach provides a better description of platelet deposition in stagnant flow where the convection-diffusion model failed to describe the deposition pattern. The NWRT method was applied to different geometries of arterial systems [48, 49]. These researchers tried to relate the NWRT to the wall shear stress, wall shear stress gradient, wall shear stress angle deviation and oscillatory level of shear stress. According to their results, larger NWRT was observed wherever the wall shear stress was high and/or the oscillatory level of the shear was low. The higher values of the NWRT also mean that particles have more time to interact with the wall, and hence, more probably adhere to the surface. However, this method lacks the accurate modeling of cell deposition since no actual attempt is made to model the adhesion at the boundaries, and only the physical presence of particles near the surface is of interest.

Marshall [50] proposed a multiple-time step numerical algorithm called the discrete-element method taking into account adhesion between the particles and the surface, and also between the particles themselves. The equation of motion was essentially the same (in the form of one-way coupling) except that he considered both translational and rotational motions of the particles. In addition, he used the soft-sphere model [46] to calculate the normal and resistant forces of collision so the solution did not need to be diluted. He assumed that Van der Waals
forces were responsible for adhesion of spherical particles and simulated the aggregation inside the pipe and channel flows. Chesnutt [51] extended the Marshall’s solution to the case of molecular binding (ligand-receptor) of spheroidal particles. However, he did not include the effect of particles on the flow. Another important shortcoming of his method was the simplified model of particle deformation. This is a general limitation of the point-volume methods in which the particles are solid or, in the best case, undergo simple deformations (as considered in the soft-sphere model). These methods are favourable whenever the deformation is not playing an important role in the problem. This characteristic makes the point-volume method the most appropriate approach for the modeling of a large number of particles in the flow with reasonable computational time [51]. However, deformation of biological cells has been shown to have a great influence on the adhesion phenomenon [12]. The complex interactions of the internal forces (due to the membrane and cell internal structures and physical properties) and external forces (like the drag force and bond force) determine the cell shape and the size of the contact area which are very crucial in explaining different behaviours observed from biological cells [12]. This led to the development of the second category of dispersed-phase numerical approaches called volume-resolved methods.

**Volume-resolved method** - In this method, the particle is considered as a capsule with a certain volume. The momentum transport equation is solved for the bulk flow and inside the particle capsule simultaneously, and the deformation of the particle is resolved [43]. The particle-flow interface, where the binding reaction occurs, is traced at any instant. Either the deterministic or probabilistic framework may be used to solve the kinetics of binding to determine the number of bonds at each time step. The bond force is then calculated (assuming that bonds behave like springs) and inserted in the momentum transport equations as the body force.

Fogelson [52] modeled the platelets as a two-dimensional fluid capsule enclosed by a thin elastic membrane. Due to computational issues in resolving the small distances between the cells, he assumed that if the platelets were closer than a threshold, they were attached to each other by a few springs. This rough approximation of cell adhesion was improved later by Agresar et al. [12] who considered the presence of the cell nucleus, diffusion of surface
molecules, and both colloidal and specific binding forces at the interface. Specific forces were obtained by solving the deterministic kinetics of reaction. Since a front-tracking method was used to resolve the interface, they had to implement an adaptive meshing method to do the refinement at the small gaps between the two cells resulting in painstaking computations.

N’Dri [53] used the immersed boundary method (IBM) which did not require the explicit tracking of the interface. Alternatively, a few marker points were used to trade the information between the grid points in the flow and the cell membrane. This greatly reduced the computational time needed to model the phenomena taking place at the nanoscale (i.e., molecular binding). The numerical approach was reasonably successful at capturing deformation and rolling of a cell (containing a nucleus) adhered to a surface. The results were also in a good agreement with the experimental data in the literature [53]. The simulation was conducted in two dimensions with the assumption that both the cell and its nucleus were Newtonian fluids. Khismatullin et al. [54] repeated the same work in three dimensions and investigated the effect of viscoelasticity of the cytoplasm and nucleus using non-Newtonian constitutive equations. They also included the deformability of the microvilli considering them as elastic bars distributed over the membrane. The addition of elasticity to the cell properties enabled them to capture the tear-drop shape observed in vivo. Tether formation and membrane disruption also happened when high shear rates were applied; while for lower rates only cell rolling occurred [54]. Their model simulated adhesion when the cell was positioned very close to the surface, at a distance of the order of an unstretched bond (i.e., 50-100 nm). They also used the surface tension model to simulate the cell membrane. Despite the thoroughness of the approach, their model did not consider any membrane rigidity against bending. Jadhav et al. [55] simulated the three-dimensional rolling of leukocytes for the Newtonian fluid inside the cell. Assuming the stochastic nature of bond formation, they used the probabilistic approach (the Monte Carlo method of Hammer et al. [26]) to obtain the number of bonds. Instead of implementing surface tension at the interface, membrane elasticity was modeled. Their results proved that for higher shear rates and stiffer membranes the bond lifetime decreases due to larger external forces sensed from the flow. However,
bending rigidity was still missing in their work. Inclusion of bending rigidity yields a more reasonable model of mammalian cell transport and deformation [34].

All of the volume-resolved methods, although more accurate in adhesion modeling, are applicable only if the number of the cells being investigated is small. This is due to fact that high resolution simulation of membrane deformation needs a long computational time. Therefore, it is not possible to use volume-resolved methods to obtain the overall picture of particle transport for more than a few pathogens. Nonetheless, it is necessary to use such methods in the small scale where the individual cell deformation and adhesion is studied. Hence, the modeling approach must be carefully selected at each step depending on the problem specifications. The methodology implemented to meet the objective of the present research is presented in Section 1.5.

1.4.3 Pathogen physical separation techniques

The objective of this research can be viewed as the design of a microseparator/microreactor for the separation and concentration of bioparticles (Cryptosporidium parvum oocysts in this case) on reactive elements. There exist different techniques to manipulate particles inside microreactors/microseparator and enhance their transport. The most important task is to find the best external driving mechanism. Different driving mechanisms can be chosen to manipulate the particles due to the effect of gravity [56], electric fields [57-59], magnetic fields [60], optics [61], acoustic waves [62], etc. However, one important design constraint is to keep the device as simple, efficient and reliable as possible so it can be used at the site of the sampling without any need for complex sampling equipment or pre-processing. In this research, two of the simplest yet most effective techniques (i.e., sedimentation and electrophoresis) are introduced and reviewed for further analysis.

*Sedimentation for pathogen transport enhancement*

Sedimentation (use of gravity) is a separation technique working based on the mass of particles involved in the process. This method has been widely used in the separation of biological cells
and biomolecules [63-65]. The use of the gravitational force is very desirable since it eliminates the need for external forces and consequently, additional equipment. The gravitational force has been used in many applications including gravitational and sedimentation field flow fractionation (GFFF) [56] and gravitational split flow thin cell fractionation (SPLITT) [64, 65]. Generally, in any field flow fractionation (FFF) method, the main idea is to separate the particles using an external field which is applied in a different direction from the main direction of the flow. Distinct particles are influenced differently by the external field due to their specific properties (such as mass in GFFF). Therefore, the particles travel at different velocities from the bulk motion of the buffer depending on their transverse position. This will create a delay in the motion of some species with respect to the others, separating them along the direction of the flow (see Fig. 1.2).

![Fig. 1.2. Schematics of a field flow fractionation (FFF) method.](image)

The general theory of field flow fractionation (FFF) methods has been extensively developed by [66]. In a recent study, Huh et al. [56] attempted to develop a gravity-driven microfluidic separator employing different rates of sedimentation for different particles. They modified their channel geometry so the hydrodynamics of the flow magnified the lateral separation of the particles. As a result, they could achieve a high-resolution separation of particles with a large size difference. In such separation methods, the gravity field is used perpendicular to the main direction of the flow of buffer. Since the settling velocity for each particle in the mixture is different, the level of deviation from the main stream also differs from one particle to another. If the mixture is given enough time by making the flow path sufficiently long, the particles are
fractionated. In the present research, gravitational field (sedimentation) is only used to separate the pathogen of interest from the main buffer stream. It is not intended to perform any particle type fractionation as single type of pathogens is analyzed in this work.

*Electrophoresis for pathogen transport enhancement*

Applying an electric field, as one of the feasible options, has been shown to work favourably for the purpose of particle manipulation [67]. Depending on the geometry of the device and the type of the applied field (AC or DC), the effect of the electric field can be categorized into electrophoresis for uniform and dielectrophoresis for nonuniform electric fields [68]. In the former, the particle charge-to-mass ratio is the important factor determining the particle motion; while in the latter, the particle polarizability is the essential parameter [68]. Dielectrophoretic transport of bioparticles has been extensively studied during the past years and is still a subject of interest in immunoassay system analyses [69-72]. Although dielectrophoresis leads to a larger driving force and higher sensitivity, electrophoresis is still widely being used for its simplicity, as only a DC electric source is required. Also, if it is applied correctly, electrophoresis has been shown to be very precise in bioparticle detection and separation [68].

In this study, electrophoresis has been selected since it requires a simple DC external power supply which can be provided by ordinary batteries. This is compatible with the design criteria which require the device to be as simple and portable as possible. Also, electrophoresis seems effective as many organisms in nature are charged (mostly negative). Therefore, it is postulated that the use of electric field might provide a strong external effect, in addition to gravity, enhancing the transport of the microorganisms toward the capture surface.

The literature on the effect of electric field on the manipulation of charged particles (either synthetic or biological) is abundant. Electrophoresis dates back to 1930’s when Tiselius [73, 74] reported the applicability of electric field on manipulation of protein molecules. Since then, many researchers from a wide range of disciplines have developed and practiced the theory in various applications. In 2003 Kassegne et al. [31] analyzed DNA hybridization on reactive
surfaces inside lab-on-a-chip devices both experimentally and numerically. By applying a rather small potential, they could achieve an ample flux of DNA molecules to the reactive surfaces. In their model, however, no chemical reaction was considered between the complementary DNA molecules that were supposed to hybridize. Also, they did not include the flow of the buffer (the effect of convection) in the transport of the sample. In 2007, Balasubramanian et al. [75] used a DC electric field inside a microfluidic device to separate and concentrate different types of bacteria and viruses (from nanometres to a few microns in diameter) in drinking water. Their values showed that a considerable capture occurred inside the trapping electric field when the bioparticles were sufficiently charged and the electric field was allowed to establish. However, no especial consideration was taken into account to hold the trapped particles. Therefore, the capture efficiency started to drop as the electric field weakened after some sampling time was passed.

Depending on how the electric field is applied to manipulate the particles, different applications have been developed. Capillary zone electrophoresis (CZE) [76], isoelectric focusing (IEF) [77], isotachophoresis [78], electric field flow fractionation (EFFF) [79, 80] and continuous flow electrophoresis (CFE) [81] are among the most common versions of electrophoresis implemented in particle manipulation and separation. In this study, the efficiency of a modified version of the electric field flow fractionation (EFFF) in separating the bioparticle of interest is examined. Basically, the EFFF method separates the particles (which can be anything from synthetic beads to biological molecules and cells) that are introduced to the separator. There is a region where the sample is pumped through an electric field which is perpendicular to the direction of the flow. Since particles possess different charge-to-mass ratios, they are differently deviated from the main direction of the stream and exit the separator at different times. If a particle is more charged, its cloud is aggregated on the surface of electrodes where the electric potential is applied. On the other hand, particles with a smaller charge are not attracted and tend to diffuse into the buffer flow. This will create a physical gap between the two species (Fig. 1.2). A sensitive detector then can be used at the end of the setup to monitor the delay caused in the motion of each species. EFFF, as one of the field flow fractionation (FFF) methods, was first introduced by Caldwell et al. [82] to separate different protein molecules.
Later on in 1993, Caldwell and Gao [79] modified their micromachined EFFF separator to improve its separation resolution. They applied their device to separate synthetic polystyrene latex particles up to 1 \( \mu m \) in diameter. Their device, however, did not show significant efficiency for large-size particles due to steric effects [79].

In this thesis, the underlying concept of EFFF is incorporated into a microfluidic capture device (with reactive binding sites at the surfaces of electrodes) to separate and trap the parasite of interest (*Cryptosporidium*) from pre-treated water. Bearing in mind that separation means capturing the particle from the fluid medium stream, not necessarily from other particles. Therefore, no “fractionation” of different species is conducted on the sample; however, once the method is developed, it can be applied to separate different particles. EFFF is selected since it makes use of transverse electric field (with respect to the flow) inside the separation channel. The transverse-direction electric field is more desirable compared to the longitudinal direction since it eliminates the need to apply very high voltages along the channel. In addition, no electroosmotic motion is created inside the channel complicating the process [68].

### 1.5 Research methodology

Some of the key questions to be answered by the current analysis are listed below:

1) What is a better device geometry and configuration of flow-capture element to reach maximum recovery?
2) What are the best potential physical separation techniques that can enhance the transport of pathogens to the capture elements?
3) What is the optimum flow rate to reach maximum sampling rate while not damaging the pathogen-antibody complex on capture elements?
4) What are the optimum adhesion parameters related to the capture molecules (such as antibody density)?
5) Do the proposed physical separation techniques practically improve the recovery of the device under experimental conditions?
6) What material would be the most suitable for fabrication of the flow cell device?
7) What sample solution properties (like pH, conductivity, etc.) are the best to reach better performances?

In order to answer these and similar questions, the following methodology is proposed.

Finding the most efficient device geometry (question 1) is not something which can be easily achieved experimentally as fabricating many different test devices is neither cost- nor time-effective. Numerical modeling is a great asset in this regard. Choosing the most efficient geometry requires a model which can best simulate the overall transport of particles in all flow paths. The continuum model (reviewed in Section 1.4.2) is used to analyze and compare the most efficient configurations since this method, although not accurate in miniaturized-scale particle-flow interactions, is very efficient in obtaining the overall particle transport/adhesion.

Device geometry is the first factor to revise and optimize; however, it is not the only one. Accordingly, the present research path is piloted to search for plausible physical separation techniques (question 2). These separation techniques involve manipulating a physical property of the pathogen (such as density and electric charge) to enhance the particle transport to the capture elements. Once the best geometry is found, numerical simulation in Eulerian framework is adopted again to investigate the effectiveness of each proposed separation technique and compare them with each other. Eulerian techniques provide the flexibility to analyze the separation in the broad perspective. Any separation technique proven to be effective this way can then be incorporated into the flow cell device.

Answering questions 3 and 4 essentially address the molecular event of binding between the cell and the reactive capture element. A fine-scale modeling of cell adhesion is needed to examine all the relevant parameters. This is why dispersed-phase models (described in Section 1.4.2) are selected. As discussed before, these methods are able to capture numerically all the details of cell-substrate adhesion for each individual cell.

In order to answer questions 5, 6 and 7, experimental setups are designed. Experimental study is used to verify the simulation and hypotheses developed in the previous steps. These
experiments are designed in such a way to confirm (or refute) the effectiveness of proposed separation technique, and also to find the optimum working conditions.

In summary, the following research sections are presented in the next chapters:

- Eulerian modeling is implemented to find the optimum geometry and effective physical separation technique (Chapters 2 and 3),
- Lagrangian dispersed-phase modeling is used to find the optimum adhesion parameters (Chapter 4),
- experiments are designed to develop the capture flow cell device and the final capture method (Chapter 5).
2 Numerical modeling of particle transport: passive separation

As mentioned in Chapter 1, the objective of this research is to design a microseparator/microreactor for the separation and concentration of particles on reactive elements. There exist different methods to manipulate particles inside microreactors/microseparator. Similar to the conventions used in the micromixing field, these methods can be categorized into passive and active groups [83]. In passive separation no physical separation technique or driving mechanism (like a force) is used to drive the particles to the target capture elements. In fact, the only parameter that can be manipulated is the geometry (topology) of the device. In active separation, on the other hand, there is a moving part or an external force (like electric field, optic source, etc.) which guides the particles. This force is harnessed to enhance transport of particles to the capture sites.

In this chapter, continuum numerical simulation is implemented to determine the optimum geometry for the flow cell device among different possible configurations (using COMSOL Multiphysics v3.5a). Since no external driving mechanism is involved, this analysis lies in the category of passive separation. The process of finding the optimum geometry is based on trial and error. Each proposed geometry is examined in terms of its separation and capture performance. Simulation helps to understand the physics of the biological capture event and consequently to offer remedies for improving the capture performance. These remedies are then implemented to create a new flow cell design which is then numerically re-examined and compared with the previous proposed designs. Many different configurations were selected in the course of this research. Each configuration has been analyzed numerically. Here, for the sake of brevity, only the most important designs are introduced and compared.

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1 Parts of Chapter 2 have been published in a journal article and conference proceedings. Reprinted with permission from [35, 108].
2.1 Theory of continuum medium transport

In this section, the theory of transport and capture of pathogens is explained. Pathogens are assumed to be a continuum medium moving with the flow of the water sample. The momentum transport equation is solved to follow the pathogens, and the convection-diffusion equation along with the first-order kinetic equation is used to obtain the adsorption (adhesion) rate of the pathogens that are close to the reactive capture surfaces.

2.1.1 Momentum transport and continuity equations

The buffer liquid is assumed to flow through a microchannel at steady state in the absence of body forces like gravity. Two-dimensional steady-state incompressible Navier-Stokes equations are used along with the continuity equation to find the flow/pathogen velocity field throughout the domain.

\[ \nabla \cdot \mathbf{u} = 0 \]  \hspace{1cm} (2.1)

\[ \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \eta \nabla^2 \mathbf{u} \] \hspace{1cm} (2.2)

In these equations, \( \mathbf{u} \) is the velocity vector, \( p \) is the pressure, \( \rho \) is the fluid medium density, and \( \eta \) is the fluid dynamic viscosity. The no-slip boundary condition is assumed at the walls. Constant velocity is specified at the inlet. The outlet is assumed to be at atmospheric pressure.

2.1.2 Mass transport equation

After the flow reaches the steady-state condition, the solute (the immersed pathogen) is released at the inlet at a specific concentration \( (c_0) \), and it is monitored after a certain sampling time period. This solute is carried by the fluid and transported to the reactive capture surfaces through diffusion and convection. The transient two-dimensional mass transport equation which is solved inside the domain (neglecting the effect of external forces) is as follows:
\[ \frac{\partial c}{\partial t} + \mathbf{u} \cdot \nabla c = D \nabla^2 c \]  

(2.3)

where \( c \) is the solute concentration in the bulk, and \( D \) is the diffusion coefficient.

For the binding chemical reaction at the reactive capture boundaries, rates of adsorption and desorption of the solute are included in the problem through a concentration flux term. The kinetic equation of the chemical reaction is used to calculate this flux.

\[ \frac{\partial c_s}{\partial t} = k_{on} c_{wall} (c_{s0} - c_s) - k_{off} c_s \]  

(2.4)

In this equation, \( c_{s0} \) is the surface concentration of the ligand (referring to the total number of the free sites available for binding), \( c_s \) is the surface concentration of the bound pathogen (referring to the number of the occupied sites), and \( c_{wall} \) is the concentration of the solute (pathogen) adjacent to the wall. \( k_{on} \) and \( k_{off} \) are forward and backward reaction rates, respectively.

At the outlet, the convective flux is specified as

\[ \mathbf{n} \cdot (-D \nabla c) = 0 \]  

(2.5)

in which \( \mathbf{n} \) is the normal vector to the boundary. All other boundaries are insulated or symmetrical, which means

\[ \mathbf{n} \cdot (-D \nabla c + c \mathbf{u}) = 0 \]  

(2.6)

For all of the equations, the change in the physical properties due to temperature variation is neglected.

2.1.3 Non-dimensional forms of the governing equations

Due to the diversity of the variables involved in the problem, non-dimensionalization allows for better understanding of the impact of each term on the output. It also helps to explain the
results in a more systematic way. The dimensionless forms of the mass transport and reaction kinetic equations are presented here [32]:

\[
\frac{\partial c^*}{\partial t^*} + \left( u^* \frac{\partial c^*}{\partial x^*} + v^* \frac{\partial c^*}{\partial y^*} \right) = \frac{1}{Pe^2} \frac{\partial^2 c^*}{\partial x^{*2}} + \frac{\partial^2 c^*}{\partial y^{*2}}
\] (2.7)

\[
\frac{\partial c_s^*}{\partial t^*} = \varepsilon Da\left[c_{wall}^*(1 - c_s^*) - K_D c_s^*\right]
\] (2.8)

where

\[
x^* = \frac{x}{hPe}, \quad y^* = \frac{y}{h}, \quad t^* = \frac{Dt}{h^2}
\] (2.9)

\[
u^* = \frac{u}{u_{avg}}, \quad \nu^* = \frac{\nu}{u_{avg}}, \quad c^* = \frac{c}{c_0}, \quad c_0^* = \frac{c_s}{c_{so}}
\] (2.10)

In the above relations, \(x^*\) and \(y^*\) are the dimensionless coordinates and \(t^*\) is the dimensionless time. \(h\) is the characteristic length (equal to the flow path size), \(u_{avg}\) is the average inlet velocity, \(Pe\) is Peclet number, \(Da\) is Damkohler number, \(\varepsilon\) is the relative adsorption capacity and \(K_D\) is the equilibrium dissociation constant [32]. Thus, the four main non-dimensional parameters governing the problem are

\[
Pe = \frac{u_{avg} h}{D}, \quad \varepsilon = \frac{c_0 h}{c_{so}}, \quad Da = \frac{k_{on} c_{so} h}{D}, \quad K_D = \frac{k_{off}}{k_{on} c_0}
\] (2.11)

The Peclet number is the ratio of the convection and diffusion strengths while Damkohler number is the relative strength of reaction at the capture surface and diffusion towards it. To compare the relative strengths of convection and reaction, \(k_{on} c_{so} l / u_{avg} h\) is used in which \(l\) is the length of the reactive area [32]. These numbers will be used later to interpret the patterns obtained from the numerical results.
2.2 COMSOL model specifications

COMSOL Multiphysics software (Incompressible Flow and Convection-Diffusion modules) is used to solve the above set of governing equations and boundary conditions. COMSOL Direct UMFPACK Stationary solver is used to solve the Navier-stokes equations with the assumption of steady-state flow. The relative tolerance for the Stationary solver is $10^{-6}$ in the simulation. To obtain the concentration profile, COMSOL Transient solver (direct UMFPACK) is selected with default relative tolerance of $10^{-4}$ and absolute tolerance of $10^{-3}$. The mesh size is selected depending on the $Pe$ number in the simulations to prevent numerical instability. A triangular mesh (created by COMSOL free mesh generator) is used throughout the domain and is refined near the reactive boundaries to capture the high gradients of concentration. The problem is sensitive to the mesh on the boundaries especially around the edges where the gradients are larger. Throughout the analysis, mesh independency was achieved by refining the grid until the average surface concentration on reactive walls did not change more than 1% of the previous run.

2.3 Model verification

The verification of the numerical model is accomplished in two steps. In the first step, the model is verified against the exact solution of a simplified form of the mass transport equation (Graetz problem). In the second step, the numerical data obtained for the mass transport inside a rectangular microchannel is verified with the experimental data obtained from the literature [84] without considering any specific simplification.

2.3.1 Model verification against the exact solution

For very large Peclet numbers at the steady-state condition with no lateral velocity in $y$ direction, Eq. 2.7 reduces to the so-called Graetz equation [32]
This equation, along with the simplified version of the boundary condition for mass flux, leads to a set of equations which can be solved analytically. Separation of variables is used to find the general solution as

\[ c^*(x^*, y^*) = \sum_{i=1} a_i G_i(\lambda_i, y) \exp\left(-\frac{\lambda_i^2 x^*}{6}\right) \]  \hspace{1cm} (2.13)

where

\[ G_i(\lambda_i, y) = \exp\left(-\frac{y^*\lambda_i}{2}\right) M\left(\frac{1}{4} - \frac{\lambda_i}{16}, \frac{1}{2}, y^*\lambda_i\right) \]  \hspace{1cm} (2.14)

and

\[ M(a, b, x^*) = \sum_{k=0}^\infty \frac{(a)_k}{(b)_k} \frac{x^{*k}}{k!} \]  \hspace{1cm} (2.15)

\( \lambda_i \) are the Eigen values. The details on the solution procedure are presented in [32]. The velocity-weighted bulk concentration at each section of the channel is calculated from the following formula:

\[ c_b = \int_0^h c u dy \]  \hspace{1cm} (2.16)

The bulk concentration can be obtained using the mass transport coefficient approach [32] as

\[ \frac{\partial c_b^*}{\partial x^*} = -Sh(c_b^* - c_w^*) \]  \hspace{1cm} (2.17)

where \( c_b^* \) is the nondimensional form of the bulk concentration \( (= \frac{c_b}{c_{b_0}} = \frac{c_b}{c_0u_{avg}^gh}) \) defined as, and \( Sh \) is the Sherwood number defined as:
\[ Sh = \frac{k h}{D} = \frac{\partial c^*}{\partial y^*}\bigg|_{\text{wall}} \]  

(2.18)

\( k \) is the mass transfer coefficient, \( h \) is the channel height and \( D \) is the diffusion coefficient. The Sherwood number for the entrance region is [32]:

\[ Sh_E = \frac{3}{\Gamma\left(\frac{1}{3}\right)} \left(\frac{3x^*}{2}\right)^{-1/3} = 0.978x^{*-1/3} \]  

(2.19)

Replacing the above equation in Eq. 2.17 and integration result in the following relation for the bulk concentration in the entrance region:

\[ c_{b,\text{entrance}}^* = \exp\left(-1.467x^2\right) \]  

(2.20)

For fully-developed region, the full solution to Eq. 2.12 is used to find the bulk concentration. Integrating the Graetz problem in the bulk (Eq. 2.12) for a rectangular microchannel with only one reactive surface (Fig. 2.1a) yields [32]

\[ \frac{\partial c_b^*}{\partial x^*} = -\left(\frac{\partial c^*}{\partial y^*}\bigg|_{\text{wall}}\right) \]  

(2.21)

Using the full solution of Eq. 2.12 and the relation for the Sherwood number (Eq. 2.18), the following relation is found for the fully-developed Sherwood number:

\[ Sh_{FD} = \frac{\lambda_1^2}{6} = 2.4304 \]  

(2.22)

where \( \lambda_1 \) is the first Eigen value in the full solution [32]. Substituting Eq. 2.22 in Eq. 2.17 and integration yield

\[ c_{b,\text{fully developed}}^* = \exp(-2.4304x^*) \]  

(2.23)

Equation 2.20 is valid in the entrance region; while Eq. 2.23 is more accurate within the fully developed region.
As it was mentioned before COMSOL Multiphysics software is used to model the mass and momentum transport (i.e., Eqs. 2.1-2.3) inside the microchannel for $Pe = 50$ and $Da \rightarrow \infty$. The Navier-Stokes and the convection–diffusion equations are assumed to be decoupled. This assumption applies to dilute solutions with no effect of the solute on the flow pattern. Thus, the Navier-Stokes equation is first solved to obtain the steady-state velocity profile inside the channel. Then, the convection–diffusion equation is solved to find the concentration profile. The result is presented in Fig. 2.1b. The range of the non-dimensional parameters considered here is expected to yield a solution close to the Graetz problem. As shown, the data points satisfactorily follow the entrance-model curve for smaller values of $x^*$ and the fully-developed curve for larger values of $x^*$. Larger values of Peclet number will result in even better agreement especially in the entrance region.
Fig. 2.1. (a) Rectangular microchannel with the reactive side at the bottom. The channel is 10 \( \mu \text{m} \) wide and 500 \( \mu \text{m} \) long. Part of the channel close to the inlet is shown here. Surface and contour plots of concentration are presented, (b) Comparison of the numerical results with the entrance and fully-developed analytical solutions for \( Pe = 50 \) and \( Da \to \infty \).
2.3.2 Model validation against experimental results

Since the experimental part of this research had not been performed before the modeling, at this step, the experimental results from [84] were used to verify the simulation. In [84] hybridization kinetics of DNA strands were tested and reported for a rectangular microchannel with a reactive spot labelled with specific ligands. The adsorption/desorption kinetics was monitored by fluorescence microscopy. The DNA solution (29 basis pairs of strands) was released at the inlet (shown in Fig. 2.2a). The channel was $10\ mm \times 1\ mm \times 10\ mm$, and the inlet velocity was $1\ mm/sec$. The flow was turned on for $50\ min$, then turned off for $310\ min$ and again turned on for the rest of the experiment. The size of the reactive spot and the ligand surface concentration were not explicitly mentioned in [84]. Here, values consistent with those used in other examples of the same reference are applied. The spot is considered to be $4\ mm$ long, and the ligand surface concentration is $1 \times 10^{-8}\ mol/m^2$. The backward reaction rate is assumed to be negligible as mentioned in [84]. The values of diffusivity ($D$), forward reaction rate ($k_{on}$) and inlet concentration ($c_0$) were obtained in [84] by fitting the numerical method to the experimental data. The same strategy is followed here.
Fig. 2.2. Validation of the numerical model: (a) rectangular microchannel with the reactive plate shown in red and (b) the fit between the experimental data (green circles) obtained for 29 basis pairs of DNA strands and the numerical results (red solid line).
Figure 2.2b shows that for the set of variables obtained from fitting \( D = 7 \times 10^{-11} \text{m}^2/\text{sec}, k_{on} = 30 \text{m}^3/(\text{mol} \cdot \text{sec}), \ c_0 = 2.9 \times 10^{-6} \text{mol}/\text{m}^3 \), the numerical curve follows the experimental data very well. The numerical values for the diffusivity and the inlet concentration are found to be the same as those presented in [84]. A better fit can be obtained using the forward reaction rate of \( k_{on} = 30 \text{m}^3/(\text{mol} \cdot \text{sec}) \) instead of that reported in [84] (i.e., \( k_{on} = 75 \text{m}^3/(\text{mol} \cdot \text{sec}) \)). The two values are still in the same order of magnitude. The difference may be due to the discrepancies existing in the assumed surface concentration or reactive surface configuration. Now that the numerical model is verified, it is used to examine the performance of the three different design configurations presented in the next section.

2.4 Geometry

Figure 2.3 shows the microfluidic device with three different geometries considered for the analysis. The geometries are the parallel-plates (PP) open channel, circular micropillars (CM), and screen plates (SP). In all cases, the design criteria that the reactive capture area and the overall channel size must be the same are met. The length of the microchannel is 235 \( \mu \text{m} \), and the height is 120 \( \mu \text{m} \). The total length of the reactive capture boundary is considered 470 \( \mu \text{m} \) for the screen-plate and parallel-plate designs. The plates are assumed to be reactive only at the front side in the screen-plate design. The gap between the plates in the screen-plate design and between the cylinders in the circular-micropillar design is 10 \( \mu \text{m} \). For the circular micropillar design, the length of the reactive capture boundary is 471.2 \( \mu \text{m} \) since, in this case, it is not possible to have exactly the same length as the others and keep the flow path width constant at 10 \( \mu \text{m} \). Thus, a length very close to the set value (i.e., 470 \( \mu \text{m} \)) is selected. Compared to the numerical error in the simulations (assumed to be 1%), this difference is not expected to have a significant impact on the results.
Fig. 2.3. Geometries of three microreactors: (a) parallel-plate open channel (PP) in which top and bottom surfaces are reactive, (b) circular micropillars (CM) in which cylinder surfaces are reactive and (c) screen plates (SP) in which only front sides of the plates are reactive.
2.5 Results and discussion

2.5.1 Device performance comparison

In the following sections, two different parameters, including capture efficiency and average surface concentration adsorbed on the reactive capture area, are used to carry out the comparison. Capture efficiency is calculated based on [32]

\[
CE = 1 - c^*_b,\text{outlet}
\]  

\(c^*_b,\text{outlet}\) is the nondimensional form of the bulk concentration at the outlet defined as

\[
c^*_b,\text{outlet} = \frac{1}{c_0u_{avg}h} \int \text{c}_{\text{outlet}}u_{\text{outlet}} \, dy
\]  

Throughout this chapter, this parameter is used to find the gain in the adsorbed concentration whenever a design factor is changed and the reactive area is unchanged.

It is clear that increasing the area leads to higher adsorbed concentrations and capture efficiencies. Therefore, there must be another criterion to scale the gain in the adsorbed solute and include the effort made for preparing the reactive surface. To meet this, average surface concentration, \(C_{s,avg}\), is determined. \(C_{s,avg}\) is interpreted as the performance of the device and obtained from the following formula for the unit depth of the channel

\[
C_{s,avg} = \frac{1}{l} \int_{\text{reactive area}} C_s \, dx
\]  

where \(l\) is the length of the reactive surface. \(C_{s,avg}\) is used whenever the reactive surface area is changed for a device.

Table 2.1 presents the numerical values of the chemical and physical parameters and the corresponding non-dimensional numbers used for the simulations. These values are within the range observed in common biological reactions [11, 21]. As mentioned before, COMSOL Multiphysics software is used to solve the governing equations in two steps. First, the Navier-
Stokes equations are solved in the domain and the steady-state velocity is obtained. Then, the transient mass transport equation is used along with the kinetic equation to derive the concentration profile of the solute in the bulk and on the surface.

Table 2.1. Numerical values of the parameters used for the simulations in Figs. 2.6-2.8 (taken form [11, 21]).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward reaction rate ($k_{on}$)</td>
<td>$10^5 , m^3/(mol\cdot sec)$</td>
</tr>
<tr>
<td>Backward reaction rate ($k_{off}$)</td>
<td>$10^{-2}, sec^{-1}$</td>
</tr>
<tr>
<td>Ligand concentration ($c_{s0}$)</td>
<td>$10^{-8}, mol/m^2$</td>
</tr>
<tr>
<td>Diffusion coefficient ($D$)</td>
<td>$10^{-11}, m^2/sec$</td>
</tr>
<tr>
<td>Inlet concentration ($c_0$)</td>
<td>$10^{-6}, mol/m^3$</td>
</tr>
<tr>
<td>Average inlet velocity ($u_{avg}$)</td>
<td>$10^{-4}, m/sec$</td>
</tr>
<tr>
<td>Peclet number ($Pe$)</td>
<td>100</td>
</tr>
<tr>
<td>Damkohler number ($Da$)</td>
<td>1000</td>
</tr>
<tr>
<td>Relative adsorption capacity ($\varepsilon$)</td>
<td>0.01</td>
</tr>
<tr>
<td>Equilibrium dissociation constant ($K_D$)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Figure 2.4 demonstrates how the three geometries introduced in Section 2.4 are meshed. The buffer liquid with the solute is allowed to flow through the channel for 5 minutes which is in the range of usual sampling times for high-throughput microfluidic devices [84]. Due to symmetry, only half of each geometry is modeled. The Reynolds numbers investigated here lie in the range of $10^{-5}$ to $10^{-3}$ (corresponding to the average velocities of 1 to 100 $\mu m/sec$) which is in the Stokes flow regime.
Figure 2.5 depicts the streamlines for two of the geometries (i.e., screen plates and circular micropillars) which are not as straightforward as the parallel-plate design. The streamlines show a laminar flow of the solution without forming any vortices in the vicinity of the reactive areas. Figures 2.6-2.8 present sample concentration plots within the domain and on the reactive surfaces for each design. After 5 minutes, the capture efficiency ($CE$) is 1.6%, 15.5% and 15.7% for the parallel-plate (PP), circular-micropillar (CM) and screen-plate (SP) designs, respectively. As expected, the efficiency significantly improved by changing the design from the open channel to the packed-bed geometry. However, for the packed-bed designs, there is no significant difference (within the numerical simulation error) between the screen plates and the circular pillars.
Fig. 2.4. View of the mesh in: (a) parallel-plate open channel, (b) circular micropillars and (c) screen plates.
Fig. 2.5. Streamlines in: (a) circular micropillars and (b) screen plates for $Re = 10^{-3}$. Edge effects are observed at the outlet far from the reactive obstacles.
Fig. 2.6. Concentration plot for the parallel-plate channel: (a) in the bulk and (b) on the reactive surface. The small overshoots happen due to the edge effect.
Fig. 2.7. Concentration plots for circular micropillar design: (a) in the bulk and (b)-(k) on the reactive surfaces for $Pe = 100$, $Da = 1000$, $K_p = 0.1$ and $\varepsilon = 0.01$. Each reactive surface is a quarter of a circle numbered in Fig. 2.7a. $x$ is measured on the perimeter of each quarter circle as shown in Fig. 2.7a.
To compare the three designs more accurately, the simulation is conducted for four different Peclet numbers. The Damkohler number is kept constant (at 1000), so the problem is in the transport-limited regime (which means the adsorption rate is sensitive to the changes in the rate of transport). Therefore, any enhancement in the transport will be signified in the final capture efficiency. This is desirable since changing the geometry directly influences the transport. An efficient geometry will easily be identified through examining the capture efficiency. $Pe$ is changed because convection and diffusion, as the two mechanisms of transport, are of great importance. The corresponding trends reveal the relative importance of each term. Figure 2.9 shows the capture efficiency of three different designs versus $Pe$ for the reactive lengths of 470 $\mu$m. For all the geometries, as $Pe$ increases, $CE$ decreases since the convection time scale becomes smaller than the diffusion time scale. As a result, solute particles are carried away by water before they diffuse to the reactive surfaces. Conversely, for smaller $Pe$, particles have enough time to adhere to the surface. Since the time scale of reaction is short (large Damkohler number), very large capture efficiency are gained for small $Pe$.

The results in Fig. 2.9 show that the capture efficiencies for the two packed-bed configurations (i.e. the screen plates and the circular pillars) are significantly larger than the capture efficiency of the parallel plates. This improvement is due to the significant reduction in the diffusion path of the solute in the bulk and also the effect of convection. The ratio of the capture efficiency of
the parallel plate design to the screen plate (or circular pillar) design monotonically increases from 1.4 to 10 as $Pe$ number increases from 1 to 100. So, the relative gain seems to be more considerable for higher Peclet numbers.

![Graph showing capture efficiency versus Peclet number for different designs with reactive length = 470 μm, $Da = 1000$, $K_D = 0.1$ and $\varepsilon = 0.01$.](image)

**Fig. 2.9. Capture efficiency versus Peclet number for different designs with reactive length = 470 μm, $Da = 1000$, $K_D = 0.1$ and $\varepsilon = 0.01$.**

To gain a better insight into the results, two parameters are introduced: the diffusion time (i.e., the time necessary to transport a particle to the reactive surface through diffusion) and the convection time (i.e., the time within which a particle travels with the bulk flow through the reactive domain). These two times are defined as
where \( l_d \) is the diffusion path which is assumed to be half of the gap between the reactive surfaces, \( l \) is the total length that a particle travels close to a reactive surface, \( u_{\text{max}} \) is assumed to be the maximum flow velocity in the device, and \( D \) is the diffusion constant. These parameters provide a rough estimate of the diffusion and convection times for a particle which is moving in the middle of the gap between the reactive plates. The ratio of these two times \((t_c/t_d)\) determines the relative capacity of the device to capture the particle. Larger values are favourable since they indicate smaller time scales of diffusion and larger efficiencies. For instance, for \( Re = 10^{-3} \) and \( Pe = 100 \), this ratio \((t_c/t_d)\) is approximately 0.02, 0.1 and 0.12 for parallel plates, circular pillars and screen plates, respectively. Comparing these values demonstrates the relative superiority of the packed-bed design with respect to the open channel. The comparison between the capture efficiencies of the screen plate (SP) and circular micropillar (CM) designs shows that their performances are almost indistinguishable (see Fig. 2.9). Considering the numerical error in the simulation, it is concluded that there is no significant gain in changing the design from the screen plates to the circular micropillars. Fabrication process may be the same in terms of difficulty for SP and CM designs. However, screen plates greatly facilitate the process of antibody immobilization and also the detection of the adsorbed particles on the reactive surfaces. Considering the good performance and ease of implementation, screen-plate design is a favourable option to use in the pathogen separation process in the laboratory. In the next section, this configuration is analyzed in terms of the effect of the design parameters.

### 2.5.2 Analysis of the screen-plate design

This section investigates the influence of the physiochemical design parameters and the geometric design parameters (including the number of the plates and reactive side preference)
on the output of the pathogen separation process for the screen-plate reactor selected in the previous section. The effects of physiochemical parameters are analyzed in terms of the nondimensional factors presented in Section 2.1.3.

**Physiochemical parameters**

Bioparticles differ in their affinity to bind to each other. Different types of ligands result in different values of the forward and backward reaction rates [5, 11, 21]. Also, they may have different diffusivities in different solutions used for in-vitro experiments. For instance, the diffusion coefficient for protein A33 used in immunoassay study is around $10^{-10} \text{ m}^2/\text{sec}$ while it is around $4 \times 10^{-11} \text{ m}^2/\text{sec}$ for DNA [32]. All the physiochemical parameters are combined in the four non-dimensional numbers (introduced in Section 2.1.3) to simplify the analysis. Among these four numbers, Damkohler and Peclet numbers are of great importance as they represent the relative strengths of reaction, convection and diffusion once the type of reactants, the ligand surface density and the inlet concentration are known. Figure 2.10 depicts the trend of the capture efficiency ($CE$) versus Damkohler number for three different values of Peclet number (keeping $\varepsilon$ and $K_D$ constant) for the time that bulk concentration has reached its steady state value (dynamic equilibrium). It is shown that the capture efficiency generally increases as $Da$ increases for any values of $Pe$. However, the rate of increase is less for larger $Da$ since the process has shifted to the transport-limited regime ($Da \to \infty$), and higher rate of reaction (with constant diffusivity) does not improve the efficiency significantly. This explains the plateau on the right part of the plots. In addition, Figure 2.10 shows that for any values of $Da$, decreasing $Pe$ augments the efficiency. This behaviour was also observed in the previous section where different designs were compared for $Da = 1000$ (Fig. 2.9). It is worth mentioning that in all the plots throughout this chapter, whenever the efficiency approaches very small values (lower than 1%), the results are not accurate because of the numerical error margin considered for the simulation.
Fig. 2.10. Capture efficiency of screen plates versus Da number for different Pe numbers ($K_D = 0.1$ and $\varepsilon = 0.01$).

**Number of reactive units**

An interesting geometric study in the screen-plate design is the investigation of the effect of the number of reactive plates on the performance of the device. Since the reactive area is changed in this case, the average surface concentration is plotted to scale the gain obtained by adding the plates. Figure 2.11 presents the nondimensional average surface concentration, $c_{s,avg}$, versus the number of units. Each reactive unit consists of two screen plates positioned consecutively. This is shown at the top right side of the figure. Changing the number of units from 1 to 2 enhances the average adsorbed concentration. However, $c_{s,avg}$ drops continuously afterwards. This means that despite the gain in the adsorbed surface concentration, the scaled
performance of the device (i.e., surface concentration per area) decreases for higher surface areas. A close look at the front side of the first reactive plate in the first unit demonstrates its low capacity to capture particles (for example, see Fig. 2.8b). This capacity significantly improves from this side to the front side of the first reactive plate in the second unit and changes less significantly from the second unit to the third one and so on (compare the areas under the curves in Fig. 2.8b, Fig. 2.8d and Fig. 2.8f). In other words, the small increase in the surface concentration because of adding downstream units (after the second unit) does not compensate the increase in the reactive area. Hence, the ratio, which is the average surface concentration, decreases. This explains why the performance is higher for two units than one unit, and is successively lower for larger unit numbers.

![Graph showing the relationship between the number of units and nondimensional average surface concentration.](image)

**Fig. 2.11.** Nondimensional average surface concentration versus number of reactive units for $Pe = 100$, $Da = 1000$, $K_D = 0.1$ and $\varepsilon = 0.01$. The single unit shown on the top right side of the plot includes two plates reactive on the front side.
Double-sided reactive surfaces

While fabricating the device, either side or both sides of the plates can be labelled depending on the experimental conditions. Although the double-sided reactive plates have the advantage of providing the same reactive area within a smaller device, the capture efficiency is still the main criterion to select the configuration. Figure 2.12 shows the capture efficiency for single-sided (SS) and double-sided (DS) devices with different reactive lengths at three Peclet numbers. This figure reveals the fact that for all the range of reactive areas investigated here, the double-sided design has lower capture efficiency compared to the single-sided design (having the same reactive area). For lower Peclet numbers (smaller diffusion time scale), the efficiencies are very close since the dominant mechanism (diffusion) works equally for both designs. As Peclet number increases, the difference between efficiencies is more significant. Thus, convection is more influential when the reactive areas are fabricated single-sided against the flow.
2.6 Conclusions

Finding the optimal device configuration to capture as many particles as possible is a crucial step in the design of microfluidic devices with flow-through channels and mass transport to reactive surfaces. The determination of the optimal configuration as well as the best range of working condition is very complicated due to the large number of parameters governing the physics of the problem. The numerical model introduced in this chapter enables the researcher to compare the performance of alternative assemblies of the same reactive area inside a fixed volume. Packed-bed designs (including screen plates and circular micropillars) were proved to enhance significantly the reaction performance over the conventional open-channel reactors.

The numerical model presented here also allows the thorough examination of the effect of design parameters on the device efficiency. The screen-plate design was selected to do the
parametric study due to good performance and ease of fabrication. It was shown that adding to the number of reactive plates does not generally increase the scaled performance (average surface concentration) of the device. In addition, labelling reactive surfaces on both sides was found to decrease the capture efficiency of the device compared to the single-sided reactive surfaces with the same reactive area. This decrease is more considerable for larger Peclet numbers. Different regimes (such as transport-limited regime and reaction-limited regime) were also observed when physiochemical parameters were changed over their usual ranges in biochemical reactions. Recognizing these regions can be useful to find out where the experiment conditions lie and how to adjust them since some changes to the parameters do not significantly contribute to the output and are worth neither the effort nor the cost.

As it was seen in the simulations presented in this chapter, for all cases except very large particle diffusivity, the capture efficiency never reached 100%. For most pathogens the diffusivity is not very large since they are relatively large in diameter. This is not desirable as even a few pathogens may cause infection. Hence, in order to gain large capture efficiencies, other remedies than the geometry/configuration must be considered. In the next chapter, possible options for such remedies are presented and discussed.
3  Numerical modeling of particle transport: active separation

Active methods as alternative approaches to increase the capture efficiency of the proposed microfluidic device are investigated in this chapter. Two different methods (sedimentation and electrophoresis) are selected among all the feasible options since they are among the simplest, most effective techniques. The theory behind these methods is well developed and they have widely been employed in many practical applications to separate different species ranging from biomolecules to biological cells [64, 66, 82].

Similar to Chapter 2, numerical simulation in Eulerian framework is used to study the effectiveness of each active method. In Chapter 2, the screen-plate configuration was determined to be the optimum geometry. Here, the analysis is conducted for part of the flow path which is located between each two plates (channel walls) where the flow streamlines are parallel and unaffected by entrance and exit regions. This will help concentrate on studying the benefit that each method introduces to the overall capture process. Once a method is proved to work accurately, it can be extended to either parallel plate or screen plate geometry.

3.1  Sedimentation for pathogen transport

3.1.1  Theory of sedimentation

Figure 3.1 depicts the two main forces that are applied on a particle moving in a flow in a gravitational field. The final path of a particle is determined through calculating the resultant of these two forces at each instant. From Fig. 3.1 it is concluded that in order to trap a particle, the capture surface must have a minimum length so that the particle does not exit the domain

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1 Parts of Chapter 3 and Appendix A have been published in a journal article and a conference proceeding. Reprinted with permission from [109, 110].
before it reaches the capture sites. This minimum length is calculated by finding the particle trajectory under the influence of both gravitational and drag forces.

![Diagram of forces](image)

**Fig. 3.1. Schematic view of two main forces applied on a particle in a gravitational field.**

Eulerian dispersed-phase approach (transient multi-component mixture flow modeling) is used to follow the bioparticles in the microfluidic device. Particles are considered to be the dispersed (solid) phase mixed in a continuous (liquid) phase with a different density. The discrete phase is exposed to gravitational forces and sediments inside the device due to its different density. At each time instant, the volume fraction of the dispersed phase is found through the following set of equations.

The momentum transport equation for the mixture is

\[ \rho \frac{\partial \mathbf{u}}{\partial t} + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} = -\nabla p - \nabla \cdot (\rho \rho_{cd} (1 - c_d) \mathbf{u}_{slip} \mathbf{u}_{slip}) + \nabla [\eta (\nabla \mathbf{u} + \nabla \mathbf{u}^T)] + \rho g \]  

(3.1)

where \( \mathbf{u} \) is the mixture velocity, \( p \) is the pressure, \( \rho \) is the mixture density, \( \mathbf{u}_{slip} \) is the relative velocity between the dispersed and the continuous phase and \( c_d \) is the dimensionless dispersed
phase mass fraction. The following equations are used to relate the mixture viscosity, mixture density and \( c_d \) to other variables.

\[
\eta = \eta_f \left(1 - \frac{\phi_d}{\phi_{max}}\right)^{-2.5\phi_{max}}
\]  
\( (3.2) \)

\[
\rho = (1 - \phi_d) \rho_f + \phi_d \rho_d
\]  
\( (3.3) \)

\[
c_d = \phi_d \frac{\rho_d}{\rho}
\]  
\( (3.4) \)

\( \eta_f \) is the dynamic viscosity of the continuous fluid phase and \( \phi_{max} \) is the maximum packing concentration in the Krieger expression (Eq. 3.2). \( \phi_d \) is the volume fraction of the dispersed phase, and \( \rho_d \) and \( \rho_f \) are the densities of the dispersed phase and the continuous phase, respectively.

The continuity equation in its original form is

\[
(\rho_f - \rho_d) \left[ \nabla \cdot (\phi_d (1 - c_d) \mathbf{u}_{slip}) \right] + \rho_f (\nabla \cdot \mathbf{u}) = 0
\]  
\( (3.5) \)

The dispersed phase transport equation is

\[
\frac{\partial \phi_d}{\partial t} + \nabla \cdot (\phi_d \mathbf{u}_d) = 0
\]  
\( (3.6) \)

where the dispersed phase velocity is

\[
\mathbf{u}_d = \mathbf{u} + (1 - c_d) \mathbf{u}_{slip}
\]  
\( (3.7) \)

The system of equations for the three main unknowns of the flow domain (\( \phi_d, \mathbf{u} \) and \( \mathbf{u}_{slip} \)) is closed knowing the mixture momentum transport equation (Eq. 3.1), continuity equation (Eq. 3.5) and dispersed phase transport equation (Eq. 3.6).
3.1.2 Geometry

A portion of the screen plate (which also resembles the two parallel plates) is used for the numerical analysis (see Fig. 3.2). This channel will resemble the region where the flow streamlines are unaffected by the inlet and outlet. The simulation is conducted in the presence of only the gravitational force (i.e., sedimentation). The length of the microchannel is 235 $\mu m$, and the height is 120 $\mu m$. The total length of the reactive capture boundary is considered 470 $\mu m$.

3.1.3 COMSOL model specifications

COMSOL Multiphysics software (Two-phase Flow module) is used to solve the system of equations in Section 3.1.1. To find the solid-phase volume fraction at each instant, COMSOL Transient solver (direct UMFPACK) is selected with default relative tolerance of $10^{-4}$ and absolute tolerance of $10^{-3}$. A triangular mesh (created by COMSOL free mesh generator) is used throughout the domain.

3.1.4 Results and discussion

Figure 3.2 presents the channel introduced in the previous section. The maximum inlet velocity is 1.25 $\mu m/sec$ in this case. The density of the solid phase is assumed to be 1050 $kg/m^3$ consistent with the values reported for Cryptosporidium oocysts in the literature [85]. The density and viscosity of the liquid phase are equal to those of water ($\sim 1000 \ kg/m^3$ and 0.001 $Pa. \ sec$). Sedimentation is the only driving force to bring the particles to the surface.

As it is shown, for this flow rate, the particles (shown in red) completely accumulate on the surface of the substrate. None of the particles leave the domain. Therefore, it can be interpreted that for the given flow rate and density difference, the reactive length (235 $\mu m$) is sufficient to act as a trapping zone. In other words, the settling velocity due to sedimentation is large enough to bring the pathogens to the capture surface. However, the corresponding liquid phase velocity is very small (i.e., in the order of a few microns per seconds). As the plot
demonstrates, 100% capture is achieved at the expense of a very low inlet flow rate which is not desirable since this reduces the overall sampling speed. Thus, approaches expediting the separation process must also be considered. In the next section, electrophoresis as another active separation process is studied.

Fig. 3.2. Simulation results for sedimentation of solid particles inside a microchannel. $\phi_{d, \text{inlet}} = 10^{-8}$, $u_{\text{in, max}} = 1.25 \, \mu m/sec$, $\rho_d - \rho_f = 50 \, kg/m^3$. Red colour shows the high dispersed-phase volume fraction.

3.2 Electrophoresis for pathogen transport

3.2.1 Theory of electrophoresis

Electrophoresis (EP) is the term referring to the use of uniform electric field for manipulation of charged particles [86]. The uniform electric field can be simply produced using a DC power supply. In electrophoresis, the natural charge of particle (such as a biomolecule or a cell) is used
along with an electric field (applied through internal or external electrodes) to drive the bioparticles to a desirable location. Figure 3.3 depicts the schematic view of the forces applied on a charged particle in a fluid-electric-gravitational field. Once the potential is applied, the bioparticles (immersed in the buffer) move towards the oppositely-charged electrodes as a result of the electrophoretic forces. As the bioparticles move across the channel height and become close to the boundaries, they may be trapped in the presence of a capture zone [31]. Depending on the orientation of the device (vertical or horizontal), the gravitational force may be favourable or neutral. The governing equations coupling the effects of the flow, electric field, mass transport and chemical reactions at the boundaries are as follows.
Fig. 3.3. Schematic view of three main forces applied on a negatively-charged particle in both electric and gravitational field for a) a horizontal device, and b) a vertical device.
**Continuity and momentum transport equations**

The fluid medium is assumed to flow through a rectangular microchannel. The external electric field is applied in transverse direction so that no electroosmotic velocity develops in the channel. Two-dimensional incompressible Navier-Stokes equations are used along with the continuity equation to find the velocity profile throughout the domain.

\[
\nabla \cdot \mathbf{u} = 0
\]

\[
\rho \frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \eta \nabla^2 \mathbf{u} + \rho_s \mathbf{E}
\]

where \( \mathbf{E} \) is the electric field strength. Gravity is neglected in the calculations to isolate the effect of electric field. This is the same as assuming a vertical device instead of a horizontal one (as shown in Fig. 3.1) with the electrophoretic forces acting instead of gravity. The no-slip boundary condition is assumed at the walls. Constant velocity is specified at the inlet. The outlet is assumed to be at atmospheric pressure.

**Mass transport equations**

It is assumed that the particle is released at the inlet at a specific concentration \( (c_0) \), and it is monitored after a certain sampling time period. This sample is carried by the fluid and transported to the reactive surfaces through diffusion and convection due to both the velocity of the carrier fluid and the electrophoretic mobility of the bioparticles [31]. The transient two-dimensional mass transport equation, which is solved inside the domain (neglecting the effect of external forces other than the electric field), is

\[
\frac{\partial c}{\partial t} + \mathbf{u} \cdot \nabla c - \nabla \cdot (\mu_e \nabla V) = D \nabla^2 c
\]

where \( c \) is the particle concentration, \( \mu \) is the electrophoretic mobility, \( V \) is the electric potential and \( D \) is the diffusion coefficient. Electrophoretic mobility is a function of the zeta potential \( (\xi) \) which itself is a measure of the surface charge of a particle in an ionic solution [87].
\[ \mu = \frac{f(r)\varepsilon_r\varepsilon_0\xi}{\eta} \]  

(3.11)

\(\varepsilon_r\) is the relative permittivity of the fluid medium, \(\varepsilon_0\) is the permittivity of the vacuum, \(\eta\) is the medium dynamic viscosity and \(f(r)\) is a constant which is a function of the particle radius and electric double layer thickness [87]. The electric double layers form around the charged particles due the accumulation of oppositely-charged ions in the fluid medium solution.

Figure 3.4 shows how zeta potential (or electrophoretic mobility from Eq. 3.11) is derived. As shown, counterions in the solution gather around the charged particle due to the electrostatic forces and reduce the effective charge sensed by any external force field. Different layers form around the particle surface. The first layer is the Stern layer in which the counterions are fixed on the surface of the charged particle and move with the particle. The second layer is the diffuse layer where the diffusion of the ions counterbalances the electrostatic forces. At some distance from the particle surface, the counterions can freely move without substantially being under the influence of the surface charge of the particle. This plane is called the slipping plane. The electric potential at the slipping plane is the zeta potential (Fig. 3.4) mentioned above. This potential is then used in Eq. 3.11 to find the electrophoretic mobility which is then substituted in Eq. 3.10 to calculate the electrophoretic flux.
Fig. 3.4. Schematic view of different layers in an electric double layer around a charged particle and definition of zeta potential.
For the chemical reaction at the reactive boundaries, the rates of adsorption and desorption of the sample are included in the problem through a concentration flux term. The kinetic equation of the chemical reaction is used to calculate this flux

\[
\frac{\partial c_s}{\partial t} = k_{on}c_{wall}(c_{s0} - c_s) - k_{off}c_s
\]  

(3.12)

where \(c_s\) is surface concentration of the particle, \(c_{wall}\) is the particle bulk concentration at the reactive surface and \(c_{s0}\) is the ligand surface concentration (capture site density). \(k_{on}\) and \(k_{off}\) are the forward and backward reaction rates. At the outlet, the convective flux is specified as

\[
n \cdot (-D \nabla c - \mu c \nabla V) = 0
\]  

(3.13)

All other boundaries are insulated, which means

\[
n \cdot (-D \nabla c - \mu c \nabla V + c u) = 0
\]  

(3.14)

where \(n\) is the normal vector. For all of the above equations, the change in the physical properties due to the temperature variation is neglected [33].

**Electrostatic equation**

Electric potential is applied on the electrodes assembled in the device to direct bioparticles towards the reactive surfaces using their natural charge. The Poisson equation is solved to derive the potential field inside the domain.

\[
\nabla^2 V = -\frac{\rho_s}{\varepsilon_0 \varepsilon_r}
\]  

(3.15)

\(\rho_s\) is the space charge density, \(\varepsilon_0\) is the permittivity of the vacuum and \(\varepsilon_r\) is the relative permittivity of the fluid medium. In this equation, the space variation of the permittivity is neglected. Device electrodes are either grounded or supplied with constant electric potentials. In addition, the space charge density, which is a function of the charge of the species carried in the buffer, is assumed to be zero in the bulk according to the assumption of electroneutrality [33]. This means that in the bulk flow, the ions (EDLs) are able to screen the charge of the
bioparticles so that the net charge of the solution is zero everywhere except in the proximity of the electrodes. If the electroneutrality assumption is violated (e.g., for very large particle concentrations where EDL overlap occurs), the simulation is much more complicated as the momentum transport, mass transport and electrostatic equations become fully-coupled. A detailed analysis of such situation is presented in Appendix A.

3.2.2 Geometry

The microfluidic channel presented in the previous section is used for the numerical analysis. The simulation is conducted in the presence of only the electrophoretic force. The length of the microchannel is $235 \mu m$, and the height is $120 \mu m$. The total length of the reactive capture boundary is considered $470 \mu m$.

3.2.3 COMSOL model specifications

COMSOL Multiphysics software (Incompressible Flow, Convection-Diffusion and Electrostatic modules) is used to solve the system of equations in Section 3.2.1. It is assumed that the flow and the electric field independently reach steady state and the fields are decoupled (i.e., the zero space charge density assumption). COMSOL Direct UMFPACK Stationary solver is used to solve the Navier-stokes equations and electrostatic equation. The relative tolerance for the Stationary solver is $10^{-6}$. To obtain the concentration profile, COMSOL Transient solver (direct UMFPACK) is selected with the default relative tolerance of $10^{-4}$ and absolute tolerance of $10^{-3}$. The mesh size is selected depending on the $Pe$ number in the simulations to prevent numerical instability (normally larger than 70000 elements were used). A triangular mesh (created by COMSOL free mesh generator) is used throughout the domain and is refined near the reactive boundaries to capture the high gradients of concentration. Throughout the analysis, mesh independency was achieved by refining the grid until the average surface concentration on reactive walls did not change more than 1% of the previous run.
3.2.4 Results and discussion

Figure 3.5 presents the simulation results for the set of parameters given in Table 3.1. These values were taken from the literature and the values related to the Cryptosporidium oocysts or similar microorganisms [21, 26, 31, 75]. As Fig. 3.5 illustrates, the bioparticle sample introduced at the inlet (shown in red) flows into the open space in between the electrodes and is attracted to the electrode with the opposite charge. The plot is presented at $t = 60 \text{ sec}$ when the dynamic equilibrium has been reached. At this time no change is observed in the appearance of the concentration profile.

Table 3.1. Numerical values of the parameters used in the particle electrophoresis simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward reaction rate ($k_{on}$)</td>
<td>$10^5 \text{ m}^3/(\text{mol. sec})$</td>
</tr>
<tr>
<td>Backward reaction rate ($k_{off}$)</td>
<td>$10^{-2} \text{ sec}^{-1}$</td>
</tr>
<tr>
<td>Ligand concentration ($c_{s0}$)</td>
<td>$5 \times 10^{-8} \text{ mol/m}^2$</td>
</tr>
<tr>
<td>Diffusion coefficient ($D$)</td>
<td>$1 \times 10^{-10} \text{ m}^2/\text{sec}$</td>
</tr>
<tr>
<td>Inlet concentration ($c_0$)</td>
<td>$1.5 \times 10^{-6} \text{ mol/m}^3$</td>
</tr>
<tr>
<td>Maximum inlet velocity ($u_{f,max}$)</td>
<td>$1.5 \times 10^{-4} \text{ m/sec}$</td>
</tr>
<tr>
<td>Electric potential ($V_0$)</td>
<td>$0.5 \text{ V}$</td>
</tr>
<tr>
<td>Electrophoretic mobility ($\mu$)</td>
<td>$1.5 \times 10^{-8} \text{ m}^2/(\text{V. sec})$</td>
</tr>
</tbody>
</table>
According to Fig. 3.5, for the given maximum velocity and applied voltage, particles cannot exit the microchannel at least until the surface is saturated (i.e., all the binding sites are occupied). The maximum inlet velocity for 0.5 \( V \) voltage difference is 150 \( \mu m/sec \). Given that the channel size is the same as for the sedimentation case, and the particle properties (such as density, charge, etc) are all similar to those of Cryptosporidium in both cases, the maximum separation flow rate for the electrophoresis is found to be larger than that obtained for sedimentation (almost 100 times). This can be enhanced even more considering the fact that the applied voltage can be increased to almost twice this value (refer to Chapter 5 for challenges regarding the maximum applied voltage). Therefore, the use of electrophoresis is expected to improve significantly the sampling rate and the total sampling volume over the sedimentation. However, the results of the current simulation are questionable due to major simplifications explained below.
3.3 Comparative study of sedimentation and electrophoresis for pathogen transport

As it will be discussed later in Chapter 5, the electrophoresis theory encounters technical hitches when applied in real experimental setups. One of the most important issues in using the electric field is the limitation in the maximum electric potential difference that can be applied between the electrodes. Due to electrochemical effects such as water electrolysis and electrode oxidation, this voltage cannot exceed a certain limit (a few volts) depending on the experimental conditions. On the other hand, it has been demonstrated in the literature that the actual electric potential difference sensed by the charged particle in the bulk flow is not any larger than one percent of the nominal field [79]. This drop is created by the formation of the electric double layers in the immediate proximity of the electrodes. Therefore, the electric potential difference considered in the simulations should not be larger than tens of millivolts. This subject will be studied later in Chapter 5.

Considering the above discussion, a comparative numerical study of sedimentation and electrophoresis separation techniques is performed based on the actual electric potential difference. The diffusion is assumed to be very small (in the order of $10^{-13}$) as large pathogens like Cryptosporidium are dealt with. To verify the magnitude of the diffusion constant Stokes-Einstein equation is used as follows:

$$D = \frac{k_B T}{6\pi \eta r} = \frac{1.38 \times 10^{-23} \times 298}{6\pi \times 10^{-3} \times 2 \times 10^{-6}} \approx 1.1 \times 10^{-13} m^2/s$$

(3.16)

The above equation provides an approximation for the diffusion constant for a particle as big as Cryptosporidium. The diffusion constant was assumed to be larger in previous simulations. Larger values do not make a significant difference in the bulk transport since the main mechanism of transport is external forces (such as gravity or electric field) in active separation. The corresponding results for the effects of sedimentation and electrophoresis are plotted in Fig. 3.6. All the geometrical and physical characteristics are identical. For the density and the charge, those of Cryptosporidium oocyst are used from the literature [85] and measurements
conducted in laboratory (refer to Table 5.1 in Chapter 5). The maximum inlet velocity is assumed to be the same for both cases (equal to 1.25 μm/sec). The electric potential difference is 5 mV for the electrophoretic transport. From the concentration profiles obtained for the two approaches one can conclude that the sedimentation and electrophoresis have similar impact on transporting the bioparticles to the reactive surface. Therefore, it is concluded that sedimentation can be as effective as electrophoresis in separating and manipulating the particles towards the reactive elements considering all the limitations and challenges it may introduce to the problem. The above fact is also shown in the experimental results presented in Chapter 5.
3.4 Conclusions

Two active methods were selected and analyzed to enhance pathogen transport. Eulerian-based numerical modeling was implemented to obtain an overall picture of transport to the reactive capture elements. It was shown numerically that both methods work favourably if the microfluidic device is designed carefully to incorporate sufficient reactive length. Electrophoresis was demonstrated to work equally as sedimentation. However, the

Fig. 3.6. Concentration plots of bioparticle separation after 300 sec using a) sedimentation and b) electrophoresis (with negligible diffusion and $\Delta V = 5 \text{ mV}$). Maximum inlet velocity is $1.25 \mu \text{m/sec}$ in both cases. The results indicate that electrophoresis and sedimentation have similar effect on transporting the bioparticles.
electrophoresis approach offers benefits to the overall separation process. For instance, by modulating the buffer solution properties, the maximum allowable electric potential difference can be increased, and the drop in the electric potential adjacent to the electrodes can be reduced. Also, larger electrophoretic mobility may be obtained with different buffer properties or for different types of bioparticles. Employing the charge-to-mass ratio of the target particles as opposed to only the mass (which is important in the sedimentation), electrophoresis introduces more specificity to the separation technique when a mixture of pathogens and other external interfering particles are dealt with. Considering all the above facts, integrating both sedimentation and electrophoresis seems to be an attractive approach to improve the transport rate of pathogens to the reactive capture elements.
Passive and active separation techniques were analyzed numerically in the previous chapters providing information about the device geometry and configuration, device dimensions and external force field characteristics. This, of course, needs to be confirmed through in situ observations. However, before conducting any experiment, first, it has to be guaranteed that the transported pathogens are trapped and held by the ligand molecules (anti-pathogen antibodies) at the capture elements. The interaction of chemical bonds and the hydrodynamics of the flow then becomes of interest in order to examine the capability of the microfluidic device in capturing besides transport. The fine-scale volume-resolved simulation of biological cell adhesion at the molecular level is presented in this chapter. Cells are considered as deformable discrete particles (long 2D cylinders) immersed in a buffer flowing in a microchannel. A very small region close to the reactive capture surface was focussed on. A coupled simulation of flow hydrodynamics, cell deformation and chemical reactions is then carried out, and the effects of different physiochemical and structural parameters (such as forward and backward reaction rates, antibody density, cell membrane receptor density, cell membrane rigidity, cell cytoplasm viscosity, etc.) are discussed. In this study, the membrane of the cell is considered as a separate layer possessing both extensional and bending rigidities. Unlike Khismatullin’s work [54] (introduced in Chapter 1) in which the membrane was modeled using surface tension, a separate layer with different resistivity against motion in different directions is believed to be a more realistic model of the cell membrane [34] and consequently a more accurate approach to characterize the adhesion phenomenon for a wide range of biological microorganisms. When the model is developed, it is tuned to incorporate the properties of Cryptosporidium oocyst and find the corresponding adhesion design parameters.
4.1 Theory of cell adhesion coupled with flow hydrodynamics

This section is divided into two subsections: i) explaining the molecular-scale kinetics of cell adhesion by assuming a first-order chemical reaction at the interface between the cell and the substrate, and ii) describing the governing equations for the fluid flow and cell motion.

4.1.1 Cell adhesion

In this section, only the specific cell adhesion is considered, i.e., the adhesion due to the chemical reaction between complementary molecules (receptors and ligands) at both surfaces of the cell and the reactive substrate [11]. This chemical reaction which is a reversible reaction occurring in both formation and breaking directions is as follows

\[ L + R \rightleftharpoons [LR] \]  (4.1)

In this equation, \( L \) stands for ligand molecules and \( R \) stands for receptor molecules. The first order reaction kinetic equation, used to describe the principle phenomena in reversible molecular binding [11, 21], follows the form

\[ \frac{dN_b}{dt} = k_f (N_r - N_b)(N_l - N_b) - k_r N_b \]  (4.2)

where \( N_b \) is the number of bonds; \( N_r \) is the number of receptor molecules; \( N_l \) is the number of ligands; and \( k_f \) and \( k_r \) are the forward and reverse reaction rates, respectively. In this study, the cell is considered as a sphere. Cylindrical microvilli (arm-like protrusions at the cell membrane) are distributed uniformly around the cell’s perimeter. Receptor molecules are assumed to be concentrated at the tip of each microvillus, and ligand molecules are distributed on the substrate similar to the model presented in [88]. At each time step, the number of the bonds formed on each microvillus depends on the forward and backward reaction rates (presenting the formation and breakage of the bond, respectively) and the availability of free binding sites on the surfaces of the cell and the substrate. Assuming chemical bonds behave like springs, the following equations are used to find the reaction rates based on the distance between complementary molecules [88]
In these equations, $k_b$ is the Boltzmann constant; $T$ is the temperature; $k_{f0}$ and $k_{r0}$ are the forward and backward reaction rates when the bond is unstretched; $\sigma_{ts}$ is the transition spring constant; $\sigma_{eq}$ is the equivalent bond/microvillus spring constant; and $\gamma$ is a factor which takes into account the event of receptor peeling from the membrane under large stretching forces [88]. $l_{\text{stretched}}$ is the stretched bond/microvillus length which is calculated at each time step knowing the new microvillus position and the contact point where the bond is initially formed; and $l_{\text{unstretched}}$ is the bond length at equilibrium without any tension and is assumed as 200 nm (i.e., 50 nm plus the microvillus natural length (i.e., 150 nm)). The above equations determine the change of the reaction under the influence of external forces from the flow. These rates are used along with the kinetic equation (Eq. 4.2) to obtain the number of bonds at each time step.

### 4.1.2 Fluid flow and cell deformation

The number of bonds ($N_b$) obtained from Eq. 4.2 is used to calculate the bond force exerted on the cell membrane. The spring force model is implemented in which a spring constant is assigned to each chemical bond. Microvilli are also considered as deformable cylindrical bars at the membrane similar to the model presented in [88]. Once a bond is formed, the equivalent spring constant is calculated assuming the bonds are springs in series.

$$\sigma_{\text{eq}} = \frac{\sigma_{\text{mv}}\sigma_{\text{bond}}}{\sigma_{\text{mv}} + \sigma_{\text{bond}}} \quad (4.5)$$

In the above equation, $\sigma_{\text{mv}}$ is the microvillus spring constant and $\sigma_{\text{bond}}$ is the bond spring constant. This equivalent constant and $N_b$ are used to determine the bond force from

$$F_b = N_b \sigma_{\text{eq}}(l_{\text{stretched}} - l_{\text{unstretched}}) \quad (4.6)$$
\( F_b \) is then inserted in the Navier-Stokes equations (Eq. 4.7) for modeling the cell membrane deformation.

\[
\rho \left( \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = -\nabla p + \nabla \cdot (\mathbf{\nabla} \mathbf{u} + (\mathbf{\nabla} \mathbf{u})^T) + F_b 
\]  

(4.7)

In the above equation, \( \mathbf{u} \) is the velocity vector, \( p \) is pressure, \( \rho \) is the cell/buffer density and \( \eta \) is the cell/buffer viscosity depending on the domain for which the momentum equation is being solved.

### 4.2 Method

The model presented here uses the mesh-free Smoothed Particle Hydrodynamics (SPH) to simulate the multi-scale problem of cell adhesion coupled with fluid flow. SPH has widely been used for different problems in physics ranging from solid mechanics to fluid flows [89]. It has also been considered to model the flow problems in very small scales [90]. SPH has a great potential to be applied in problems with large deformations and complex geometries due to its mesh-free nature. Hosseini and Feng [34] were among the first who used the same method to model deformation of red blood cells moving through contractions in microchannels. However, no adhesion was included in their model as the focus was mainly on the dynamics of the motion. Compared to the Eulerian mesh-based approaches, SPH significantly facilitates tracking the interfaces and large deformations due to its Lagrangian nature. It also reduces the time needed to model cell motion and adhesion inside complicated geometries in dynamic adhesion. Moreover, the capability of the SPH method to model various structural elements inside the cell makes it very promising for the future modifications.

In SPH, the domain is discretized into particles (like nodes in mesh-based methods). Physical properties (such as mass, density, velocity, temperature, etc.) are assigned to each particle. The properties of each particle are updated at each time step by summation over the neighbouring particles. These physical properties and their derivatives are discretized using the integral property of the Dirac delta function (for any function like \( f \) at a point like \( x_0 \))
\[
    f(x_0) = \int_{-\infty}^{\infty} f(x)\delta(x - x_0)\,dx
\]

(4.8)

Considering the above property, the following approximation can be used in the situation where the finite number of points around the point of interest, \(x_0\), is considered.

\[
    f(x_0) \approx \sum_{i=1}^{N} f(x_i) w(x_i - x_0) \Delta x
\]

(4.9)

where, \(w\) is a weight function with specific properties [89]. Equivalently in SPH, each point can be considered as a particle and \(f\) can be any physical property of that particle. The derivative of \(f\) will result in [89]

\[
    \nabla f(x_0) \approx \sum_{i=1}^{N} f(x_i) \nabla w(x_i - x_0) \Delta x
\]

(4.10)

By assuming a support domain, i.e. a fictitious domain with a finite range around the point of interest, the summation can be conducted over a finite number of neighbouring particles in the vicinity of \(x_0\). Also the weight function, \(w\), must be defined in such a manner that a constant factor is assigned to the effect of each particle depending on its distance from \(x_0\). The piecewise cubic weight function (in the following form) is used for the present study [89].

\[
    W(R, h) = \frac{15}{7\pi h^2} \begin{cases}
        \frac{2}{3} R^2 + \frac{1}{2} R^3 & 0 \leq R < 1, \\
        \frac{1}{6} (2 - R)^3 & 1 \leq R < 2, \\
        0 & R \geq 2.
    \end{cases}
\]

(4.11)

In these relations, \(R = r/h\) in which \(r\) is the distance between each neighbouring particle and the particle of interest, and \(h\) is the SPH smoothing length determining the support domain size [89].

The SPH approximation of a function and its derivatives are used to discretize the governing equations. All the time derivatives are kept at the left hand side and are integrated over each time step to obtain the new positions and properties of each SPH particle. Here the final SPH-
discretized forms of the continuity (summation density) and Navier-Stokes equations are presented.

\[ \rho_i = \sum_{j=1}^{N} m_j W_{ij} \]  
\[ \frac{d u_i}{d t} = - \sum_{j=1}^{N} m_j \left( \frac{p_j}{\rho_j^2} + \frac{p_i}{\rho_i^2} \right) \nabla_i W_{ij} + \sum_{j=1}^{N} \frac{m_j}{\rho_i \rho_j} (\eta_j + \eta_i) (u_i - u_j) \left( \frac{1}{r_{ij}} \frac{\partial W_{ij}}{\partial r_i} \right) + F_{b,i} \]

where \( j \) is the index representing the neighbour particle. \( r_{ij} \) is the distance between each two particles, \( W_{ij} \) is the value of weight function for \( r_{ij} \), and \( m_j \) is the mass of particle \( j \). The first term on the right hand side calculates the pressure term in the Navier-Stokes equations while the second term calculates the viscous forces. The detailed implementation of SPH can be found in [89].

In this study, five types of SPH particles (not to be mistaken with the physical bioparticle) are used to discretize the domain (see Fig. 4.1). The first type represents the solid walls at the top and bottom of the microchannel. The second type discretizes the fluid domain which is the buffer in which the cell moves and deforms. The third and fourth types represent the cell cytoplasm and cell membrane, respectively. Finally, the fifth type is used to specify the periodic boundary conditions at the left and right sides of the microchannel.
For the first type of SPH particles, i.e. solid boundaries, the method introduced by Hosseini et al. [34] is used. For each particle, a virtual velocity is obtained by considering its image in the buffer with respect to the boundary and interpolating among all the neighbouring SPH buffer particles. The opposite sign of this image velocity is then used in the governing equations. This will create a virtual force that prevents the fluid particle from penetrating the walls. This force resembles the no-slip boundary conditions. The image velocity has been shown to represent accurately the boundary conditions [34].

Navier-Stokes equations are discretized over SPH particles for the fluid, cell cytoplasm and cell membrane. The only term that is added to the momentum equations is the bond force which is applied only for the membrane particles. To find this term, the micorvilli are distributed uniformly around the cell membrane, and the number of bonds is derived for each of them. Then, the force is calculated based on Eq. 4.6. This force is converted to two point forces at the two neighbour membrane particles using a linear extrapolation. In addition to these forces, extensional and bending spring forces between each two particles at the membrane are implemented to model the membrane structure and maintain the overall stability of the cell shape. Then, based on the membrane dilation and bending, the extensional and bending forces are calculated and applied at the SPH membrane particle positions. Figure 4.2 shows the...
schematic of the springs between the membrane particles. For the extensional springs, the force is calculated based on a constant and the elongation of the distance between each two membrane particles at each instant. This constant is chosen in a way to conserve the area of the cell so no artificial shrinkage or dilation occurs. For the bending resistivity, the curvature is calculated using three immediate neighbour membrane particles. As a result, the force is calculated based on the deviation of curvature from its value at natural cell shape (see the details in [34]). The periodic boundary conditions are used for SPH boundary particles. In essence, each SPH particle at the end of the channel is imaged to a SPH particle at the inlet which is about to enter the fluid domain.

![Diagram](image)

**Fig. 4.2. Schematic of extensional and bending springs at the cell membrane.**

### 4.3 Computer program specifications

A 2D numerical program written in FORTRAN is used to implement the model described. Two dimensional models for cell simulation have proven to work satisfactorily, although not as accurate as three dimensional models [53]. In 2D, cells are considered as long cylinders. This is not a realistic model of cells in general; however, it provides an upper limit for the parameters under study, e.g., the critical detachment force. For the same reason, 2D simulation is sufficient to provide conservative values for the current analysis.
Total number of particles was changed from 1000 (20×50) to 4000 (40×100) to investigate the independency of the results with respect to the numerical nodes. The cell average velocity was calculated with 4000 SPH particles, and it did not vary more than 1% of the value obtained for a lower grid resolution (1000 SPH particles). Thousand real fluid particles were found to provide sufficiently accurate numerical results. Since conventional quasi-incompressible SPH is used here, an explicit time advance scheme was implemented to integrate the data over time. The distance between each two SPH particles (Δx) then determines the time steps according to the following equations (convection and diffusion time constraints):

\[ \Delta t < \frac{\Delta x}{U}, \quad \Delta t < \frac{\Delta x^2}{\eta} \]  

(4.14)

where \( U \) is the characteristic velocity (e.g., inlet velocity) and \( \eta \) is dynamic viscosity. Depending on the viscosity, time steps as small as \( 10^{-9} \text{ sec} \) were used. All the simulations were performed on a workstation with Intel(R) Core(TM)2 Duo Processor E8400. The simulation time varied from 20 minutes to five days depending on the specified parameters.

### 4.4 Validation

In order to validate the numerical program for cell motion and deformation, the cell is positioned in the middle of the microchannel containing a two-dimensional Poiseuille flow. The cell center of mass velocity (\( U_c \)) measured by the program is compared to the analytical equation obtained by [91]

\[ \frac{U_c}{U_{f, max}} = 1 - \frac{\eta_r}{2 + 3\eta_r} \left( \frac{2a}{h} \right)^2 \]  

(4.15)

where \( a \) is the particle radius, \( h \) is the channel height, \( \eta_r \) is the ratio of cytoplasm viscosity to surrounding fluid (buffer) viscosity, and \( U_{f, max} \) is the fluid centerline velocity. For \( \eta_r = 1 \), \( h = 10 \mu m \) and \( a = 2 \mu m \), the snapshots of the cell motion in the middle of the microchannel are presented in Fig. 4.3. Different snapshots are shown using dimensionless time (\( t^* \)) which is defined as
where \( \dot{\gamma} \) is the shear rate at the wall. As shown, the cell deforms to the shape of two-dimensional Poiseuille velocity profile in the middle of the channel. Based on this simulation, the velocity is calculated to be 0.93 which is in agreement with the value of 0.968 obtained from Eq. 4.15.
Fig. 4.3. Snapshots of cell motion in the middle of a microchannel with \( \eta_r = 1, a = 2 \mu m \) and \( v_{\text{max}} = 1 \text{ cm/sec} \) at a) \( t^* = 0 \), b) \( t^* = 1 \), c) \( t^* = 2 \), d) \( t^* = 3 \) and e) \( t^* = 4 \).

As another validation, the model is compared to the results of the numerical simulation developed by Griggs et al. [92]. For this comparison, the cell is positioned at a vertical distance of 3 \( \mu m \) from the middle of the channel (Fig. 4.4). For the present simulation, \( y_{c,\text{initial}}/h = 0.3 \) where \( y_{c,\text{initial}} \) is the \( y \)-coordinate of cell center at \( t^* = 0 \sec \) and \( h \) is the channel height (\( x_{c,\text{initial}} = 12 \mu m, y_{c,\text{initial}} = 3 \mu m \)). The center of mass velocities obtained from the simulation presented in this study and that in [92] are 0.74 and 0.77, respectively. The difference between these values is less than 4\% which is acceptable within the range of numerical errors. In the next section, the developed program is used to model the cell motion and deformation while adhesion occurs at the cell-substrate interface.
4.5 Results and discussion

As discussed in Section 4.1, there are many different parameters which determine the behaviour of a cell near a reactive substrate. Examples of these parameters include forward and backward reaction rates, receptor and ligand densities, fluid flow rates, membrane rigidity, and cell cytoplasm properties. In this section, the effect of important parameters which have not been considered thoroughly in the literature will be studied using the simulation described above. The results are presented to allow identification of the effect of each parameter on the overall regime of cell motion from firm adhesion to rolling and/or detachment.

4.5.1 Cell adhesion: general patterns

This section demonstrates the effect of different factors on cell adhesion behaviour. The most essential dimensionless parameters governing the hydrodynamics and kinetics of cell adhesion are summarized in Table 4.1. These dimensionless parameters were obtained from the work of Hammer et al. [26] who did not consider the cell deformation since only solid spheres were studied. Here, two different dimensionless parameters, viscosity ratio and bending/bond rigidity ratio, are introduced. These two ratios include the effect of non-solid characteristics of the cell in the simulation. Hereafter, all the results are presented and discussed in terms of these dimensionless parameters in order to provide a clear picture of cell motion/adhesion pattern.
Table 4.1. Dimensionless parameters of cell hydrodynamics and adhesion [26].

<table>
<thead>
<tr>
<th>Dimensionless parameters</th>
<th>Expression</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>$\frac{\sigma_{eq}}{6\pi \eta_{medium} \dot{\gamma} r}$</td>
<td>Bond spring energy/fluid energy</td>
</tr>
<tr>
<td>$F_{\sigma}$</td>
<td>$\frac{\sigma_{eq} - \sigma_{ts}}{\sigma_{eq}}$</td>
<td>Fractional spring slippage</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>$\frac{k_{f0} N_i}{k_{r0}}$</td>
<td>Equilibrium constant</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$\frac{k_{r0}}{\dot{\gamma}}$</td>
<td>Backward reaction rate/fluid flow rate</td>
</tr>
<tr>
<td>$\chi$</td>
<td>$\frac{k_{f0} N_i}{\dot{\gamma}}$</td>
<td>Forward reaction rate/fluid flow rate</td>
</tr>
<tr>
<td>$\eta_r$</td>
<td>$\frac{\eta_{cytoplasm}}{\eta_{medium}}$</td>
<td>Cytoplasm viscosity/buffer viscosity</td>
</tr>
<tr>
<td>$\theta$</td>
<td>$\frac{\sigma_{bend}}{\sigma_{eq}}$</td>
<td>Bending rigidity/bond rigidity</td>
</tr>
</tbody>
</table>

Figure 4.5a shows a cell positioned close to a reactive surface inside a microchannel. The microchannel height is 10 $\mu m$ and the length is 25 $\mu m$. The cell diameter is 4 $\mu m$. The unstretched bond length, microvillus length and microvillus radius are $l_{\text{unstretched}} = 50 nm$, $l_{mv} = 150 nm$ and $r_{mv} = 100 nm$, respectively. These geometrical variables are kept constant throughout this chapter. For each simulation, SPH particles inside the cell are generated randomly and allowed to reach numerical equilibrium so that there is no significant internal motion at $t^* = 0$. The current configuration of the cell-buffer yields the density of the cell slightly larger than that of the buffer (up to 10% larger) which is similar to real biological cells [26]. As the first step, the cell is supposed to have the same viscosity as the buffer, i.e. 0.001 Pa. sec. The maximum inlet velocity is 0.1 cm/sec resulting in a Reynolds number of 0.01 and a
shear rate of 400 sec$^{-1}$ at the wall. The bending spring constant, bond spring constant, microvillus constant and transition constant (in Eqs. 4.4 and 4.5) are $3 \times 10^{-18}$ N$m$, $5 \times 10^{-4}$ N/m, $2 \times 10^{-4}$ N/m and $1 \times 10^{-4}$ N/m, respectively, consistent with the values reported in the literature for biological particles [34, 88]. The forward and backward reaction rates are assumed to be $1 \times 10^{-13}$ m$^2$/(molec.sec) and $1 \times 10^{-4}$ sec$^{-1}$, respectively. The receptor and ligand densities are both equal to $1 \times 10^{15}$ molec/m$^2$. For the specific receptor density and the radius of microvillus considered, the number of receptors on the tip of each microvillus becomes 31. For this set of variables, the dimensionless parameters are $\alpha = 9$, $F_o = 0.3$, $\kappa = 10^6$, $\beta = 2.5 \times 10^{-7}$, $\chi = 0.25$, $\eta_r = 1$ and $\theta = 2 \times 10^{-14}$ which are all in the range frequently observed in the literature [26]. Figure 4.5 presents the snapshot of cell motion for the above parameters. As it is shown, the cell flattens on the surface due to rather large strength of the forward rate and intermediate rigidity of the membrane. As the membrane moves close to the surface, the fast rate of bond formation leads to rapid binding and attracts the SPH particles towards the substrate. The contact at the interface tends to be large in this case due to the combined effects of the shear flow and intermediate membrane rigidity against bending. For this set of kinetic parameters, the chemical bonds are strong enough to hold the cell on the surface against the shear from the flow. The flattened shape of the cell is similar to that observed experimentally [93]. Figure 4.5f shows a snapshot of large Jurkat cell (22 $\mu$m in diameter) attached to an EL monolayer [93]. The shear rate at the wall is reported to be 257.7 sec$^{-1}$. The overall shape of the cell is very similar to that obtained numerically by the present model even without changing the kinetic or hydrodynamic parameters to match the numerical model to the experimental results. It is expected that for the same dimensions and flow rate, cell shape will accurately resemble the overall shape observed in the experiment by modifying parameters like bending rigidity.
Fig. 4.5. Snapshots of cell adhesion with $\dot{\gamma} = 400$ sec$^{-1}$, $\alpha = 9$, $F_\sigma = 0.3$, $\kappa = 10^6$, $\beta = 2.5 \times 10^{-7}$, $\chi = 0.25$, $n_p = 1$ and $\theta = 2 \times 10^{-14}$ at a) $t^* = t\dot{\gamma} = 0$, b) $t^* = 1$, c) $t^* = 2$, d) $t^* = 3$ and e) $t^* = 4$. X and Y are the coordinates. For simplicity only SPH membrane particles are shown. f) Snapshot of a Jurkat cell adhered to an EI monolayer under the shear rate of 257.7 sec$^{-1}$ (Reprinted with permission from [93]).

Figure 4.6 depicts two snapshots of the same cell under the same conditions except for the flow rate. The maximum velocity is changed to 10 times the previous value. The figure compares the final deformation of the cell for the two flow rates at $t^* = t\dot{\gamma} = 4$. For both cases, the cell is attached to the substrate. However, in the case of large shear, the deformation of the cell is
more significant, and cell adhesion occurs after a sequence of pulsating motions in the form of “stop-and-go” from the initial start point. The large deformation results in a smaller contact area between the cell and the substrate. Although the contact area seems to include only a few membrane particles, the number of bonds and their strengths are enough to hold the cell on the surface even after it moves a small distance along the channel wall.

![Graph showing cell adhesion at different flow rates](image)

**Fig. 4.6.** Comparison of cell adhesion at two different flow rates $\dot{\gamma} = 400$ and 4000 sec$^{-1}$. Other parameters are the same as Fig. 4.5.

The effect of the ligand (anti-pathogen antibody) density on cell deformation and adhesion has also been studied (see Fig. 4.7). In real experiments, once the cell of interest is selected, there are only a few variables (such as flow rate, ligand density, and channel dimensions) that can be modulated independently to study the cell adhesion phenomenon. Changing ligand density is one of the simplest and at the same time most effective variables to observe different regimes of cell adhesion. As it is seen in Fig. 4.7, a 10-fold increase in the ligand, while keeping other variables constant, changes the regime from detachment to firm adhesion resulting in a large contact area (which is an important parameter when the substrate is intended to act as a trapping zone for the biological cell). In this case, the optimal design criteria in terms of required capture molecule density versus the flow rate can be obtained numerically to satisfy the design rules.
Fig. 4.7. Comparison of cell adhesion for two different ligand densities at \( t^* = 7 \) for \( \dot{\gamma} = 4000 \text{ sec}^{-1} \) and \( \theta = 2 \times 10^{-13} \). All other parameters are the same as Fig. 4.5. The cell initial position at \( t^* = 0 \) is also displayed in black. The small disconnection in the membrane line demonstrates the rotation of the cell while moving away from its initial position.

Another interesting effect in cell adhesion is bending rigidity which can be observed by comparing the shape of the blue cell in Fig. 4.6 and red cell in Fig. 4.7 (both obtained at the shear rate of \( \dot{\gamma} = 4000 \text{ sec}^{-1} \)). The result shows that the large rigidity of the red cell causes the cell to be less deformable against shear from the flow, and consequently, to have less tendency to keep its contact with the substrate. This results in detachment from the surface. This effect is further investigated in Section 4.5.2.

Similar simulations can be presented for the effects of other variables, like the reaction rates. In order to summarize the cell response to all these parameters, state diagrams have been introduced [28, 29]. As discussed before, the state diagrams presented in the literature do not usually include effects such as cell deformation. The proposed methodology in this chapter addresses this issue through adding the cytoplasm viscosity and membrane rigidity, enabling the cell to demonstrate various patterns from the solid-like behaviour to the very deformable liquid-like motion. Instead of presenting a state diagram, this chapter focuses on the effects of the structural parameters (like cytoplasm viscosity and membrane rigidity) on cell deformation and the final cell response.
4.5.2 Effect of bending rigidity

In this section the effect of bending rigidity on the behaviour of the cell is studied. In order to compare the results, the dimensionless number $\theta$ is used (see Table 4.1). This number is the ratio of membrane bending rigidity to chemical bond rigidity and plays an important role in determining the overall shape of the cell. The larger the membrane rigidity, the more the solid-like behaviour of the cell under the shear flow. Figure 4.8 shows the adhesion patterns for different values of membrane bending rigidity. The cell center is initially at $x = 1.2 \times 10^{-5}$ m. The results show that for the lowest rigidity value (Fig. 4.8a), the cell is deformed significantly and detached from the surface moving towards the exit. For a larger value of rigidity (Fig. 4.8b), there is still significant deformation of the membrane. However, the cell lies down on the surface and flattens. This creates strong binding between the cell and the substrate, resulting in cell adhesion to the channel wall. Further increase in the value of bending rigidity leads to detachment and free motion of the cell (Fig. 4.8c). The above pattern can be interpreted as follows: when bending rigidity is small, the large deformation causes the cell to follow the flow pattern and lose its ability to form a sufficient area of contact with the channel wall. For intermediate rigidity (in Fig. 4.8b), however, a small degree of deformation creates enough interaction area between the complementary molecules due to the combined effects of rigidity and shear. For very large rigidity, the cell projected area against the shear is larger while the tendency for deformation is smaller. This results in a having a larger drag force, and hence a reduced contact area which causes rolling or detachment of the cell. For a specific cell viscosity, there seems to be a certain range of rigidity for which the cell is trapped on the substrate. Lower rigidity tends to deform the cell so that it follows the flow pattern, and its contact area reduces to almost a contact point. For larger rigidities, cell tends to behave like a solid sphere with a small contact area causing it either to roll or detach from the substrate. This is an important conclusion which seems to have drawn less attention in previous studies due to undervaluing or miscalculating the deformation parameters.
Fig. 4.8. Cell adhesion patterns at $t^* = 4$ for $\dot{\gamma} = 400 \, \text{sec}^{-1}$ with a) $\theta = 2 \times 10^{-15}$, b) $\theta = 2 \times 10^{-14}$ and c) $\theta = 2 \times 10^{-13}$. Other parameters are $\alpha = 9, F_\sigma = 0.3, \kappa = 10^6, \beta = 2.5 \times 10^{-7}, \chi = 0.25$ and $\eta_r = 1$. 
4.5.3 Effect of cytoplasm viscosity

In addition to membrane rigidity, the viscosity of the cytoplasm is also important in the stability of a biological cell structure. Different viscosities may result in different deformation patterns and contact areas. Viscosity can also change the area projected against the shear flow. Figure 4.9 shows the significance of cytoplasm viscosity. In this simulation all the parameters are kept the same as those used in Fig. 4.8a. The membrane rigidity is very small. Therefore, the effect of viscosity will be more pronounced. As it is seen in this figure, a ten-fold increase in the viscosity value totally alters the cell behaviour from freely-moving (with the flow) to firmly adhered (to the reactive substrate). In essence, the larger viscosity of the cytoplasm enables the cell to resist against the flow and deform slowly so that receptor molecules on the tips of microvilli have enough time to react with ligand molecules and form bonds.

From Fig. 4.8 and Fig. 4.9, it is concluded that the response of a cell in different biological events of adhesion completely depends on its combined effects of structural parameters including both cytoplasm viscosity and membrane bending rigidity. These two parameters govern the overall deformation behaviour. Different values of either rigidity or viscosity may result in a shift from one adhesion regime to another. Even if the regime does not change, the deformation may be completely different. For instance, increasing the viscosity leads to firm adhesion (Fig. 4.9), similar to the behaviour observed by increasing the bending rigidity (Fig. 4.8b). However, the final shape of the cell and its deformation for the above two cases are totally different. Therefore, assuming that all other parameters are constant, an accurate prediction of cell deformation and adhesion pattern requires a reasonably precise estimation of such structural parameters.
Fig. 4.9. Effect of increasing cytoplasm viscosity on cell adhesion \((t^* = 4, \dot{\gamma} = 400 \text{ sec}^{-1})\). All other parameters are the same as Fig. 4.8.

4.6 Adhesion design parameters for Cryptosporidium capture

Considering the characteristics of a Cryptosporidium oocyst as being an almost spherical cell surrounded by a hard shell, it is assumed that the above numerical model will be sufficiently accurate if a large bending rigidity is selected for the cell membrane \((\theta = 2 \times 10^{-13})\). The viscosity is assumed to be that of water for both the buffer and the cell cytoplasm \((\eta_r = 1)\). The maximum shear rate used in the simulation \((4000 \text{ sec}^{-1})\) is assumed as the maximum shear possibly encountered in the proximity of the capture elements. This value is in the order of commonly-observed shear rates in biological events \([88]\). It will be ensured that for none of the experimental tests performed the shear rate exceeds the above margin. No direct measurement of forward and backward reaction rates, and cell receptor density were found in the literature for Cryptosporidium. Here, they are assumed to be within the range observed for similar biological organisms \((\alpha = 9, F_0 = 0.3, \kappa = 10^6, \beta = 2.5 \times 10^{-7}, \chi = 0.25)\) \([26]\). The simulation presented in Fig. 4.7 qualitatively determines the minimum antibody density required for the oocysts to be captured. As it is seen, for the antibody densities smaller than \(10^{15} \text{ molec/m}^2\), pathogens detach and move towards the outlet without being captured. However, a ten-fold increase results in firm adhesion and complete capture of the same cell. Therefore, a minimum antibody density of \(10^{16} \text{ molec/m}^2\) is used in all the experimental runs conducted in the real flow cell device. This minimum density is, however, found assuming
hypothetical values for the receptor density (number of cell binding sites) and reaction rates from the literature [26].

4.7 Conclusions

Cell adhesion phenomenon is of great importance due to its major application in biotechnology and human health. Developing a tool to model such phenomenon facilitates the study of essential physics occurring in real-life biological events. In this chapter, a two-dimensional numerical program was used to simulate the transport and adhesion of a biological cell inside a microchannel. A mesh-free method (Smoothed Particle Hydrodynamics) was implemented, for the first time, to conduct the simulations to investigate the effects of fundamental parameters in detail. It was shown that changing any of these parameters may lead to a totally different behaviour in the adhesion pattern and cause a cell to alter its state from firm adhesion to rolling or detachment. It was also demonstrated that structural parameters such as cytoplasm viscosity and membrane rigidity play crucial roles in determining the cell-surface contact area and specific adhesion. Rigidity was modulated to resemble solid-like cells, as used in previous studies to find the adhesion state diagrams. It was then modified over a wide range of values to examine the sensitivity of the final cell response with respect to the membrane stiffness. Modifying membrane rigidity from small to large values resulted in completely different adhesion patterns than those reported in the previous studies [26]. Cell cytoplasm viscosity was also demonstrated to have significant contribution in cell deformation. For the parameter values analyzed in this study, increase in the viscosity value resulted in the formation of a large contact area between the cell and the reactive substrate which consequently caused the cell to adhere firmly to the substrate.

In summary, the numerical method presented in this chapter offer a more accurate model of cell behaviour by including both rigid and deformable shells, and consequently, a more realistic model of mammalian cells. The present numerical model determined the minimum antibody density for the Cryptosporidium capture to be $10^{16}$ molec/m$^2$ under the simulated experimental conditions without any need for painstaking experimental replicates. This is a
significant achievement since it helps to focus the experimental phase only on the examination of the transport mechanisms and eliminate any concerns about the capture. In other words, there is no need to find the optimized adhesion parameters including antibody density experimentally. The only remaining task is to verify experimentally if the active separation techniques (sedimentation and electrophoresis) presented in Chapter 3 work favourably in the microfluidic capture device.
5 Particle transport and capture in the microfluidic capture device

As discussed before, the main purpose of this study is to design a microfluidic device to separate and concentrate as many pathogens as possible from a continuous flow. The main methodology to achieve this purpose is to utilize physiochemical properties of the target pathogens to manipulate them inside the fluid flow towards the capture elements. In this chapter, the applicability and effectiveness of the active separation techniques, presented in Chapter 3, to manipulate the pathogens inside the microfluidic device is tested experimentally. The theory behind each method was explained in Chapter 3. In the following sections, the corresponding experimental results are presented for each technique. Finally, the device performance for each technique is compared, and the most practical and efficient method is selected for the separation process.

5.1 Sedimentation: experimental study

5.1.1 Materials and method

Experimental device

A portion of the screen-plate design similar to that selected in Chapter 3 (which resembles two parallel electrodes) is used as the flow cell to study experimentally the theory of sedimentation (see Fig. 5.1). The device was composed of two decks; one made from steel and another from Plexiglas. Two holes (inlet and outlet) were drilled all the way through the thickness of the steel deck. A thin rubber gasket (120 μm), acting as a spacer, was sandwiched between the steel deck and a gold slide. The gold slide (CA134, purchased from EMF Corp.) was fixed using the Plexiglas deck. As a result, a rectangular microchannel was formed inside the gap between the gold slide and the steel deck (see Fig. 5.1). The dimensions of the rectangular microchannel
were 6.5 cm * 1 cm * 120 μm. Using silicone tubing (1/16 in. ID) and connectors, the inlet hole was connected to a syringe pump (KD Scientific, KDS 220). The syringe pump was used to modulate the flow rate. The outlet tube (connected to the outlet hole) was placed into 15 ml collection tubes to collect the effluent (Fig. 5.1c).
Fig. 5.1. Experimental flow cell device for sedimentation test: a) a schematic, b) fabricated parts and c) the assembled device.
Sample preparation

Heat-killed *Cryptosporidium* oocysts (10^6 oocysts/ml) were purchased from Waterborne Inc. In order to stain the oocysts, 160 μl of the sample (nominally containing 4 × 10^4 oocysts) was mixed thoroughly with the same amount of the DAPI (4',6-diamidino-2-phenylindole) solution and incubated for half an hour at 37°C. The sample was then added to 39.68 ml DI water so the nominal final concentration became approximately 1000 oocysts/ml.

Gold coated slides were cleaned with Piranha solution (i.e., a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2)) and rinsed thoroughly with DI water before use. Mouse anti-*Cryptosporidium* IgG antibodies (purchased from Waterborne Inc.) were deposited on the surface of the gold slides and allowed to settle over night at 4°C. For more details on antibody selection and preparation the reader is referred to [3]. For the sake of simplicity, a sufficiently large antibody surface concentration of 4 × 10^{16} molec/m^2 (consistent with the value obtained numerically in Chapter 4) was selected so there would be an abundance of capture sites. This value also lies at the top of the range of the antibody density value reported in the literature [26].

Method

Two different techniques for the counting and analysis of the effluent particles were used. Figure 5.2 shows the experimental setups corresponding to each technique. The oocyst sample was kept inside a glass syringe above the inlet tube. A mixer was used to make constantly a vortex inside the syringe so that the oocysts did not sediment before flowing through the device. All the tubing was kept as short and vertical as possible. The outlet tube was connected to a 5 ml plastic syringe (Becton-Dickinson) which was mounted on the syringe pump. For each run, the flow rate was set at the syringe pump. A sample of 1.5 ml (almost five times the volume of the whole experimental line) was drawn into the device from the glass syringe and collected inside the plastic syringe. In the first version of particle analysis setup, i.e. direct counting (Fig. 5.2a), the effluent inside the plastic syringe was tested in terms of the number of oocysts. Membrane counting and direct epifluorescence microscopy were used for effluent
counting (refer to [3] for more details). The number of oocysts, which did not sediment and exited the device, was found for each flow rate. In the second version (Fig. 5.2b), microflow imaging was used, immediately after the flow cell, to monitor the oocysts which left without interaction. The software for the particle imager (Brightwell Technologies Inc.) provided the graphs and data necessary for analysis.

![Flowchart](image)

Fig. 5.2. Flowcharts of the two versions of the experimental setup used for sedimentation experiment.

### 5.1.2 Experimental results and discussion

During initial studies, the setup depicted in Fig. 5.2a was used. The concentrations of oocysts in the effluent (i.e., not captured) were found for different flow rates using membrane counting (see Fig. 5.3). As shown, when the flow rate was less than 1 \( ml/hr \), the number counted in the effluent was not significant compared to the numbers found for the flow rates of 100 \( ml/hr \) and 10 \( ml/hr \). The higher flow rate pushes more particles out of the device. The concentration
at 1 ml/hr was less than 2.5% of the concentration at 100 ml/hr which is the closest to the sample initial concentration (500 oocysts/ml) inside the glass syringe. Therefore, the critical flow rate for which the majority of the particles (more than 95% assumed here) are deposited on the surface is found to be 1 ml/hr for the present flow cell device. It is worth mentioning that for the current setup and the maximum flow rate used here (100 ml/hr), the shear rate at the wall is

\[
\dot{\gamma} = \frac{4u_{f,max}}{h} = \frac{4 \times 1.5 \times u_{f,avg}}{h} = \frac{6q_{in}}{A_ch}
\]  \hspace{1cm} (5.1)

where \(q_{in}\) is the inlet flow rate and \(A_c\) is the cross-sectional area of the microchannel. Knowing the channel dimensions, the shear rate becomes

\[
\dot{\gamma} = \frac{6q_{in}}{A_ch} = \frac{6 \times 100 \text{ cm}^3/\text{sec}}{1\text{ cm} \times 0.012\text{ cm}} \approx 14 \text{ sec}^{-1}
\]  \hspace{1cm} (5.2)

This is two orders of magnitude smaller than what was considered in Chapter 4 as the maximum applied shear rate (4000 sec\(^{-1}\)).
Fig. 5.3. Concentration of *Cryptosporidium* oocysts in the effluent for different flow rates. Nominal inlet concentration was 500 oocysts/ml.

To observe the effect of sedimentation against the negative control (no-sedimentation case), the setup shown in Fig. 5.2b was employed. A flow imaging microscope (Brightwell Technologies Inc.) was placed in between the flow cell device and the syringe pump to count the number of effluent oocysts at each time and produce the corresponding plots. Two different cases were examined. In the first case, the experimental device was placed horizontally; while, in the second case, it was fixed vertically so the gravitational force was in the same direction as the flow (no sedimentation towards the reactive surfaces). For both cases, the device was operated at 1 ml/hr with the same initial oocyst concentration. Figure 5.4 presents the cumulative number of oocysts at each time obtained for the two cases. The difference in the results signifies the influence of sedimentation (gravitational force) in trapping the oocysts. When the device was horizontally placed, most of the oocysts remained inside the setup; whereas for the vertical case, they exited the device after traversing the length of the chamber. These results were repeated for different flow rates and with different initial numbers of oocysts. For all the experiments conducted with the horizontal device the oocysts were effectively trapped at 1 ml/hr.
Fig. 5.4. Accumulative number of oocysts versus time for horizontal (diamonds) and vertical (squares) flow cell devices at the 1 ml/hr inlet flow rate. The initial plateau is observed due to the void volume of the device, and it determines the time it takes for the particles to enter the detector instrument.

To compare the experimental results with the numerical simulation, Fig. 3.2 in Section 3.1.4 is considered here again. For the flow rate of 0.06 µl/min (equivalent to the maximum velocity of 1.25 µm/sec in the simulation), the minimum required length \( l \) was 150 µm. Assuming the drag force, moving the particle along the flow direction, is linearly dependent on the velocity (based on Stokes’ law, \( F_d = 6\pi\mu r U \)), it is estimated that with such a length of the experimental flow cell device (5.5 cm between the inlet and outlet holes) the velocity can be increased to 450 µm/sec (22 µl/min =1.32 ml/hr). This flow rate is in agreement with the values presented in Fig. 5.3 and Fig. 5.4.

An alternative way to find the maximum tolerable velocity theoretically is to calculate the different time scales of transport in the problem. For a particle (dispersed phase) with radius \( r \) and density \( \rho_d \) settling inside a fluid medium with a density of \( \rho_f \) and dynamic viscosity \( \eta \), the terminal settling velocity is
where \( g = 9.81 \text{ m/sec}^2 \) is the specific gravity. This equation is valid for small Reynolds numbers \((<<1)\). Equation 5.3 shows that if a \textit{Cryptosporidium} oocysts with \( \rho_d \approx 1050 \text{ kg/m}^3 \) and \( r = 2.5 \mu\text{m} \) [3] is moving in water with \( \rho_f = 1000 \text{ kg/m}^3 \) and \( \eta = 0.001 \text{ kg/m}.\text{sec} \), the oocyst settling velocity becomes 0.681 \( \mu\text{m/sec} \). In other words, if a \textit{Cryptosporidium} oocyst is given one hour to settle inside water, it falls around 2.5 \text{ mm} along the gravity direction. This displacement can be implemented to drive oocysts along the channel height all the way down to the reactive surface. The sedimentation time scale \((t_{\text{sed}})\) is obtained through dividing the height of the channel (in this case \( h = 120 \mu\text{m} \)) by the settling velocity \((u_{\text{set}} = 0.681 \mu\text{m/sec})\).

\[
t_{\text{sed}} = \frac{h}{u_{\text{set}}} = 176 \text{ sec}
\]  

(5.4)

On the other hand, there must be a continuous flow of medium inside the device since a certain volume of water (often much larger than the device volume) needs to be sampled. As a result, the oocysts move in the direction of the flow due to the drag force exerted from the medium. The minimum hydrodynamic drag time \((t_{\text{drag}})\) is

\[
t_{\text{drag}} = \frac{l}{u_{f,\text{max}}}
\]

(5.5)

where \( u_{f,\text{max}} \) is the medium maximum velocity and \( l \) is the effective length of the flow cell device. Equation 5.5 has been obtained assuming that the particle moves with the maximum velocity in the flow field. In order to capture the particles on the surface, the sedimentation time must be smaller than the drag time so that the particle reaches the substrate before exiting the device. Given that \( l = 5.5 \text{ cm} \) and \( t_{\text{sed}} < t_{\text{drag}} \) \((176 \text{ sec} < \frac{5.5 \text{ cm}}{u_{f,\text{max}}})\),

\[
u_{f,\text{max}} < 0.03 \frac{\text{cm}}{\text{sec}}
\]

(5.6)

\( u_{f,\text{max}} \) must be smaller than 300 \( \mu\text{m/sec} \). This velocity is a conservative limit since in the calculations, the particle was assumed to have the maximum velocity during the travel time.
This is not necessarily true as particles move in different vertical positions at each instant and consequently, acquire different horizontal velocities always smaller than the maximum velocity. Nonetheless, the velocity obtained through the time scale calculations (300 μm/sec) is in the same range of that obtained through the continuum simulation (450 μm/sec). This velocity determines the critical flow rate (~1 ml/hr) larger than which the sedimentation rate is not enough to capture oocysts. This critical flow rate is consistent with the value found experimentally. Considering the fact that there might be small discrepancies between the parameters assumed in the simulation (like the oocyst diameter or density) and the real values, the numerical values are in close agreement with the experimental results. It is worth mentioning that it is not expected to find many external particles as large as Cryptosporidium oocysts in finished water samples (such as tap water). Therefore, the level of interference in sedimentation and capture is expected to be very low. Even if there are interfering particles, the anti-Crypto antibodies provide sufficient specificity in capturing the target oocysts and not other external particles.

5.2 Electrophoresis: experimental study

5.2.1 Materials and method

Device description

Two different experimental devices were used for the electrophoresis tests are shown in Fig. 5.5. The first device was the same as the one used for the sedimentation tests. The second configuration was similar on all counts except for the top part which, in this version, was made of a second gold slide (purchased from EMF Corp.) creating a gold-gold electrode combination. The inlet and outlet holes were connected to silicone laboratory tubing (1/16 in. ID). The inlet was connected to a syringe pump (KD Scientific, KDS 220) to modulate the flow rate at which the fluid medium flowed into the device.
Fig. 5.5. Two versions of the electrophoretic microfluidic device: a) steel-gold and b) gold-gold electrode combination.
Sample preparation

Different concentrations of DAPI-stained oocyst were prepared according to the method explained in Section 5.1.1. In order to prove the concept of electrophoresis, runs were performed using both real oocysts and also synthetic microspheres made of polystyrene (with average diameter of 5 μm and density of 1.06 g/cm³ purchased from Bangs Laboratory Inc.) as surrogate particles (Fig. 5.6). These microspheres are charged as they are functionalized with carboxyl groups. They are visible under the light which made them easy to observe using direct microscopy.

Method

The experimental setup is shown in Fig. 5.7. The particle (oocyst or microsphere) sample was kept inside a glass syringe above the inlet tube. A mixer was used to make constantly a vortex inside the syringe so the particles did not sediment before they arrived at the device. The outlet tube was connected to a microflow imaging system (Brightwell Technologies Inc.) and then a syringe pump (KD Scientific, KDS 220) at the end of the line with a mounted 5 ml plastic syringe
(Becton-Dickinson Co.). The electrodes (gold and steel) were connected to an external DC power supply (BK Precision 1673). For each run, the flow rate was set at the syringe pump and the electric current was measured using a sensitive multimeter (Amprobe, 37XR). The particles detected by flow microscopy (particle analyzer) were monitored using the software incorporated in the instrument. For selective runs, the effluent inside the 5-ml plastic syringe was also analyzed using membrane counting and direct eipfluorescence microscopy. At the end, the number of particles exited the device and not being trapped was found for different flow rates and applied voltages.
Fig. 5.7. Experimental setup for electrophoresis analysis.
5.2.2 Experimental results and discussion

The experimental phase of the research was designed in such a way to verify the conclusions of the numerical study in Section 3.3. The inlet flow rate was set at 1 ml/hr. This flow rate was shown, both numerically and experimentally, to work efficiently for the sedimentation case. Figure 3.6 (Chapter 3) demonstrated the equality of performance for both sedimentation and electrophoresis (under the same conditions) and actual values of the electric potential difference. Therefore, the selected flow rate should work for the electrophoresis method as well. An alternative approach to reach to this hypothesis is as follows.

In addition to the COSMOL simulation results presented in Section 3.2, a similar analysis to the one presented in Section 5.1.2 can be used here to calculate different time scales for electrophoretic and hydrodynamic drag transport. The electrophoretic migration time \( t_e \) is obtained through dividing the height of the channel \( (h = 120 \mu m) \) by the electrophoretic velocity.

\[
t_e = \frac{h}{u_e} \tag{5.7}
\]

where electrophoretic velocity \( u_e \) is

\[
u_e = \mu E \tag{5.8}
\]

\( \mu \) is the electrophoretic mobility and \( E \) is the magnitude of the electric field strength. The hydrodynamic drag time is

\[
t_{drag} = \frac{l}{u_{f,max}} \tag{5.9}
\]

In order for the electric field to capture the charged particle, the electrophoretic time must be smaller than the hydrodynamic drag time \( t_e < t_{drag} \). Cryptosporidium oocysts were sent to Particle Characterization Laboratories, Inc. (Novato, US) to measure their electrophoretic mobility for different solution properties (see Table 5.1). Based on the measurements, an average electrophoretic mobility of \( -1.5 \times 10^{-8} m^2/V sec^{-1} \) was used in the calculations.
There are practical limitations for the voltage which can be applied to the bare electrodes in contact with water (refer to the discussion in Section 5.2.3). To conduct a reasonable estimation, the reduced effective voltage was used to calculate the actual electrophoretic velocity of the oocyst. The actual effective voltage difference is assumed to be only around one percent of the maximum applicable value (0.01 × 1V = 0.01 V = 10 mV). The electrophoretic velocity corresponding to this effective voltage is

\[ u_e = \mu E = \frac{\Delta V}{h} \]

\[ = 1.5 \times 10^{-8} \times \frac{0.01}{1.2 \times 10^{-4}} = 1.25 \mu m/sec \]  

and the electrophoretic migration time is

\[ t_e = \frac{h}{u_e} = \frac{120}{1.25} = 96 \text{ sec} \]  

(5.10)

(5.11)

On the other hand, the minimum hydrodynamic drag time \((t_d)\) is

\[ t_{drag} = \frac{l}{u_{f,max}} = \frac{5.5 \text{ cm}}{u_{f,max}} \]  

(5.12)

where \(u_{f,max}\) is the fluid medium maximum velocity. In order to capture the particles on the surface, the electrophoretic time must be smaller than the drag time. Given that \(l = 5.5 \text{ cm}\) and \(t_e < t_{drag} \) \(96 \text{ sec} < \frac{5500 \mu m}{u_{f,max}}\)

\[ u_{f,max} < 573 \mu m/sec \]  

(5.13)

which is twice the velocity obtained for sedimentation. This was expected since the electrophoretic velocity of Cryptosporidium oocyst is almost twice its settling velocity. Apart from the fact that the particle is assumed to have the maximum drag velocity, the actual applied voltage sensed by the particle is very small. This velocity determines the critical flow rate \((2.45 ml/hr)\) larger than which the electrophoretic force is not strong enough to separate
and hold the oocysts. The selected flow rate for electrophoresis experiment (1 ml/hr) is smaller than this critical value.
### Table 5.1. Oocyst mobility measurements for different pH and conductivity values as reported by Particle Characterization Laboratories, Inc. (Novato, US).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mobility (μm/sec)/(V/cm)</th>
<th>Zeta Potential (mV)</th>
<th>Conductance (μS)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>BI-ZR3; -53+/-3 mV</td>
<td>-4.43E+00</td>
<td>-56.69</td>
<td>368</td>
<td>6.4</td>
</tr>
<tr>
<td>BI-ZR3; -53+/-3 mV</td>
<td>-3.92E+00</td>
<td>-55.25</td>
<td>337</td>
<td>6.4</td>
</tr>
<tr>
<td>BI-ZR3; -53+/-3 mV</td>
<td>-4.08E+00</td>
<td>-57.42</td>
<td>319</td>
<td>6.4</td>
</tr>
<tr>
<td>0.22 um filtered DI RO H₂O</td>
<td>7.66E-02</td>
<td>1.08</td>
<td>12</td>
<td>6.55</td>
</tr>
<tr>
<td>0.22 um filtered DI RO H₂O</td>
<td>-6.84E-02</td>
<td>-0.96</td>
<td>24</td>
<td>6.55</td>
</tr>
<tr>
<td>0.22 um filtered DI RO H₂O</td>
<td>-9.17E-02</td>
<td>-1.29</td>
<td>25</td>
<td>6.55</td>
</tr>
<tr>
<td>CV in DI RO H₂O</td>
<td>-1.66E+00</td>
<td>-23.38</td>
<td>103</td>
<td>6.88</td>
</tr>
<tr>
<td>CV in DI RO H₂O</td>
<td>-1.74E+00</td>
<td>-24.51</td>
<td>104</td>
<td>6.88</td>
</tr>
<tr>
<td>CV in DI RO H₂O</td>
<td>-1.54E+00</td>
<td>-21.70</td>
<td>104</td>
<td>6.88</td>
</tr>
<tr>
<td>0.22 um filt. 1 mM KCl</td>
<td>-1.12E-01</td>
<td>-1.57</td>
<td>317</td>
<td>5.76</td>
</tr>
<tr>
<td>CV in 0.22 um filt. 1 mM KCl</td>
<td>-1.19E+00</td>
<td>-16.82</td>
<td>378</td>
<td>5.76</td>
</tr>
<tr>
<td>CV in 0.22 um filt. 1 mM KCl</td>
<td>-1.25E+00</td>
<td>-17.54</td>
<td>386</td>
<td>5.76</td>
</tr>
<tr>
<td>CV in 0.22 um filt. 1 mM KCl</td>
<td>-1.19E+00</td>
<td>-16.79</td>
<td>386</td>
<td>5.76</td>
</tr>
<tr>
<td>0.22 um filt. 10 mM KCl</td>
<td>1.09E-01</td>
<td>1.54</td>
<td>2841</td>
<td>5.76</td>
</tr>
<tr>
<td>CV in 0.22 um filt. 10 mM KCl</td>
<td>-2.04E+00</td>
<td>-28.72</td>
<td>2904</td>
<td>5.76</td>
</tr>
</tbody>
</table>

---

3 Conductance (S) is Conductivity (S/m) multiplied by the working distance between the electrodes (m). Working distance was reported to be 0.5 cm.

4 BI-ZR3; -53+/-3 mV: standard reference particles to calibrate the instrument

5 0.22 um filtered DI RO H₂O: purified water by reverse osmosis and filtered through 0.22 micron hole-size membrane

6 CV: Cryptosporidium oocysts
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mobility ((\mu m/sec)/(V/cm))</th>
<th>Zeta Potential ((mV))</th>
<th>Conductance (^3) ((\mu S))</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV in 0.22 um filt. 10 mM KCl</td>
<td>-1.86E+00</td>
<td>-26.19</td>
<td>2912</td>
<td>5.76</td>
</tr>
<tr>
<td>CV in 0.22 um filt. 10 mM KCl</td>
<td>-2.04E+00</td>
<td>-28.74</td>
<td>2917</td>
<td>5.76</td>
</tr>
<tr>
<td>DI RO H2O, pH 3.03 w/ HCl (^7)</td>
<td>-4.11E-01</td>
<td>-5.78</td>
<td>1204</td>
<td>3.03</td>
</tr>
<tr>
<td>CV in H2O, pH 3.03 w/ HCl</td>
<td>-4.38E-01</td>
<td>-6.17</td>
<td>1179</td>
<td>3.03</td>
</tr>
<tr>
<td>CV in H2O, pH 3.03 w/ HCl</td>
<td>-4.36E-01</td>
<td>-6.14</td>
<td>1043</td>
<td>3.03</td>
</tr>
<tr>
<td>CV in H2O, pH 3.03 w/ HCl</td>
<td>-4.26E-01</td>
<td>-6.00</td>
<td>1000</td>
<td>3.03</td>
</tr>
<tr>
<td>DI RO H2O, pH 10.00 w/KOH</td>
<td>-8.61E-02</td>
<td>-1.21</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>CV in H2O, pH 10.00 w/KOH</td>
<td>-2.30E+00</td>
<td>-32.32</td>
<td>119</td>
<td>10</td>
</tr>
<tr>
<td>CV in H2O, pH 10.00 w/KOH</td>
<td>-2.26E+00</td>
<td>-31.87</td>
<td>119</td>
<td>10</td>
</tr>
<tr>
<td>CV in H2O, pH 10.00 w/KOH</td>
<td>-2.43E+00</td>
<td>-34.22</td>
<td>118</td>
<td>10</td>
</tr>
<tr>
<td>0.01wt% Tween</td>
<td>-3.24E-01</td>
<td>-4.56</td>
<td>18</td>
<td>7.85</td>
</tr>
<tr>
<td>CV in 0.01wt% Tween (^8)</td>
<td>-1.50E+00</td>
<td>-21.08</td>
<td>88</td>
<td>7.85</td>
</tr>
<tr>
<td>CV in 0.01wt% Tween (^8)</td>
<td>-1.58E+00</td>
<td>-22.18</td>
<td>90</td>
<td>7.85</td>
</tr>
<tr>
<td>CV in 0.01wt% Tween (^8)</td>
<td>-1.66E+00</td>
<td>-23.36</td>
<td>91</td>
<td>7.85</td>
</tr>
</tbody>
</table>

\(^7\) The count rate for this control, like all the controls, was very small. Upon adding the sample the reported count rated jumped many fold. Thus this incidental value actually is associated with a very high experimental variance whereas the CV data collected under the same conditions had much tighter reproducibility.

\(^8\) wt% : percentage mass fraction
The electrophoresis experiment is designed and conducted similarly to the sedimentation experiment except for the fact that the electric potential is applied to the steel-gold or gold-gold electrodes. Since the electric field is applied perpendicularly to the direction of the flow, it is expected that the electrophoretic forces would bring the particle to the reactive electrodes as if there was gravity attracting them in the horizontal direction. Since the gravity was able to hold the particles in the horizontal device, the electrophoresis force must also be able to separate and hold them in the vertical case as well. Figure 5.8 presents the schematics of the condition described above. In both Fig. 5.8a and Fig. 5.8b, the side view of the microchannel is shown. As seen in Fig. 5.8a, some of the charged particles move in high-velocity regions (around the centerline of the channel) while others travel in low-velocity regions close to the channel walls. When the power supply is off, particles move along the streamlines and exit the device depending on their initial position where they enter the channel. As soon as the electric field is applied (Fig. 5.8b), the particles approach the oppositely-charged electrode (i.e., gold). They either attach to the reactive gold surface or keep moving in the low velocity regions next to the walls of the device. This causes a delay in the motion of particles which consequently delays their exit from the device compared to the case where they are moving under the zero-potential condition.

![Figure 5.8](image-url)

*Fig. 5.8. Schematic view of particle accumulation on oppositely-charged reactive electrodes; a) power supply off, b) power supply on.*
The microfluidic device was placed vertically so that the effect of gravity was removed from the analysis. The syringe pump was set at 1 ml/hr, low enough to observe the effect of the electric field. Oocysts were prepared in 10 mM KCl solution with the nominal concentration of 3 \times 10^5 oocysts/ml. Sample pH and conductivity were measured to be 7.8 and 1340 \mu S/cm, respectively. A multimeter (Amprobe, 37XR) was used to monitor the electric current. Depending on the properties of the solution used for sampling (such as conductivity), the electric current flowing between the electrodes may differ considerably. The electric current is crucial since it is a measure of the electric field strength sensed by the particles in the bulk phase. Figure 5.9 shows the measured electric current versus time for the case that steel-gold electrodes were used. The electric current reported in the literature [75] for the electrophoresis in micron scales is almost three or four times larger than what was obtained here. The current for the present case was still small even when samples with larger conductivities were used. The electric current in the gold-gold configuration was also analyzed in terms of electrophoretic manipulation efficiency. The values were significantly larger than before and similar to the currents reported in [75]. In addition, gold-gold electrodes provided a better compatibility in terms of electrochemical effects, explained in Section 5.2.3. Therefore, for the sake of brevity, only the results obtained for the gold-gold configuration are presented here.
Two different sets of experiments were performed. In the first set, the electric field was not present, and the particles were freely moving with the flow (as shown in Fig. 5.8a). In the second set, however, the DC power supply was turned on. The applied voltage difference was $1.2 \, V \ (E = 10 \, kV/m)$. The steady state current for this case was measured to be $\sim 18 \, \mu A$. Five replicates were conducted for each set, and the results obtained from the flow microscopy (particle analyzer) were plotted and compared. Figure 5.10 shows a representative output graph from the flow microscopy. This graph demonstrates the number of particles counted in 0.1-minute time intervals. For each replicate, a similar graph was obtained and the data were used to find the pattern of the particles exiting the microfluidic device. Figure 5.11 presents the average cumulative counts of five runs (with standard deviations) for both cases of zero and nonzero electric potential. Considering the error bars, no meaningful difference is observed between the two cases. This is in contrast with what was expected from the theory. The above
experiment was repeated for different ranges of pH, from 4 to 10 (adjusted using HCl and KOH), and conductivities, from a few tens to a few thousands (adjusted using different monovalent salts such as NaCl and KCl). Also, different oocyst initial concentrations (up to a million per millilitre and down to a few hundred per millilitre) were used to address issues such as particle crowding. Finally, electric potentials, varied in both magnitude and direction, were examined along with different flow rates. To prevent repetition, the results for any of the above conditions are not shown here. In summary, there was no difference (within the order of statistical errors) between the results of zero and nonzero electric potential cases.

Fig. 5.10. Representative output graph of instantaneous number of particles (exiting the device) versus time obtained by flow microscopy (Brightwell Technologies Inc.). Each number is counted in a 0.1-minute time interval. Flow rate is 1 ml/hr.
Fig. 5.11. Average of five replicates for zero and nonzero electric potential runs. Flow rate is 1 ml/hr, $pH = 7.8$ and $\sigma = 1340 \mu S/cm$. The applied voltage is 1.2 V. The initial plateau is observed due to the void volume of the device, and it determines the time it takes for the particles to enter the detector instrument.

In addition to the oocysts, visible polystyrene microspheres (measured to be more charged than the oocysts as presented in Table 5.2) were used as the target particles. Still, the difference was not statistically significant. Different possible scenarios can be postulated to cause the discrepancy between the theory and the experiment. Some of the most important issues are discussed in the next section and the proposed remedies (if any) are presented.
Table 5.2. Polystyrene microspheres mobility as reported by Particle Characterization Laboratories, Inc. (Novato, US).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mobility ($\mu m/sec)/(V/cm)$</th>
<th>Zeta Potential (mV)</th>
<th>Conductance ($\mu S$)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RED in 0.22 um filt. diluent$^9$</td>
<td>-2.69E+00</td>
<td>-34.47</td>
<td>255</td>
<td>4.29</td>
</tr>
<tr>
<td>RED in 0.22 um filt. diluent</td>
<td>-2.80E+00</td>
<td>-35.85</td>
<td>255</td>
<td>4.29</td>
</tr>
<tr>
<td>RED in 0.22 um filt. diluent</td>
<td>-2.51E+00</td>
<td>-32.18</td>
<td>258</td>
<td>4.29</td>
</tr>
</tbody>
</table>

5.2.3 Challenges and troubleshooting

*Cryptosporidium/surrogate particles charge*

Depending on the host source of the oocysts, their charge may vary significantly. As seen in Table 5.1, the oocysts present a small-to-average zeta potential (up to 35 mV). Although not very large, this charge should be enough for the pathogen to be manipulated under an applied electric field. For the red polystyrene microspheres (Bangs Laboratories Ltd.), the same measurements were performed for a specific pH and conductance which thought to be the worst case scenario for the electrophoretic migration (Table 5.2), but. The results confirm that surrogate particles are generally more charged than the oocysts and better indicators for testing the applicability of electrophoresis. All the runs in Section 5.2.2 were replicated using both oocysts and surrogate particles to eliminate the concern about the neutrality or insignificant charge of *Cryptosporidium* oocysts.

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$^9$ RED: red polystyrene microspheres
**Electric double layer formation**

Similar to the charged particles (as discussed in Section 3.2.1), the formation of the electric double layer occurs at the surfaces of the electrodes. As a result, the effective electric field in the bulk solution is reduced (see Fig. 5.12). Counterions in the solution approach the oppositely-charged electrodes and screen the surface charge of the metals. This decreases the effective electric strength inside the bulk (sometimes down to less than one percent of the nominal field [79]). This drop is a function of the solution properties (pH and conductivity) [79]. Very high ionic solutions are not desirable since the surface charge of the electrodes becomes completely screened so that there will not be any significant electric potential difference in the bulk. The range of conductivities used in electrophoresis usually lies between 10 μS/cm and 10000 μS/cm [79]. Monitoring the electric current inside the channel helps to obtain an estimate of the field strength in the bulk (as shown in Fig. 5.9). Different conductivities were investigated in the experimental runs. For each run, the current was monitored and compared with the reported values in the literature for similar samples [75]. It was found that steel-gold electrode configuration did not perform as good as the gold-gold electrode configuration. Also, for the present setup, the best electric current was obtained for the conductivities in the range between 50 μS/cm and 1500 μS/cm for which Cryptosporidium oocysts possess a reasonable electric charge.

![Diagram of electric double layer formation](image)

**Fig. 5.12. Voltage drop at the EDLs close to the electrodes.**
Nonetheless, EDL formation poses a limitation on the minimum amount of the electric potential which can be applied to the electrodes. For very small amounts, practically no electrophoretic force is generated inside the solution. One may think that the voltage can be increased to compensate for the effect of EDLs. However, there are also limitations on the maximum applied voltage as explained in the next section.

**Electrode oxidation potential**

Metals possess an oxidation potential at which they start to release ions into a solution where electrons are exchanged. As the gold surface is in direct contact with water in the microfluidic device, its oxidation potential becomes a challenge and limits the maximum voltage which can be applied to the electrodes. Once the gold electrode starts to oxidize, it releases gold ions into the solution. These heavy metal ions may interfere with the solution and the electric field, or adsorb to the surface of charged particles. This can even lead to charge reversal in the worst conditions. The reason to select gold is its rather high oxidation potential compared to other metals, delaying the ion release phenomenon. It is also reported to be biocompatible which means it causes the least damage to the microorganisms [75]. The oxidation potential of gold is around 1.5 V to 1.6 V. Beyond this voltage, gold starts to oxidize. Close to this voltage, the chromium beneath the gold surface on the slide also starts to diffuse out to the solution (electroplating). Apart from the change in the appearance (Fig. 5.13), the fluctuations in the electric current were an indication of the occurrence of electroplating and gold oxidation. This maximum allowed voltage was found with trial and error, and all the tests were run under this marginal value (1.5 V).
Fig. 5.13. Gold oxidation and slide electroplating at a large voltage differences (2 V). Back of the slide is shown.

Electrolysis of water

Since the electrodes are in contact with water, electrolysis is inevitable at the interface. Formation of hydrogen and oxygen bubbles, as a result of electrolysis, may cause severe interference with the bulk flow and degrade the device performance. The chemical reactions are

\[
\text{Cathode (reduction): } 2 \text{H}_2\text{O}(l) + 2e^- \rightarrow \text{H}_2(g) + 2 \text{OH}^{-}(aq) \\
\text{Anode (oxidation): } 4 \text{OH}^{-}(aq) \rightarrow \text{O}_2(g) + 2 \text{H}_2\text{O}(l) + 4 e^- \tag{5.14}
\]

Water electrolysis also limits the maximum voltage which could be applied to the device. With this maximum voltage was found to be around 1.3-1.4 V for the present device configuration with a slight variation depending on the buffer solution properties. Beyond this limit, large bubbles were formed and released into the flow. The formation of bubbles was confirmed with real time observation through a preliminary design similar to the real experimental setup. However, for the real setup, no real-time observation of the electrode surface could be performed. Therefore, there was no way to determine whether microbubbles formed at the
electrodes. If microbubbles form, they will adversely affect the motion of particles in the bulk and close to the electrode surfaces.

Sample buffer properties

Electrophoretic mobility of a charged particle is a function of buffer properties such as pH and conductivity [87]. The electrophoretic mobility may approach zero or even reverse its sign when the solution properties change [94, 95]. As a practical application, this concept has been used in isoelectrophoresis where particles are exposed to a pH gradient and concentrated at their zero-mobility (isoelectric) point [58]. The isoelectric point occurs as a result of the excess presence of H⁺ ions which neutralize the negatively-charged chemical groups at the surface of the particles. Conductivity and pH were measured every time the flow cell experiments were conducted, both for the influent and the effluent. The measurements were compared with those obtained from Characterization Laboratories, Inc. to ensure particle charges are not affected during the separation process.

In Fig. 5.14, the electrophoretic mobility of oocysts is shown with relation to both pH and conductivity. The data were not sufficient to obtain an accurate surface plot. However, several conclusions can be drawn regarding the sensitivity of mobility with respect to variation of pH or conductivity. Figure 5.14b depicts the mobility versus pH for different conductivities. Although conductivity changes in each case, it is reasonable to infer that the absolute value of the mobility generally decreases as the pH approaches the acidic region. This is in agreement with the results reported in [94]. In the range investigated, the isoelectric point of the oocysts was not reached.

A similar plot was obtained for conductivity (see Fig. 5.14c). Neglecting the effect of pH, the mobility shows a significant change over a wide range of conductivity. Generally, the magnitude of mobility decreases when the conductivity is increased. This is due to the formation of strong thin double layers around charged particles. For maximum conductivity (far-right point on the graph), however, the trend is reversed. This seems to be due to the very large pH value (basic
solution). The best working condition was found to be in the range between 100 $\mu S/cm$ and 1000 $\mu S/cm$ for conductivity and around 7 and 10 for pH.
The pH is the most important factor in determining the mobility. For each run, it was confirmed that the pH of the solution did not change drastically from the influent to the effluent by measuring it before and after sampling. For all cases it was ensured that the measured pH was far enough from the isoelectric point where the particles act as if they were neutral. It is usually
assumed that when there is no significant pH change between the inlet and outlet, most likely, no charge neutralization due to pH occurs [75]. Nonetheless, no direct and real time measurement of solution pH was performed inside the microfluidic device as the sample was being tested. Therefore, no information is available about the possibility of having a pH gradient across the height of the microchannel. Any local change of pH inside the device could easily modify the mobility of the particle and interfere with the separation process.

In order to study the effect of pH independently, two data points with very similar conductivities and different pH values were selected. Figure 5.15 demonstrates the corresponding plot with the standard deviations calculated over three replicates. As expected, the mobility magnitude increases with increasing pH towards the basic region. The difference is significant considering the error bars. A similar type of graph could not be presented for conductivity using the available data.

![Figure 5.15. Effect of pH on mobility for constant conductivity (200 \( \mu S/cm \)).](image-url)
Presence of chemicals

Microspheres and oocysts are provided in a storage solution containing detergents such as Tween 20 to prevent hydrophobic interactions and agglomeration of the particles. Such detergents are occasionally reported to neutralize the charged particles. The mobility of oocysts was determined by Particle Characterization Laboratory (Table 5.1) and is plotted in Fig. 5.16. Adding Tween 20 to the solution had an insignificant effect on the charge of the oocysts.

![Graph showing the effect of Tween on electrophoretic mobility of the oocysts.](image)

**Fig. 5.16. Effect of Tween on electrophoretic mobility of the oocysts.**

5.3 Conclusions

The experimental results presented in the previous sections demonstrated that for any specific device dimensions, there was a critical flow rate below which the majority of particles did not leave the domain due to the gravitational field effect. For the current flow cell device, this critical flow rate was around 1 ml/hr assuming that the accepted limit for the capture
efficiency was larger than 95% of the inlet concentration. This flow rate is equivalent to 24 ml in 24 hours. If 10 litres of water needs to be sampled in 24 hours, 417 similar microchannels need to be fabricated and placed parallel to each other to provide enough sampling channels. This rather large number is an inevitable consequence of the low sampling rate of sedimentation. Although the magnitude of the capture efficiency can be quite satisfactory, the low sampling rate is one of the main shortcomings of using sedimentation alone which demands a long sampling time and/or large capture surfaces (i.e., bigger devices). To make it worse, the presence of external particles as dense as Cryptosporidium oocysts may interfere with the sedimentation process and cause the capture sites to be blocked. This issue is not severe if treated drinking water is being monitored for the presence of parasites. Considering all the above facts, any alternative method which is able to provide capture of organisms with a faster flow rate is worth investigating.

Theoretically, electrophoresis was expected to work favourably for particle separation. It was also expected that through using the electric field, the sampling rate could have been increased due to the stronger effect of electrophoretic forces (compared to that of the gravitational force). However, no success was achieved when the electric field was implemented in the experimental phase. The unsuccessful attempt is postulated to be due to the new challenges (such as water electrolysis and electrode polarization) that accompany the use of the electric fields. Some of the most important challenges were discussed and remedies were examined. It is concluded that electrophoresis for the separation and concentration of such large oocysts requires further investigation. Forces ignored in the present study, such as the steric repulsion between substrates and large particles, may need to be considered rigorously [79]. Also, extra efforts must be made to incorporate more effective remedies to the introduced experimental challenges. These remedies may remove the undesirable effects of harmful chemical reactions (such as water electrolysis at the electrodes) and produce a more efficient electric field for particle manipulation.
6 Conclusions and future work

6.1 Conclusions

Despite the recent progress in water quality monitoring and detection methods, the presence of pathogens such as Cryptosporidium parvum in drinking water remains a major threat to human health. The conventional detection method (EPA 1623) has been shown to be unreliable and inefficient in many circumstances. It also requires conducting complicated time-consuming processes such as magnetic separation. A microfluidic design was proposed to replace the separation and concentration steps in the current EPA 1623 method for the detection of Cryptosporidium oocysts in drinking water. The proposed device was intended to substitute the filtration and magnetic separation steps and hence, reduce complexity and unreliability of the EPA 1623 method. The underlying mechanisms in the proposed design involved separation of the pathogen by physical means and then capture them on functionalized reactive surfaces containing conjugate anti-pathogen antibodies.

In order to achieve an efficient reliable separation technique, the physics of pathogen transport and adhesion inside the microfluidic device were studied. Two different approaches were undertaken. First, the topology of the device (configuration) was examined to maximize the passive transport of pathogens to the reactive surfaces. This was performed through continuum numerical simulation of many different configurations and comparing the capture efficiencies. Three representative designs, believed to be the most effective ones, were selected and analyzed. The most efficient design was then found for the chosen parameters.

However, passive separation was found to be inefficient under most circumstances as the majority of pathogens exited the device without being captured. After the optimum configuration was selected, the possibility of adding any physical separation mechanism (active separation) was investigated in detail. Two different methods (sedimentation and electrophoresis) were chosen. Continuum numerical simulation was used again to compare the
effectiveness and benefits of each method. Based on the numerical modeling, it was found that both methods (especially electrophoresis) would work favourably towards enhancing the transport of pathogens to the reactive surface.

For both active and passive separation techniques, a continuum model of cell adhesion was included at the reactive elements neglecting the effect of flow hydrodynamics and fluid/particle interactions (cell deformation). Such phenomena have significant influence on the final behaviour of a biological cell when it is in contact with a surface equipped with conjugate molecules. A detailed molecular scale dispersed-phase simulation of cell adhesion was presented for the small gap close to the reactive elements. Cell physical characteristics (membrane rigidity, cytoplasm viscosity and density, membrane binding molecules density, etc) were selected for the simulation. The modeling provided a minimum antibody density required for the capture of the Cryptosporidium oocysts under maximum shear rates encountered in real experimental conditions. This simulation eliminates the need to conduct painstaking replicates to find the optimum antibody density and helps to focus on the examination of other important mechanisms such as selecting an effective transport method.

Finally, both sedimentation and electrophoresis were implemented experimentally for a fabricated microfluidic test cell. The numerical results obtained for sedimentation were in agreement with the experimental results. However, the experimental results for electrophoresis deviated from the simulations. Expected sources of this discrepancy (electrolysis of water, bubble formation, the large size of Cryptosporidium oocysts, etc.) were discussed, and some remedies were proposed. Nonetheless, the electrophoresis needs to be investigated further in terms of the feasibility of the approach. It was proposed in Chapter 5 that using the sedimentation technique and mass production of hundreds of microchannels will result in a separator flow cell device with a large recovery (95% depending on the flow rate compared to the average 50% reported for EPA 1623) and still a reasonable size. Then, this device can be flushed by means of a dissociation agent with low pH (similar to the agent used in EPA) to remove the captured oocysts and detect them under the microscope. In addition,
higher precision results obtained indicates the higher repeatability of the sedimentation technique over the EPA method in which filtration step introduces great unreliability.

The contributions of the present research can be summarized as follows:

- The significance of the assumption of electroneutrality and its effect on electrophoretic transport of highly-concentrated particle samples were investigated for the first time. It was shown that this assumption may lead to considerable errors under specific circumstances (Appendix A).
- A multiscale mesh-free numerical program was developed, for the first time, to simulate the coupled biological phenomena and hydrodynamic events which occur in cell adhesion. The numerical results were in great agreement with experimental results. Unique features of SPH facilitate the simulation of complex cell shapes in arbitrary channel geometries. The numerical simulation in this research can be easily modified to simulate the adhesion of other pathogens such as *Giardia*, if needed. Ultimately, such modeling can assist the development of flow cell devices that are able to capture different types of pathogen on their corresponding capture elements containing their conjugate antibodies.
- Membrane bending rigidity was incorporated in the cell adhesion simulation for the first time, providing more realistic models for different adhesion regimes introduced in previous adhesion studies.
- Sedimentation was successfully used in a microfluidic device with labelled reactive surfaces, to separate *Cryptosporidium* oocysts from pre-treated water.
- The applicability of electrophoresis was examined in detail for separation of *Cryptosporidium* oocysts. Oocysts electrostatic properties were thoroughly investigated with the focus on electrophoresis application in cell manipulation.
- For proof of concept, a microfluidic design was developed to replace filtration and IMS steps in EPA 1623 method using sedimentation as the driving mechanism to separate pathogens from water. The proposed design provided better recovery with higher precision.
6.2 Proposed future work

- Implementation of other external driving mechanisms such as non-uniform electric field (dielectrophoresis) can be the subject of future study. Rearranging the electrodes to create an electric field gradient helps to use other oocyst physical properties (such as permittivity) that can result in stronger driving forces. However, this has to be considered along with the complexity and the cost it may introduce to the fabrication process and the entire detection method.

- Design and fabrication of an integrated prototype including several microchannels is recommended for performing a pilot study. This will determine the applicability of the proposed design for sampling large amount of water in limited time intervals.

- Integrating new automated detection methods, other than the direct fluorescence microscopy, is required to reduce the detection time and human error during the process. There have been studies to develop optic-based methods as they are among the fastest and easiest method to implement. Incorporating such methods into the current separator will result in a very short detection time period, desirable for taking prompt action against the spread of the pathogens.

- Direct observation of flow/cell interaction and also cell/surface interaction will be beneficial to provide a realistic picture of cell adhesion close to the reactive elements. This can be used along with the SPH numerical simulation to obtain a more accurate estimation of oocyst physiological properties (structural properties, adhesion parameters, etc). Also, it is possible to observe effects such microbubble formation and pH gradient (using pH indicator solutions) inside the microchannel and propose remedies to prevent such phenomena.
Bibliography


Appendix A. Effect of electroneutrality assumption

In this section, the effect of electroneutrality (zero space charge density) on the efficiency of the separation is analyzed in a rectangular microchannel. It is shown that the electroneutrality assumption can drastically influence the final adsorbed concentration depending on the device configuration for very high sample concentrations. Average adsorbed surface concentration and capture efficiency are compared as measures of the performance of the device for a wide range of physiochemical parameters. The sensitivity of the simulation with respect to the ionic concentration of the buffer is investigated. It is also discussed how the electric field and nonzero space charge density alter the bulk concentration profile and the velocity field inside the microchannel.

A.1 Theory

The governing equations for the mass and momentum transport are basically similar to those presented in Chapters 2 and 3 with the difference that a body force is added to the Navier-Stokes equations due to the presence of an electric field.

A.1.1 Momentum transport and continuity equation

The buffer liquid is assumed to flow through a rectangular microchannel in the presence of a body force exerted due to an external electric field. The external electric field is applied in the transverse direction so that no electroosmotic velocity develops in the channel. Two-dimensional incompressible Navier-Stokes equations are used along with the continuity equation to find the velocity profile throughout the domain.

\[ \nabla \cdot \mathbf{u} = 0 \]

(A.1)

\[^1\text{Parts of Chapter 3 and Appendix A have been published in a journal article and a conference proceeding. Reprinted with permission from [109, 110].}\]
The no-slip boundary condition is assumed at the walls. A parabolic velocity profile is specified at the inlet with a known average velocity. The outlet is assumed to be at the atmospheric pressure.

### A.1.2 Mass transport equation

The transient two-dimensional mass transport equation which is solved inside the domain (neglecting the effect of external forces other than the electric field) is

\[
\frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \eta \nabla^2 \mathbf{u} + \rho_s E
\]  

(A.2)

Boundary conditions are the same as those discussed in Chapter 3. For all of the above equations, the change in the physical properties due to the temperature variation is neglected. This assumption is valid for small sampling time periods and low conductivity buffers which is the case in this study [33, 96]. The field strengths used in this study is in the order of 10-100 V/cm which is far below the critical value (2500 V/cm) introduced in the work of [96]. The reader is referred to a series of studies conducted by [69], [33] and [70]-[72] for more details on the effect of electrothermal phenomena when they are not negligible.

### A.1.3 Electrostatic equation

Electric potential is applied on the electrodes assembled in the device to use the natural charge of the particles and direct them towards the reactive surfaces. The Poisson equation is solved to derive the potential field inside the domain.

\[
\nabla^2 V = -\frac{\rho_s}{\varepsilon_0 \varepsilon_r}
\]  

(A.4)
In this equation, the space variation of the permittivity is neglected. Device electrodes are either grounded or supplied with constant electric potentials. The space charge density is a function of the charge of the species carried in the buffer and can be obtained from

\[ \rho_s = \sum_{i=1}^{N} c_i F z_i \]  

(A.5)

where \( c_i \) is the species concentration, \( z_i \) is its valence and \( F \) is the Faraday’s constant. The electric charge of the electrode surface causes the counterions in the buffer to travel towards the electrodes and form an electric double layer [33]. In this study, it is assumed that the surfaces of the electrodes are coated with polymers (permanent coating) or covered with chemical compounds (dynamic coating) so that the surface zeta potential is suppressed and no electroosmotic flow develops close to the boundaries [97]. This assumption helps to eliminate the electric field due to the surface charge and focus only on the effect of electrophoresis (originating from the applied electric field) on the separation. These types of surface treatments are commonly observed in capillary electrophoresis applications where electroosmosis may have unfavorable effects and is intended to be removed [98, 99]. Electric double layer (EDL) also forms around the charged biomolecules due to ion migration to their surfaces. The higher the ion concentration in the bulk, the thinner the EDL thickness. The electric double layer which forms around each individual particle reduces its effective net charge. For a high ionic concentration, the ions from a compact layer and screen the charge of the particle so that the bulk solution becomes neutralized [33]. However, for very low ionic and high sample concentrations, there are not enough counterions to neutralize completely the charge of the particles. In this case, the EDLs around the molecules overlap before they vanish into the bulk. This results in an uncompensated charge and consequently a nonzero space charge density in the bulk solution. In other words, the electroneutrality assumption (i.e., zero bulk space charge density) considered usually in electrophoresis analyses may not be true for low ionic solutions. In the present study, this effect has been included in the calculations through the valence of the charged particle. In essence, the actual valence used to simulate the particle migration is chosen to be only a percentage of the nominal one. This partial valence (charge) due to the
counterion effect has been discussed before in the works of Manning [100], and Dwyer and Bloomfield [101] for different applications of DNA molecules. The partial charge demonstrates the effective net charge of the particle and is estimated through a rough calculation of the electric potential and electrostatic charge of the particle as discussed in the next section. The resultant nonzero space charge density complicates the analysis as it couples the Navier-Stokes equations and the mass transport equation with the electrostatic equation (Eq. A.6). In the following sections, the significance of this assumption is investigated in detail.

To carry out the comparison between different cases analyzed in this thesis, the average surface concentration \( (c_{s,\text{avg}}) \) adsorbed on the reactive area is determined and compared for different cases. \( c_{s,\text{avg}} \) is interpreted as the performance of the device (see Chapter 2).

### A.1.4 Electroneutrality

In most electrophoretic and electroosmotic analyses it is assumed that there is an imbalance in the concentration of the ions in close proximity to the charged surfaces [33]. This imbalance is restricted to the double layer on the order of nanometers, and everywhere outside this layer, electroneutrality is assumed to be valid. However, this assumption has been proved to be invalid for small dimensions of the channel and buffer confinements [102] in which the EDLs formed around charged surfaces overlap to some extent. There have been some studies on the modeling of this phenomenon in nanochannels or solutions containing only ions. However, there is little effort made toward the investigation of the validity of this assumption in the buffers containing a sample and ions together.

Dwyer and Bloomfield [101] discussed that the net charge of biomolecules (DNA in their case) sensed in ionic solutions is lower than what it is supposed to be theoretically. The counterions gathered around the biomolecule are responsible for this charge reduction. In all previous studies [31, 33, 69-72, 103-105] this effect has been neglected by assuming the complete neutralization and zero space charge density in the electrostatic equation. The underlying assumption in all these studies is that the sample concentration is very small (much less than a thousandth) compared to the ion concentration of the buffer so that there are enough ions to
screen the sample molecule charges [31, 106]. Here, an approach is adopted to examine the validity of this assumption for the cases that the ion concentration is still larger than, yet more comparable to, the sample concentration (1000 times or smaller). A particle, 100 \text{ nm} \text{ in diameter}, is placed in a solution containing positive and negative ions (e.g., Na$^+$ and Cl$^-$). To reduce the computational cost, only a small box of the solution (3×3×1 \text{ \mu m}) is considered as shown in Fig. A.1. The ion concentration is kept low at 1×10^{-3} \text{ mol/m}^3. Although this ion concentration is still large compared to the sample concentration, it lies at the bottom range of the observed concentrations in bioseparation processes [106]. In essence, the lower the ionic strengths of the buffer, the larger the electrophoretic mobility.

\[ =100 \text{ micron/sec} \]

\[ \text{Min: } 0 \text{ to Max: } 14.276 \]

\[ \text{Min: } 4.663e^{-11} \text{ to Max: } 14.0 \]

**Fig. A.1. Space charge density for a charged particle inside a symmetric solution.**

In Fig. A.1 the buffer flows from left to right with the velocity of 1×10^{-4} \text{ m/sec} at the left boundary. No viscous stress is assumed at the right boundary, and slip boundary condition is assumed at the top and the bottom boundaries to allow the fluid to flow smoothly between the imaginary simulation boxes which form the entire buffer liquid by repetition. The surface
charge of the biomolecule is assumed to be -20e (e is the electron charge). This surface charge is used to calculate the surface potential of the particle using the following equation

\[ \psi_0 = \frac{Q_0}{4\pi \varepsilon_0 \varepsilon_r d} \]  \hspace{1cm} (A.7)

The surface potential becomes around -7 mV. All other boundaries are at 0 V since they are assumed to be far away from the particle surface. The ions are introduced at the left boundary with the specified concentration \((1 \times 10^{-3} \text{ mol/m}^3)\), and convective flux is considered at the right boundary. Other boundaries are insulated or symmetry. Using COMSOL Multiphysics software, 2D mass, momentum and electrostatic equations are solved simultaneously. As a result of the electrostatic force between opposite charges, positive ions gather around the bioparticle and negative ions repel. Figure A.1 shows the space charge density contours and surface plot. A smaller box \((1.3 \times 1.3 \times 1 \mu m)\) with dash sides is shown in this figure in the middle of the bigger box surrounding the particle. This box (with one particle inside) corresponds to the sample concentration of \(1 \times 10^{-6} \text{ mol/m}^3\) for one micron depth of the channel. This concentration lies in the usual range of biomolecule concentrations in bioseparation processes [31]. As shown in this figure, the charge density does not approach zero in the areas near the boundaries of the small box. Considering the fact that, in reality, there will be another particle in the proximity of the small box at such sample concentration, it is concluded that for this certain ionic concentration, the charge of the particle is not completely neutralized and there will be EDL overlap between neighbouring particles. This simulation, although very rough, proves that it is not always true to assume electroneutrality especially for low ionic strength. To consider this uncompensated charge in the continuum simulation of particle transport, an approach similar to the one introduced by Chatterjee [103] is taken in a sense that a net effective charge (and net electrophoretic mobility) is defined for the ensemble of ions and the sample altogether. If, instead of distinct particles, one considers the small box as a continuum medium, a nonzero partial charge for the solution (containing the particle and the ions as a whole) must be included. To estimate this partial charge, the electric potential of the EDL for spherical particles is used from Crow et al. [46]. This potential is given by
\[ \psi = \psi_0 \frac{a}{r} \exp[-\kappa(r - a)] \]  

(A.8)

where \( \psi_0 \) is the surface potential, \( a \) is the particle radius, \( r \) is the radial distance and \( \kappa \) is Debye-Huckel length. \( \psi_0 \) can be related to \( Q_0 \), the surface charge of the particle, using Eq. A.7.

Here, the maximum value of \( r \) is equal to half of the dimension of the small box considered in Fig. A.1 (denoted as \( r_m \)). This small box is used to determine the sample concentration. Therefore, it is possible to relate the maximum radial distance, \( r_m \), to the sample concentration, \( c \). For one micron depth, the volume of the simulation box with \( 2r_m \times 2r_m \) dimensions is

\[
\text{volume} = (2r_m) \times 10^{-9} \times (2r_m) \times 10^{-9} \times 1 \\ \times 10^{-6} = 4r_m^2 \times 10^{-24} \text{ m}^3
\]

(A.9)

For one particle inside this box, the sample concentration would be

\[
c = \frac{1}{4r_m^2 \times 10^{-24} \times 6.022 \times 10^{23}} = \frac{0.41}{r_m^2} \text{ mol/m}^3
\]

(A.10)

So, the maximum radial distance is

\[ r_m = \sqrt{\frac{0.41}{c}} \]

(A.11)

Using Eq. A.8, a relation can be found between the electric potential at \( r_m \) and the sample concentration

\[ \psi_m = \psi_0 \frac{a}{\sqrt{\frac{0.41}{c}}} \exp[-\kappa \left( \sqrt{\frac{0.41}{c}} - a \right)] \]

(A.12)

Debye-Huckel length for water at room temperature is
\[ \kappa^{-1}(nm) = \frac{0.304}{\sqrt{1/2 \sum c_i x_i^2}} \]  

(A.13)

In this equation, \( c_i \) is the ion concentration in terms of \( \text{mol/lit} \). For example, for the particle shown in Fig. A.1, \( \kappa^{-1} \) becomes 304 nm.

The electric potential at the edge of the small box (\( \psi_m \)) is only a small portion of the surface electric potential (\( \psi_0 \)). As a result, the entire box can be replaced with a large particle of radius \( r_m \) with a lower surface potential equal to \( \psi_m \) (and equivalently a lower surface charge of \( Q_m \)). Hence, instead of \( Q_0 \), a new charge like \( Q_m \) is considered.

\[
\frac{Q_m}{Q_0} = \frac{r_m \psi_m}{a \psi_0} = \exp[-\kappa(r_m - a)]
\]

\[
= \exp \left[ -\kappa \left( \frac{0.41}{c} - a \right) \right]
\]

(A.14)

The new partial charge after including the effect of the ions can be calculated as a function of \( c \), sample concentration, at each point. Later on, this partial charge will be reflected in the valence (\( z \)) in the electrostatic equation to calculate the space charge density from Eq. A.5, and the mass transport equation will not be solved for the ions inside the buffer. In other words, the effect of the ions will be considered indirectly in the simulation through the fractional charge. This method is an approximate way to find the partial charge since Eq. A.8 is derived when zero electric potential is used as a boundary condition far from the charged particle.

It is worth mentioning that the primary purpose of this study is to investigate the importance of the electroneutrality assumption. The nonzero space charge density complicates the governing equations significantly. Therefore, the following simplifications have been considered in the simulation to reduce the computation time:

1) The nonspecific adsorption and diffusion of particles to the reactive surface (as presented by Das et al. [104]) are not considered.

2) No ion exchange occurs at the electrodes in contact with the buffer.
3) The electrostatic repulsion force originated from the possible interactions of EDLs of different particles is neglected. A detailed analysis of the validity of this assumption will be presented in the following sections.

A.2 Experimental validation

In this section, the validity of the nonelectroneutrality assumption is examined against the experimental data presented by Kassegne et al. [31]. COMSOL Multiphysics software is used to solve the coupled 2D mass transport and electrostatic equations. The DNA hybridization microchip introduced in the works of Kassegne et al. [31] and Das et al. [33] is analyzed here and the results for both the zero and nonzero space charge density are compared with the experiment. The diffusion coefficient and the mobility are assumed to be the same as the values used by Kassegne et al. [31] (which are $2\times10^{-11}$ $m^2/sec$ and $1.5\times10^{-8}$ $m^2/V.sec$, respectively). Figure A.2 shows the cylindrical chip that consists of an anode and a ring-shaped cathode at its center and perimeter, respectively.
Fig. A.2. a) Schematic of the microchip used by Kassegne et al. [31]. Anode diameter is 80 microns. Cathode ring is 40 microns wide. b) Comparison of the experimental data with numerical simulation. c) Error percentage of the two cases (zero and nonzero space charge density).

Figure A.2b compares the accumulated DNA concentration relative to the maximum accumulated DNA at $t = t_{\text{max}}$ at the anode for both the zero and nonzero space charge density. As it is clear, the numerical data is in very good agreement with the experimental results. However, the numerical results obtained based on the nonzero space charge density assumption demonstrate a better agreement - both in terms of values and pattern - with the experiment. The results with the zero charge density does not show the plateau observed at the end of the experiment while the results of nonzero density demonstrate a better trend. The errors are compared for the two cases in Fig. A.2c. Detailed examination of both cases confirmed the difference in the observed trends is due to the change in the electric field as a result of charge accumulation inside the chip and close to the electrodes. The contour plots of the electric potential are presented in Fig. A.3. As it is seen, the difference is more profound in the middle of the simulation box resulting a larger gradient and flux on top of the anode.

![Graph showing error percentage comparison](image-url)
Fig. A.3. Electric potential contours for the cases of a) zero and b) nonzero space charge density.

This example shows the effect of the nonzero-density assumption in a simple microfluidic chip used for DNA hybridization with no external flow of the buffer. However, in most applications, a microchip is developed for the separation and detection of particle in a continuous flow of the sample to reach higher throughputs. Optimizing the separation efficiency of such devices is a
non-trivial task as many different parameters become involved. In the next section, the influence of the applied electric field and the electroneutrality assumption on the separation efficiency of a continuous flow microchannel is investigated.

### A.3 Geometry

Figure A.4 shows the parallel-plates (PP) open channel with the reactive surface considered for the electrokinetic analysis. In all simulation cases (conducted with and without applied voltage, and with and without zero space charge density), the design criteria that the reactive surface area and the overall channel size must be the same are met. That means the channel size is always $120 \, \mu m \times 235 \, \mu m$. Both the top and bottom surfaces are labeled with ligand molecules. Thus, the total length of the reactive boundary becomes $470 \, \mu m$ for all simulations.

![Parallel-plates (PP) open channel with reactive surfaces](image)

**Fig. A.4.** Open channel parallel plate (PP) in which top and bottom surfaces are reactive.

### A.4 Results

In this section, the electrokinetics of the particle separation process is analyzed for the geometry introduced above. Table A.1 presents the numerical values of the chemical and physical parameters used for the simulations. COMSOL Multiphysics software is used to solve the governing equations. The two-dimensional Navier-Stokes equations are coupled with the mass transport and electrostatic equations and solved simultaneously whenever the electroneutrality assumption does not hold. Triangular mesh is used throughout the domain.
and is refined near the reactive boundaries to capture the high gradients of the concentration. Meshing the boundaries is very crucial especially around the reactive surfaces where the capture occurs. The mesh independency has been investigated with the margin of 1% change in the numerical results. The buffer liquid with the sample is allowed to flow through the channel for one minute which is in the range of the usual sampling times for high throughput microfluidic devices [31]. In the next section, the significance of adding electric field to the process is examined.

Table A.1. Values of the parameters used in the simulations for non-electroneutral condition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward reaction rate ($k_{on}$)</td>
<td>$10^5$ m$^3$/ (mol. sec)</td>
</tr>
<tr>
<td>Backward reaction rate ($k_{off}$)</td>
<td>$10^{-2}$ sec$^{-1}$</td>
</tr>
<tr>
<td>Ligand concentration ($c_{s0}$)</td>
<td>$5 \times 10^{-8}$ mol/m$^2$</td>
</tr>
<tr>
<td>Diffusion coefficient ($D$)</td>
<td>$1 \times 10^{-10}$ m$^2$/sec</td>
</tr>
<tr>
<td>Inlet concentration ($C_0$)</td>
<td>$1.5 \times 10^{-6}$ mol/m$^3$</td>
</tr>
<tr>
<td>Average inlet velocity ($u_{avg}$)</td>
<td>$10^{-4}$ m/sec</td>
</tr>
<tr>
<td>Electric potential ($V_0$)</td>
<td>0.5 V</td>
</tr>
<tr>
<td>Electrophoretic mobility ($\mu$)</td>
<td>$1.5 \times 10^{-8}$ m$^2$/V. sec</td>
</tr>
</tbody>
</table>

A.4.1 Effect of the electric field on biomolecule adsorption

Figure A.5 compares the surface plots of the particle bulk concentration with and without the electric field. The same plots have been presented in Chapter 3. Here they are repeated for the sake of comparison between the electroneutral and non-electroneutral cases. When solving the electrostatic equation, the space charge density was assumed to be zero. In this case, no body force due to the applied electric field is included in the Navier-Stokes equations, and the electrostatics is decoupled from the mass transport. The results show that the particles are
deviated and directed towards the positive-voltage electrode due to their inherent negative charges.

Fig. A.5. Sample bulk concentration in PP a) without and b) with the electric field (zero space charge density) at t=60 sec.

During the sampling period, the electrophoretic mobility of particles brings them close to the reactive surface. As a result, the device will eventually have a larger average adsorbed surface concentration with respect to the passive parallel-plate device (with no electric field applied). Figure A.6 depicts the difference in the adsorbed surface concentration for the two cases (i.e., with and without the electric field) at both the top and bottom surfaces. The average surface
concentration for the top and bottom surface increases from $5.17 \times 10^{-10} \text{ mol/m}^2$ to $2.35 \times 10^{-9} \text{ mol/m}^2$ by applying voltage at the electrodes. This implies around 4.5 fold increase in the rate of adsorption. The capture efficiency for the active microchannel is around 98.6%, which is about 5.5 times the capture efficiency of the passive case (17.8%).

Fig. A.6. Sample adsorbed surface concentration at the top and bottom surfaces a) without and b) with the electric field at $t=60 \text{ sec}$. 

(a) 

(b)
A.4.2 Effect of the nonzero space charge density on particle adsorption

Here, the same channel is considered with the same properties (as presented in Table A.1). However, the valence calculated through Eq. A.14 is used in Eq. A.5 to find the space charge density. This density, which is a function of sample concentration, is inserted at the right hand side of Eq. A.4 to compute the electric field which is coupled with the mass and momentum transport. Figure A.7 depicts the effect of the nonzero space charge density on the electric potential contours. It can be seen that the electric potential contours are more compact near the bottom of the channel at the inlet for the nonzero charge density (Fig. A.7b).

![Electric potential contours for a) zero and b) nonzero space charge density.](image)

Fig. A.7. Electric potential contours for a) zero and b) nonzero space charge density.
Figure A.8 compares the surface plots of the electrophoretic flux for the zero and nonzero space charge densities. As expected, wherever higher gradients of potential exists (i.e., near the bottom surface at the inlet) larger electrophoretic flux is observed. Conversely, near the middle of the inlet, where the potential gradient is lower, smaller flux exists. The distribution of the negative space charge density changes the electric lines in a way that a minimum potential appears at the inlet. This minimum potential causes some of the particles to migrate towards the top surface as it produces a favorable electric potential in that direction (see Fig. A.8b).
Fig. A.8. Electrophoretic flux with a) zero and b) nonzero space charge density (arrows show the relative magnitude and direction of the electrophoretic flux) at $t = 60$ sec.
The corresponding surface plot of the bulk concentration is presented in Fig. A.9. As expected, the large electrophoretic flux at the bottom and small flux in the middle modify the concentration plot so that there seems to be a wider profile of particles at different concentrations. In other words, the relatively small flux in the middle delays the particle motion towards the reactive surface. The large flux at the bottom, on the other hand, accelerates the transport of particles at the bottom of the channel to the reactive surface. These effects eliminate the sharp concentration profile edge that exists in Fig. A.5b for the case of the zero space charge density.

![Figure A.9](image.png)

**Fig. A.9. Sample bulk concentration with the nonzero space charge density at t=60 sec.**

Figure A.10 compares the adsorbed surface concentration at the top and bottom surfaces of the channel for the zero and nonzero space charge densities. For the latter, the larger electrophoretic flux manifests itself in the higher surface concentration at the inlet (on the bottom surface) which gradually decreases as the particles move towards the outlet. The average surface concentration for the top and bottom surfaces is $2.35 \times 10^{-9} \text{ mol/m}^2$ for the zero charge density and $2.4 \times 10^{-9} \text{ mol/m}^2$ for the nonzero charge density. It is worth mentioning that, due to the electric field pattern, some of the particles are deviated towards the top surface (grounded electrode) for nonzero charge density as it is shown in Fig. A.8b.
From Figs. A.6-A.10 it is concluded that the capture efficiency of the device is a function of the electrical properties as well as the geometry. For instance, if the channel was shorter, the number of biomolecules exiting the outlet would be different for the zero and nonzero charge density. This is demonstrated in Fig. A.11 where the capture efficiencies of the two cases are compared at different vertical cross sections along the channel. As it is seen, depending on the length of the reactive surface, different values are observed for different charge densities. For shorter reactive lengths, the capture efficiency (CE) of the nonzero density case is larger than
the zero charge density. However, the two efficiencies are in the same order as the channel becomes longer. The nonzero CE increases more gradually towards the complete capture as expected.

![Graph showing capture efficiency comparison at different cross sections for zero and nonzero charge density.](image)

**Fig. A.11.** Capture efficiency comparison at different cross sections for zero and nonzero charge density.

### A.4.3 Effect of the electric potential and flow rate on capture efficiency

Figure A.12 reveals the transient behaviour of the sample adsorption on both the top and bottom surfaces for the three cases (zero potential, zero density and nonzero density). The end time (60 sec) is significantly lower than the time needed for surface saturation. Therefore, the plots present the initial rate of adsorption for each case. As it is seen, the rate of adsorption at the bottom surface is the highest for the zero density case while at the top surface it becomes the lowest.
Fig. A.12. Surface occupation of binding sites with respect to time at the a) top surface and b) bottom surface for no electric field, zero charge density and nonzero charge density. Ligand concentration is $5\times10^{-8}$ mol/m$^2$.

The behavior of the system under different electric potential differences is also worth studying as migration due to the electric field is predominant here. All the reactions take place in the transport-limited regime since Damkohler number is $k_{on}c_0\tau/D=1.8\times10^5$ [35]. The three main
mechanisms, governing the sample transport, are diffusion, convection and electromigration. Since Peclet number is large \((Pe = u_{avg} h/D = 120)\), the convective transport is more dominant compared to the diffusion except for the areas very close to the reactive surfaces. Thus, for the sake of brevity, only the effect of the relative strengths of convective and electromigrative transport are compared here. This will imply the effect of the potential difference on the surface adsorption for certain slow rates. The dimensionless number introduced in Barz [105] is used \((\Lambda = \mu V / u_{avg} h)\). Figure A.13 depicts the capture efficiency for both zero and nonzero space charge density versus \(\Lambda\) numbers. For large \(\Lambda\) (equivalent to large potentials at constant flow rates), the capture efficiency is larger, and there is no significant difference between the two cases as both are able to capture all the sample molecules in the given space. However, at small \(\Lambda\), the capture efficiency of the zero density case is much smaller. This deviation is due to the fact that for nonzero charge density there exists a large potential difference inside the domain due to the charge of the sample introduced at the inlet (e.g., see Fig. A.7b). The potential extremum at the inlet is responsible for the large capture efficiency even at low applied electrical potentials.

![Figure A.13. Capture efficiency versus \(\Lambda\) for zero and nonzero space charge density.](image-url)
A.4.4 Effect of ion concentration on surface adsorption

The sensitivity of the results to the ion concentration in the buffer is examined in Fig. A.14. Adsorbed surface concentration is solved for three different ion concentrations using the nonzero space charge approach and compared with the result of the zero space charge density for $\Delta V=0.5$ V. As it can be seen, for the ion concentration above $1 \times 10^{-1}$ mol/m$^3$ the results are indistinguishable from the zero density case. Therefore, for the specific conditions under which the simulation is run this buffer concentration is the threshold below which the nonzero space charge density causes a significant deviation with respect to the zero density.

![Graph of surface concentration for different ion concentrations in buffer solution.](image)

**Fig. A.14. Surface concentration for different ion concentrations in buffer solution.**

A.4.5 Effect of nonzero charge density on velocity field

The nonzero space charge density also changes the velocity profile considerably (see Fig. A.15). As it is shown in this figure, there is a significant deviation from the parallel velocity profile occurring in the case of the zero space charge density. The magnitude of this deviation
becomes more significant for stronger fields. This deviation increases the convection of biomolecules to the surface due to the body force which appears in Eq. A.2.

![Fig. A.15. Velocity field of the buffer with nonzero charge density.](image)

### A.4.6 Effect of neglecting the EDL repulsion force on biomolecule adsorption

Throughout this study, it was assumed that the EDLs overlap so the charge of the sample molecule is not neutralized. However, the overlap of EDLs is expected to introduce an extra repulsion force as discussed in the literature [107]. Here an approach is taken to confirm EDL repulsion is not significant compared to the electrostatic force present in the analysis. According to Hunter [107], for small values of $\kappa\alpha$, the repulsive force between two spheres with overlapped EDLs is obtained through the following equation

$$F_R \approx 2\pi\varepsilon a\psi_0^2\kappa e\exp(-\kappa H)$$  \hspace{1cm} (A.15)

where $H$ presents the minimum distance between the surfaces of the two spheres. Using the above equation, the repulsive force is obtained for $\psi_0=7$ mV and $\kappa^{-1}=304$ nm. $H$ is selected based on the dimensions shown in Fig. A.1 by considering another small box at one side of the presented dash box.
\[ H = 2(r_m - a) = 2 \left( \sqrt{\frac{0.41}{c} - a} \right) \]  

(A.16)

In this relation, \( r_m \) is replaced from Eq. A.11. Now, the electrostatic force due to the external electric field is calculated and compared with the repulsive force. It is known that

\[ F_E \approx E Q_0 \]  

(A.17)

where \( E \) is the electric field strength and \( Q_0 \) is the surface charge. The surface charge is used since it is assumed that the shear plane lies at a distance very close to the diffuse layer. Hence, the zeta potential (i.e., the potential at the shear plane) is close to the surface potential. Using the relation between \( \psi_0 \) and \( Q_0 \), the force ratio becomes

\[
\frac{F_R}{F_E} = \frac{2\pi \varepsilon_0 \kappa \exp(-\kappa H)}{E Q_0} = \frac{\psi_0}{2E^\kappa \exp(-\kappa H)}
\]

\[
= \frac{\psi_0}{2E^\kappa} \exp \left[ -2\kappa \left( \sqrt{\frac{0.41}{c} - a} \right) \right]
\]  

(A.18)

Figure A.16 presents the surface plot of the force ratio ranges of 0.01 < \( \frac{F_R}{F_E} \) < 0.1. The electric potential is 0.5 V between the electrodes (outside this range is shown in white color). Except for a small area at the inlet, the repulsive force is one to two orders of magnitude smaller than the electrostatic force. In most part of the channel (except near the outlet), the nonzero space charge density is influencing the electric lines and deviating them from being parallel while only in a small portion at the inlet the error introduced through neglecting of the repulsive force is observed. Thus, this assumption is expected to cause some errors at the inlet. However, the simulation still remains valid for almost the entire domain with a reasonable accuracy. Including the repulsive force in the mass transport equation (Eq. A.3) can add higher accuracy. This modification will be considered as the future work.
Microfluidics devices with flow-through channels and mass transport to reactive surfaces have been considered in numerous applications in recent chemical and biological research. The performance of such devices strongly depends on the conditions under which they run. It is an intricate task to find the optimal working conditions due to the variety of the parameters that govern the physics of the problem [35]. This study presented numerical simulation which allows the thorough examination of the effect of electrophoretic properties of the sample on the device efficiency. It was demonstrated that electric field can significantly enhance the capturing capability of the device, and this significance depends on the reactive surface configuration. However, unlike past studies, it was shown that the electroneutrality assumption is not necessarily true especially for low ionic concentrations for which charge screening of the sample molecule does not happen effectively. In fact, the results of this study show that the nonzero space charge plays an important role in bioparticle capturing, flow and electric field patterns for low ionic concentrations. The electric lines are significantly influenced by a nonzero
term in the Poisson electrostatic equation. Consequently, this alters the bioparticle electrophoretic migration paths towards the reactive surfaces. In addition, a nonzero space charge density appears as a body force in the Navier-Stokes equations which changes the convective motion of the target bioparticle. All these facts suggest that the electroneutrality assumption must be used with caution whenever low ionic strengths of the buffer are encountered in detection and separation processes.