CFD ANALYSIS OF EDTA-CACL2 REACTION IN A MICROFLUIDIC CHANNEL TO AID IN DESIGN OF NOVEL CALORIMETER DEVICE

A Thesis Presented
by
Justin Reiter

to
The Department of Mechanical and Industrial Engineering

in partial fulfillment of the requirements
for the degree of

Master of Science
in the field of
Mechanical Engineering
Thermoﬂuids

Northeastern University
Boston, Massachusetts

January 2015
ABSTRACT

Calorimetry is a valuable tool in pharmaceutical, biochemical and clinical diagnostic application areas. Thermodynamic quantities such as enthalpy of reaction are an indication of the efficacy of the interaction between active compounds. This information can enable acceleration of the drug development process by facilitating proper selection of the most promising compounds for later stages of development. A microfluidic calorimeter is being developed which offers reduced compound consumption, shorter experiment time, and higher throughput. The calorimeter employs a novel optical sensor based on the extraordinary optical transmission (EOT) of light through nanohole arrays (NHAs) which offers fine temperature and spatial resolution. Thermodynamic quantities such as enthalpy of reaction and binding constant can then be extracted from the temperature and concentration measurements.

A 3-D computational fluid dynamics (CFD) simulation was performed with the commercial package ANSYS Fluent to understand the momentum, heat and mass transport within the device for the reaction of calcium chloride with EDTA. A simplified reaction model for the formation of the calcium-EDTA complex was developed. The temperature and concentration fields for several different cases were analyzed to assess the viability of the device and determine the effect of changing the flow rate and reactant concentration. The original intent was to mix 1mM CaCl2 solution with 0.1mM EDTA at a flow rate of 5 ul/min. It was found that this set of parameters did not produce a large enough temperature change within the device, limiting its use as a calorimeter. It was then found that higher heat release can be achieved by increasing reactant concentration or flow rate, at the expense of reactant quantity consumed per experiment. The flow rate is also directly related to the width of the diffusion region, the distribution of the chemical reaction heat source, and the shape of the resulting thermal plume.
ACKNOWLEDGEMENTS

First and foremost I would like to thank my advisor, Professor Gregory Kowalski, for granting me this opportunity and supporting me throughout this project. He has been a mentor to me during my Northeastern education, both undergraduate and graduate. His guiding voice and limitless patience kept me grounded despite seemingly endless challenges and setbacks. I have learned more about this topic, engineering and research in general, and myself during this experience than I ever thought possible, and I owe it all to him. I would like to thank Mehmet Sen and Masoud Modaresifar for their valuable time and insight, as well as performing the work that made my research possible. I would also like to thank Professor Reza Sheikhi and Professor Barry Satvat for the valuable insight they provided in the areas of CFD and chemical thermodynamics. Finally, I would like to thank Tom Olson and Payam Parsinejad for supporting the computer technology required to perform this research.

I owe the world to my parents, Carolyn and Lance, for giving me the opportunity to attend Northeastern and encouraging me to challenge myself and pursue excellence in this work and throughout my academic career. The technical challenges of this project were only exceeded by the emotional ones, and I would have been lost without Christina Scilingo. She steadfastly supported me throughout this process, giving me the strength to keep going when all I wanted to do was give up. I know that you have sacrificed a lot for me over these last few months, and I am eternally grateful.
# TABLE OF CONTENTS

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

2. Background ................................................................................................................. 1

   2.1 Microfluidic Flow Characteristics ................................................................. 2

   2.2 Extraordinary Optical Transmission .............................................................. 7

   2.3 EDTA-CaCl$_2$ Reaction ............................................................................... 8

      2.3.1 Coordination Chemistry ....................................................................... 10

      2.3.2 Complex Ion Formation ..................................................................... 11

      2.3.3 Buffers ............................................................................................... 15

      2.3.4 Chemical Kinetics ............................................................................ 17

3. Problem Setup ........................................................................................................... 21

   3.1 Experimental Parameters .............................................................................. 22

   3.2 Reaction System ........................................................................................... 23

4. Numerical Simulation ............................................................................................... 26

   4.1 Geometry and Boundary Conditions ........................................................... 26

   4.2 Computational Domain ................................................................................ 28

   4.3 Models .......................................................................................................... 30

   4.4 Solution Methods ....................................................................................... 34

5. Results and Discussion ............................................................................................. 35

   5.1 Case 1: $Q=5\mu$L/min, $C_{Ca}=1\text{mM}$, $C_{EDTA}=0.1\text{mM}$ .................. 35

   5.2 Case 2: $Q=5\mu$L/min, $C_{Ca}=100\text{mM}$, $C_{EDTA}=100\text{mM}$ .......... 40

   5.3 Case 3: $Q=2.5\mu$L/min, $C_{Ca}=1\text{mM}$, $C_{Edta}=0.1\text{mM}$ .............. 42

   5.4 Case 4: $Q=500\mu$L/min, $C_{Ca}=1\text{mM}$, $C_{Edta}=1\text{mM}$ .............. 44

6. Conclusions ............................................................................................................... 45

7. Future Work .............................................................................................................. 46

8. Appendices ................................................................................................................. 48

9. References ................................................................................................................. 56
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T-sensor geometry</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>T-sensor diagram [1]</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Typical velocity profile within a microfluidic channel of high aspect ratio [5]</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Effect of distance along channel on concentration profile [5]. a) For distances small compared to the channel height, the concentration profile has 3D character. b) For distances greater than the channel height, the profile is uniform.</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Schematic of temperature sensor using a nanohole array and SPR/EOT technology [3]</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Protonated H4EDTA [12]</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>Deprotonated EDTA with carboxyl lone pairs more visible [13]</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>Metal-EDTA complex</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>EDTA Equilibrium Speciation</td>
<td>13</td>
</tr>
<tr>
<td>10</td>
<td>Ca-EDTA reaction system</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>Graph of observed binding enthalpy vs. buffer ionization enthalpy obtained using ITC calorimetry [21]</td>
<td>17</td>
</tr>
<tr>
<td>12</td>
<td>Flow channel</td>
<td>26</td>
</tr>
<tr>
<td>13</td>
<td>Plot of CaEDTA Concentration in Z-Direction for Mesh Independence</td>
<td>29</td>
</tr>
<tr>
<td>14</td>
<td>Plot of CaEDTA Concentration in X-Direction for Mesh Independence</td>
<td>29</td>
</tr>
<tr>
<td>15</td>
<td>Plot of CaEDTA Concentration in Y-Direction for Mesh Independence</td>
<td>30</td>
</tr>
<tr>
<td>16</td>
<td>XY Plane Mesh Cross-Section</td>
<td>30</td>
</tr>
<tr>
<td>17</td>
<td>Reaction 1 (forward reaction) inputs</td>
<td>32</td>
</tr>
<tr>
<td>18</td>
<td>Reaction 2 (reverse reaction) inputs</td>
<td>32</td>
</tr>
<tr>
<td>19</td>
<td>Residuals plot</td>
<td>34</td>
</tr>
<tr>
<td>20</td>
<td>XZ-Midplane Velocity Contours, Q=5 uL/min</td>
<td>35</td>
</tr>
<tr>
<td>21</td>
<td>X-direction velocity profiles at different distances along channel length, Q=5 ul/min</td>
<td>36</td>
</tr>
<tr>
<td>22</td>
<td>Y-direction velocity profiles at different distances along channel length, Q=5 ul/min</td>
<td>36</td>
</tr>
<tr>
<td>23</td>
<td>Fully developed velocity profile, Q=5 ul/min</td>
<td>37</td>
</tr>
<tr>
<td>24</td>
<td>Contour Plots of CaEDTA Concentration at different z locations. From top to bottom, z=0.25mm, 0.5mm, 1mm, 2.5mm, 5mm, 7mm. Q=5uL/min, Cca=1mM, Cedta=0.1mM</td>
<td>38</td>
</tr>
</tbody>
</table>
Figure 25: Averaged CaEDTA concentration, Q=5ul/min, Cca=1mM, Cedta=0.1mM... 39
Figure 26: Averaged temperature, Q=5 uL/min, Cca=1mM, Cedta=0.1mM ............... 39
Figure 27: Averaged Heat of Reaction, Q=5ul/tmin, Cca=1mM, Cedta=0.1mM......... 39
Figure 28: EOT signal data from EDTA-CaCl2 experiment, run 1 [1]; Z1=0.9mm, Z2=2.2mm. ....................................................................................................................... 40
Figure 29: EOT signal data from EDTA-CaCl2 experiment, run 2 [1]; Z1=0.9mm, Z2=2.2mm. ....................................................................................................................... 40
Figure 31: Contour Plots of CaEDTA Concentration at different z locations. From top to bottom, z=0.25mm, 0.5mm, 1mm, 2.5mm, 5mm, 7mm; Q=5uL/min, Cca=100mM, Cedta=100mM .................................................................................................................. 41
Figure 32: Averaged CaEDTA concentration, Q=5ul/min, Cca=100mM, Cedta=100mM .................................................................................................................. 41
Figure 33: Averaged temperature, Q=5uL/min, Cca=100mM, Cedta=100mM .......... 41
Figure 34: Averaged heat of reaction, Q=5uL/min, Cca=100mM, Cedta=100mM ....... 41
Figure 35: Contour Plots of CaEDTA Concentration at different z locations. From top to bottom, z=0.25mm, 0.5mm, 1mm, 2.5mm, 5mm, 7mm. Q=2.5uL/min, Cca=1mM, Cedta=0.1mM ................................................................................................................... 42
Figure 36: Averaged CaEDTA concentration, Q=2.5uL/min, Cca=1mM, Cedta=0.1mM ................................................................................................................... 42
Figure 37: Averaged temperature, Q=2.5uL/min, Cca=1mM, Cedta=0.1mM .......... 43
Figure 38: Averaged heat of reaction, Q=2.5uL/min, Cca=1mM, Cedta=0.1mM ....... 43
Figure 39: Contour Plots of CaEDTA Concentration at different z locations. From top to bottom, z=0.25mm, 0.5mm, 1mm, 2.5mm, 5mm, 7mm. Q=500uL/min, Cca=1mM, Cedta=1mM ...................................................................................................................... 44
Figure 40: Averaged CaEDTA concentration, Q=500uL/min, Cca=1mM, Cedta=1mM. 44
Figure 41: Averaged temperature, Q=500uL/min, Cca=1mM, Cedta=1mM ............. 44
Figure 42: Averaged heat of reaction, Q=500uL/min, Cca=1mM, Cedta=1mM ........... 44
LIST OF TABLES

Table 1: EDTA Acid Dissociation Constants ................................................................. 12
Table 2: EDTA Formation Complexes ................................................................. 14
Table 3: Outer sphere stability constants and effective formation rate constants ..... 20
Table 4: Characteristic values ................................................................................. 21
Table 5: Damkohler numbers .................................................................................. 21
Table 6: Chemical species concentration [24] ......................................................... 22
Table 7: MicroCal Test Kit Results [24] ................................................................. 22
Table 8: Equilibrium Distribution of EDTA at pH=6 ............................................ 24
Table 9: Reaction constants .................................................................................... 25
Table 10: Channel geometry. *Reduced from 5mm to reduce computational cost .... 26
Table 11: Boundary conditions ............................................................................... 27
Table 12: Default boundary condition values ......................................................... 27
Table 13: Inlet species calculations ....................................................................... 28
Table 14: Material properties ............................................................................... 33
1 Introduction

Microfluidics has been widely used in many disciplines to increase reaction speed and measurement sensitivity while reducing sample volume and device size. Current research in the Department of Mechanical and Industrial Engineering is developing a microscale calorimeter that uses surface plasmon resonance technology for measuring temperature and concentration fields in a fluid. By analyzing and understanding the thermochemical heat and mass transfer processes in the microchannel, one can obtain valuable information such as the enthalpy of reaction, entropy, Gibbs free energy, binding, and equilibrium constants [1]. This thermodynamic information can be used in the decision making process of the pharmaceutical industry to accelerate drug development. This current research aids in the design of a microfluidic calorimeter by understanding the momentum, heat and mass transfer within the device. FLUENT, a commercial computational fluid dynamics (CFD) package, was used to simulate mixing of solutions of EDTA and CaCl$_2$. The results are used to assess the viability of the device geometry, flow conditions, and reaction characteristics and optimize calorimetry performance.

2 Background

Microfluidic channels utilizing low co-flow of reagents in low Reynold’s number situations have been studied extensively in their application to mass transport and calorimetry. Despite the extensive attention, most studies involve development of simplified analytical and numerical models based on the assumption that the flow in the vertical direction can be ignored and the problem simplified to a 2D case. This work does not make the same assumption and examines the problem in 3D using the ANSYS Fluent CFD package.

This embodiment of the coflow microfluidic calorimeter utilizes surface plasmon resonance and extraordinary optical transmission phenomena to achieve excellent temperature and concentration measurement resolution and sensitivity. Previous work done at Northeastern University has established the feasibility of such an approach ([2], [3]). By precise measurements of temperature and concentration within the channel, important thermodynamic quantities such as Gibbs free energy change of reaction, heat of reaction, entropy change of reaction, and binding constant can be determined. The
Gibbs free energy of the reaction is the maximum amount of energy that can be extracted from the reaction system. The Gibbs free energy can be decomposed into the enthalpy of reaction and entropy of reaction. Enthalpy of reaction is the net heat release due to the chemical reaction and is representative of the interaction between the reactant molecules. Entropy of reaction represents the energy lost to irreversibility during the binding of the molecules. The equilibrium constant is a measure of the stability of the resulting complex.

The reaction of interest is the reaction between calcium chloride and ethylenediaminetetraacetic acid (EDTA). This reaction was chosen because it is commonly used in isothermal scanning calorimetry calibration procedures and has well established values of binding constant and enthalpy of reaction.

The purpose of this investigation is to predict the heat and mass transport that will occur within the microchannel for a specific chemical reaction. The commercial CFD package ANSYS Fluent was used to solve the heat and mass transport equations and determine velocity, temperature and concentration fields within the microchannel. This information can be used to optimize the calorimeter design and as a reference for obtained experimental data.

2.1 Microfluidic Flow Characteristics

Use of microfluidic flow devices has garnered attention from many researchers due to the numerous benefits it offers for calorimetric applications. The small size of these devices keeps flows laminar even at high flow velocities, allowing controlled mixing of reactants and simplified analytical and numerical modeling. The small size also reduces the amount of chemical reagents required and enables high testing throughput [4].
A typical microfluidic flow channel utilizing a T-sensor configuration is shown in Figure 1.

The channel height is $h$, the channel width is $w$, and the channel length is $L$. The Reynold’s number of the flow is given by

$$Re = \frac{\rho V D_h}{\mu} \quad (1)$$

Where $\rho$ is the density of the fluid, $V$ is the flow velocity, $D_h$ is the hydraulic diameter of the channel, and $\mu$ is the viscosity of the fluid. The hydraulic diameter is given by

$$D_h = \frac{4A}{p} \quad (2)$$

Where $A$ is the cross-sectional area of the channel and $p$ is the perimeter of the channel cross-section. Channel widths $w$ and heights $h$ are typically on the order of 100 $\mu m$ and the values for properties of water at 25°C result in $Re$ on the order of 1. This is safely within the laminar flow regime.

The present microcalorimeter is a co-flow device utilizing a T-sensor configuration as shown in Figure 2: T-sensor diagram. Two input streams containing species A and B are injected through the arms of the T-sensor and mix in the main channel. Mixing is due to diffusion of the chemical species throughout the channel, as the laminar flow conditions prevent mixing due to convection or turbulence. The extent of the diffusion is controlled by the diffusion coefficients of the species and the residence time, which is controlled by the channel geometry and flow rate [5]. Due to the laminar nature of the flow, the residence time at different points in the channel is a
function of only the distance from the beginning of the channel and the flow velocity [5]. This allows for precise control of the mixing of the two streams.

Figure 2 shows a schematic of the mass diffusion within the T-sensor. Species A and B enter through the two inlets, and mix and react within the main channel. This produces a "plume" containing the product of their reaction AB, shown with the dotted lines, as it is formed via chemical reaction and spreads throughout the channel via diffusion. A thermal plume is also produced, as energy is absorbed or released by the reaction. For an exothermic reaction, heat is released and will produce a temperature rise within the flow. The magnitude of the temperature rise within this plume and its shape are governed by the molar flow rate of reactants and the enthalpy of reaction. This temperature rise can be measured and used to determine calorimetric quantities such as the heat of reaction.

![Figure 2: T-sensor diagram](image)

The Peclet number, Pe, is the ratio of the advective transport rate to the diffusive transport rate and is indicative of the relative importance of convection and diffusion in a flow. A Peclet number can be calculated for both the mass and thermal transport within the flow. The mass transfer Peclet number $Pe_m$ is calculated using (3), while the thermal Peclet number $Pe_{th}$ is calculated using (4).

$$Pe_m = \frac{L_c \nu}{D} = ReSc$$  \hspace{1cm} (3)

$$Pe_{th} = \frac{L_c \nu}{\alpha} = RePr$$  \hspace{1cm} (4)

$L_c$ is the characteristic length, $D$ is the diffusion coefficient of the mass being transported in the fluid, $Sc$ is the Schmidt number for the mass in the fluid, $\alpha$ is the thermal...
diffusivity of the fluid, and $Pr$ is the Prandtl number for the fluid. For this type of problem, the thermal diffusivity is typically higher than the diffusion coefficient, resulting in a Peclet thermal number that is several orders of magnitude smaller than the mass transport Peclet number. This indicates that heat spreads through the flow by diffusion much more quickly than mass does. As a result, the shape of the thermal plume within the flow is different than that of the mass diffusion plume.

Two assumptions are generally made to allow for simplified modeling the flow. The first is that the flow is fully developed throughout the channel. At low Reynold’s numbers, the entrance length required for fully developed flow, $L_e$, has been shown to be dependent only on geometry and approximately equal to the channel width $w$ [6]. Since the channel is generally much longer than it is wide, fully developed flow is a reasonable assumption. The second assumption involves reducing the problem from 3D to 2D by assuming the flow velocity, concentration and temperature fields are uniform over the y-direction. This assumption is often validated by the geometry of the channel which produces flow that is very uniform across the channel. Previous studies have shown that flow in a rectangular duct with high aspect ratio produces a velocity profile that is Poiseuille-like over the channel height $h$ and approaching plug flow over the channel width $w$ [5] as shown in Figure 3.
Figure 3: Typical velocity profile within a microfluidic channel of high aspect ratio [5].

The flow is slower at the tops and bottoms of the channel, meaning the residence time of the two species in those regions is higher. This results in an increased diffusion distance in the x-direction, producing a “butterfly effect” as shown in Figure 4a [5]. This has been shown to be true for distances $z < Pe \times h$ [6], [7]. For distances $z > Pe \times h$, the molecules will also have diffused in the y-direction and the concentration profile becomes more uniform, as shown in Figure 4b [5].
The small geometry of most microfluidic channels makes the fully developed and 2D assumptions appropriate. This enables simplified analytical and numerical models.

2.2 Extraordinary Optical Transmission

The novelty of the proposed device is its temperature and concentration sensing technology. Previous versions of the device have used optical means such as detecting changes in fluorescence ([5] [6]) to resolve the concentration and field within the channel. Temperature field sensing has been accomplished using fluxmeters [4], thermopiles [8], and infrared thermography [9].

The current embodiment measures temperature and concentration optically via the transmission of light through a nanohole array (NHA). The theory behind this technique has been developed in detail and experimentally proven to be a viable measurement technique in previous works ([3] [10]). The basics of the technique and the implications of using it are discussed here.

Extraordinary optical transmission (EOT) refers to the transmission of light through sub-wavelength holes in metallic films that exceeds the amount predicted by classical diffraction theory. This increased transmission can be attributed to the excitation of surface plasmons on the metallic film at their resonant frequencies, causing energy to be transmitted through the hole more effectively [11]. The exact mechanism by which surface plasmon resonance (SPR) transfers energy is not agreed upon, but the end result is the transmission of light which depends on the dielectric properties of the material.
above the aperture. This enables construction of a sensor using a sensing dielectric layer, a metallic film with a NHA, and a substrate dielectric layer as shown in Figure 5.

Figure 5: Schematic of temperature sensor using a nanohole array and SPR/EOT technology [3]

The EOT signal measured on the backside of the dielectric substrate represents the amount of light transmitted through the NHA, which depends on the dielectric properties of the sensing layer above the NHA. The dielectric properties are related to the temperature and chemical composition of the material. Therefore, measuring changes in light transmittance through a NHA with constant temperature or composition allows determination of the other quantity [3]. The small size of the NHAs allows for excellent spatial resolution, and the temperature resolution is on the order of $10^{-3}$ K.

As discussed in section 3.1, the thermal and mass diffusion Peclet numbers are different by several orders of magnitude for most microfluidic flow regimes. This will produce temperature and species concentration profiles of different shapes. Proper design of the microchannel can enable one of these quantities to be made uniform, while the other is measured. It could even be possible for thermal information (e.g. heat of reaction) and concentration information (e.g. equilibrium constant) to be determined with the same device. The aim of the present work is to generate flow temperature and concentration profiles which can be analyzed to determine whether the given geometry, flow conditions and reaction characteristics are suitable for measurement using the EOT technique.

2.3 EDTA-CaCl₂ Reaction

Ethylenediaminetetraacetic acid (EDTA) is a water-soluble solid with chemical formula $\text{C}_{10}\text{H}_{16}\text{N}_{2}\text{O}_8$ that has found numerous uses due to its ability to act as a chelating
agent and form very stable complexes. Its structure is shown in more detail in Figure 6 and Figure 7.

![Protonated H⁴EDTA](image)

**Figure 6: Protonated H⁴EDTA** [12]

![Deprotonated EDTA⁻⁴](image)

**Figure 7: Deprotonated EDTA⁻⁴ with carboxyl lone pairs more visible** [13]

It is used in cleaning applications to reduce water hardness by binding calcium and magnesium ions, as well as in medical applications to sequester toxic heavy metals such as mercury and lead. As a chelating agent that forms very stable, stoichiometric complexes with a variety of metals, EDTA is also widely used in titration applications such as isothermal titration calorimetry to determine binding constants and thermodynamic information about reactions.

Calcium is an alkaline earth metal and the 5th most abundant element by mass in the earth’s crust, and the 5th most abundant dissolved ion in seawater. Due to this natural abundance, it is frequently involved in reactions with EDTA in natural waters. This had led to many experimental investigations involving calcium and EDTA and there are established values for the equilibrium constant of their coordination complex.
2.3.1 **Coordination Chemistry**

When calcium and EDTA react, they form a coordination complex. A coordination complex consists of a central atom or ion, normally a metal, known as the coordination center. The coordination center is surrounded by an array of bound ions, known as ligands. The number of ligands bound to the coordination center is known as the coordination number. Ligands generally bind to the coordination center via coordinate bonds, in which the ligand donates an electron pair to the bond. The ligand can therefore be considered a Lewis base, and the metal ion a Lewis acid as it accepts the electron pair [14].

Ligands can be characterized by their denticity, which is the number of times that a ligand bonds to a metal through non-contiguous donor sites. For example, ammonia (NH$_3$) has one lone pair of electrons available on the nitrogen atom, making it monodentate. Up to six ammonia ligands can bond to a calcium ion, each at one site each. EDTA has six lone pairs of electrons, making it hexadentate. One EDTA molecule can fill all of the binding sites on the calcium ion. Complexes of ligands with higher denticity are more stable than complexes formed from numerous monodentate ligands [14]. This increased stability is known as the chelate effect and results from favorable entropic changes related to the liberation of six hydrating water molecules from around the metal ion [15].

EDTA is hexadentate and has six lone pairs of electrons available for binding. Four of these pairs belong to the carboxyl (OH) groups, while two belong to the amine (N) groups. Upon complex formation, the hydrogens are released and the lone pairs are used to bind the metal ion. A representation of the resulting structure are shown in Figure 8.

![Figure 8: Metal-EDTA complex](image-url)
2.3.2 Complex Ion Formation

Reactions involving EDTA and a metal ion cannot be represented simply by the equation [16]

\[ M^{+v} + Y^{-u} \rightarrow MY^{v-u} \]  \hspace{1cm} (5)

Where \( M \) represents the metal ion, \( v \) is the charge of the metal ion, \( Y \) represents EDTA, \( u \) is the charge of the EDTA ion and \( MY \) represents the metal-EDTA complex. \( Y \) will be used to represent EDTA in the subsequent development for brevity. (5) is not valid because of the varying stages of protonation of the EDTA molecule. As shown in Figure 6 and Figure 7, EDTA can be fully protonated (\( H_6EDTA \)), fully deprotonated (\( EDTA^{-4} \)), or at an intermediate stage with between one and three protons. The deprotonation or ionization of any of these forms can be described using the general equation [16]

\[ H_jY^{j-u} \Leftrightarrow H_{j-1}Y^{j-u-1} + H^+ \hspace{1cm} j = 1 \text{ to } 4 \]  \hspace{1cm} (6)

Where \( H \) is a hydrogen atom/proton and \( j \) is the number of protons. The complete family of reactions describing EDTA protonation/ionization is shown below in (7a) through (7f)

\[ H_6Y^{+2} \leftrightarrow H_5Y^{+1} + H^+ \]  \hspace{1cm} (7a)
\[ H_5Y^{+1} \leftrightarrow H_4Y + H^+ \]  \hspace{1cm} (7b)
\[ H_4Y \leftrightarrow H_3Y^{-1} + H^+ \]  \hspace{1cm} (7c)
\[ H_3Y^{-1} \leftrightarrow H_2Y^{-2} + H^+ \]  \hspace{1cm} (7d)
\[ H_2Y^{-2} \leftrightarrow HY^{-3} + H^+ \]  \hspace{1cm} (7e)
\[ HY^{-3} \leftrightarrow Y^{-4} + H^+ \]  \hspace{1cm} (7f)

Each of these reactions constitutes a dissociation or ionization reaction, and therefore has a characteristic acid dissociation constant which relates the equilibrium concentration of the reactants and products to the hydrogen ion concentration as shown below in (8) [14]

\[ K_a = \frac{[H^+][H_{j-1}Y^{j-u-1}]}{[H_jY^{j-u}]} \]  \hspace{1cm} (8)

Where \( K_a \) is the acid dissociation constant, \([H^+]\) is the hydrogen ion concentration, \([H_{j-1}Z]\) is the concentration of the deprotonated EDTA form, and \([H_jY]\) is the concentration of the protonated EDTA form. The pK notation is often used in acid-base equilibrium chemistry due to the fact that dissociation constants can vary over several orders of magnitude and is related to the value of \( K \) by (9) [14]

\[ pK_a = -\log K_a \]  \hspace{1cm} (9)
The relative concentration of these different forms depends on the pH of the solution. Each of the dissociation reactions (7a)-(7f) have an acid dissociation constant, \( pK_a \), which are listed below in Table 1.

<table>
<thead>
<tr>
<th>Proton Number</th>
<th>Group</th>
<th>( pK_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>pK_{1a}</td>
<td>Carboxyl</td>
<td>1.15</td>
</tr>
<tr>
<td>pK_{2a}</td>
<td>Carboxyl</td>
<td>1.15</td>
</tr>
<tr>
<td>pK_{3a}</td>
<td>Carboxyl</td>
<td>2.12</td>
</tr>
<tr>
<td>pK_{4a}</td>
<td>Carboxyl</td>
<td>2.57</td>
</tr>
<tr>
<td>pK_{5a}</td>
<td>Amine</td>
<td>6.16</td>
</tr>
<tr>
<td>pK_{6a}</td>
<td>Amine</td>
<td>10.26</td>
</tr>
</tbody>
</table>

Using the dissociation constants shown in the table and (8), the composition of an EDTA solution as a function of pH can be calculated. The total EDTA concentration must be equal to the sum of the concentrations of the different forms, as shown in (10).

\[
[Y]_t = [Y^{-4}] + [HY^{-3}] + [H_2Y^{-2}] + [H_3Y^{-1}] + [H_4Y] + [H_5Y^+1] + [H_6Y^+2]
\]

(10)

Where \([Y]_t\) is the total EDTA concentration, and brackets denote concentration of the enclosed species. \(Y^{-4}\) is chosen as the working form, and all other terms on the right hand side of (10) are expressed in terms of \([Y^{-4}]\) using (8). For example, \([HY^{-3}]\) is related to \([Y^{-4}]\) through

\[
K_{6a} = \frac{[H^+][Y^{-4}]}{[HY^{-3}]}
\]

(11a)

Which can be rearranged to obtain

\[
[HY^{-3}] = \frac{[H^+][Y^{-4}]}{K_{6a}}
\]

(11b)

\([HY^{-3}]\) is then related to \([H_2Y^{-2}]\) through

\[
K_{5a} = \frac{[H^+][HY^{-3}]}{[H_2Y^{-2}]}
\]

(11c)

Which can be rearranged and combined with equation X to obtain

\[
[H_2Y^{-2}] = \frac{[H^+][Y^{-4}]}{K_{6a}K_{5a}}
\]

(11d)

This procedure can be repeated for all forms, resulting in the general equation

\[
[H_jY^{j-u}] = \frac{[H^+][Y^{-4}]}{\prod_{i=j}^{u} K_{ia}}
\]

(12)

Substituting (12) into (10) and factoring out \([Y^{-4}]\) yields
\[
[Y]_t = [Y^{-4}] \left\{ 1 + \frac{[H^+]}{K_{6a}} + \frac{[H^+]^2}{K_{5a}K_{6a}} + \frac{[H^+]^3}{K_{4a}K_{5a}K_{6a}} + \frac{[H^+]^4}{K_{3a}K_{4a}K_{5a}K_{6a}} + \frac{[H^+]^5}{K_{2a}K_{3a}K_{4a}K_{5a}K_{6a}} + \frac{[H^+]^6}{K_{1a}K_{2a}K_{3a}K_{4a}K_{5a}K_{6a}} \right\}
\]  
(13)

Finally, multiplying the right hand side by \(\frac{K_{1a}K_{2a}K_{3a}K_{4a}K_{5a}K_{6a}}{K_{1a}K_{2a}K_{3a}K_{4a}K_{5a}K_{6a}}\) and solving for \(\frac{[Y^{-4}]}{[Y]_t}\) yields

\[
\frac{[Y^{-4}]}{[Y]_t} = \alpha_{Y^{-4}} = \frac{K_{1a}K_{2a}K_{3a}K_{4a}K_{5a}K_{6a}}{[H^+]^6 + K_{1a}[H^+]^5 + K_{1a}K_{2a}[H^+]^4 + K_{1a}K_{2a}K_{3a}[H^+]^3 + K_{1a}K_{2a}K_{3a}K_{4a}[H^+]^2 + K_{1a}K_{2a}K_{3a}K_{4a}K_{5a}[H^+] + K_{1a}K_{2a}K_{3a}K_{4a}K_{5a}K_{6a}}
\]  
(14)

Where \(\alpha_{Y^{-4}}\) is the fraction of total EDTA in the \(Y^{-4}\) form. Once \(\alpha_{Y^{-4}}\) is obtained, unit total concentration is assumed and the fractions of the other EDTA forms can be calculated sequentially using (8). This procedure was performed for the full pH range from 0 to 14 using a MATLAB program, shown in Appendix A. The result is shown in Figure 9 below.

---

**Figure 9: EDTA Equilibrium Speciation**

Of these forms of EDTA, only two play a significant role in forming a complex with the metal ion, \([Z]\) and \([HZ]\) [17] [18]. The reaction proceeds primarily through the \([Z]\) form, but also reacts with \([HZ]\) to a lesser degree. The two complexes are also related by association/dissociation with a hydrogen ion as well. These reactions are shown below in (15a)-(15c), and their associated formation constants are shown in (16a)-(16c). Values for the formation constants are given in Table 2.

\[
Ca^{+2} + EDTA^{-4} \leftrightarrow CaEDTA^{-2}
\]  
(15a)
\[ Ca^{+2} + HEDTA^{-3} \leftrightarrow HCaEDTA^{-1} \]  \hspace{1cm} (15b)

\[ HCaEDTA^{-1} \leftrightarrow H^+ + CaEDTA^{-2} \]  \hspace{1cm} (15c)

\[ K^M_{MY} = \frac{[CaEDTA^{-2}]}{[Ca^{+2}][EDTA^{-3}]} \]  \hspace{1cm} (16a)

\[ K^M_{MY} = \frac{[HCaEDTA^{-1}]}{[Ca^{+2}][HEDTA^{-3}]} \]  \hspace{1cm} (16b)

\[ K^M_{MY} = \frac{[HCaEDTA^{-1}]}{[H^+][CaEDTA^{-2}]} \]  \hspace{1cm} (16c)

**Table 2: EDTA Formation Complexes**

<table>
<thead>
<tr>
<th>K</th>
<th>log K</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K^M_{MY} )</td>
<td>10.28</td>
</tr>
<tr>
<td>( K^M_{HY} )</td>
<td>3.53</td>
</tr>
<tr>
<td>( K^M_{MY} )</td>
<td>3.47</td>
</tr>
</tbody>
</table>

Together, equations (7a)-(7f) and (15a)-(15c) fully describe the reaction system. The reaction system is shown schematically in Figure 10.
Implicit in each of reactions (15a) and (15b) is the ligand exchange reaction of the hydration layer of water molecules with the EDTA molecule, which would be written as shown below in (17) [19]

\[
Ca^{+2} + EDTA^{-4} \leftrightarrow CaEDTA^{-2}
\]

\[
Ca^{+2} + HEDTA^{-3} \leftrightarrow CaHEDTA^{-2}
\]

\[
H_2EDTA^{-2}
\]

\[
H_3EDTA^{-1}
\]

\[
H_4EDTA
\]

\[
H_5EDTA^{+1}
\]

\[
H_6EDTA^{+2}
\]

Figure 10: Ca-EDTA reaction system

It is the release of the six water molecules and the associated entropy increase that results in the added stability of the chelate effect. The mechanism of water removal from the complex is discussed later in Section 2.3.4 describing chemical kinetics.

Many of the aforementioned reactions have hydrogen ions as a product. The release of the hydrogen ions would result in an increase of solution pH, further complicating the analysis, if not for the use of a buffer.

2.3.3 Buffers

Buffer solutions are aqueous solutions containing a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid [14]. The presence of these
buffering agents helps to maintain solution pH at a desired level despite addition of small amounts of strong acids or bases. The buffering agent used in the current investigation is 2-(N-morpholino)ethanesulfonic acid, commonly known as MES. It was developed by Good et. al. in the 1960s with the goal of having pKₐ between 6 and 8, high solubility in water, and minimal salt effects [20]. MES has a pKₐ value of 6.15, meaning that it maintains solution pH in the vicinity of 6.15.

An ideal buffer would maintain solution pH while not interacting with any of the components of interest in the study. In reality, however, the buffer can be protonated or ionized. It has been shown that the ionization of the buffer can contribute greatly to observed enthalpies of reaction [15] [21]. The ionization of the buffer can be represented by Eq. 14 below

\[ H^+ + MES \leftrightarrow H(MES)^+ \] (18)

where \( MES \) represents the MES buffer and \( H(MES)^+ \) represents the protonated buffer. In addition to being ionized, the buffer can also form a complex with the calcium ion, as described by Eq. 15 below

\[ Ca^{+2} + MES \leftrightarrow Ca(MES)^{+2} \] (19)

where \( Ca(MES)^{+2} \) represents the calcium-buffer complex. MES buffer was specifically developed to have minimal complex formation with metal ions, and experimental data shows a very low formation constant (log K=0.7) for calcium-MES complex [21], meaning very little the complex is formed. As a result, the interaction of metal and buffer is negligible and is neglected in the analysis.

The heat release observed in the device, \( \Delta H_{obs} \), is therefore the sum of the intrinsic binding enthalpy of the calcium-EDTA complex, \( \Delta H_b \), and the ionization enthalpy of the buffer, \( \Delta H_{ion} \), as shown in (16) [15] [21].

\[ \Delta H_{obs} = \Delta H_b + n\Delta H_{ion} \] (20)

Where \( n \) is the number of hydrogen ions released per mole of complex formed. This value changes based on the pH of the solution, which affects which EDTA forms are most prevalent and therefore how many hydrogen ions must be released to form the CaEDTA complex. For example, at pH of 4, \( H_2EDTA \) is the dominant EDTA form, and each CaEDTA complex formed would involve the release of 2 protons (\( n=2 \)), leading to a high enthalpy of ionization. At a pH of 11, however, fully deprotonated \( EDTA^{-4} \) is the dominant EDTA form, and no protons are released upon formation of the CaEDTA complex. This leads to a much lower enthalpy of ionization, and therefore a different
observed enthalpy change. The influence of ionization enthalpy of the buffer is shown in Figure 11.

![Graph of observed binding enthalpy vs. buffer ionization enthalpy obtained using ITC calorimetry](image)

The enthalpy associated with the ionization of the buffer is significant and must be considered when analyzing the observed enthalpy change to determine the intrinsic binding enthalpy.

### 2.3.4 Chemical Kinetics

Chemical kinetics is the study of the rates of chemical reactions and the mechanisms by which reactions take place. Knowledge of the kinetics of the reactions involved is essential in order to resolve the behavior of the system within the mixing region. For a reaction of the form given below by (21a), with an equilibrium constant as shown in (21b) [14]

$$aA + bB \rightarrow cC \quad (21a)$$

$$K_{eq} = \frac{[C]}{[A][B]} \quad (21b)$$

The rate of the reaction is equal to the rate at which the concentrations of the reactants and products change, as shown in Equation 17 [14]

$$r = -\frac{1}{a} \frac{d[A]}{dt} = -\frac{1}{b} \frac{d[B]}{dt} = \frac{1}{c} \frac{d[C]}{dt} \quad (22)$$
Where \( r \) is the rate of the reaction and \( \frac{d[i]}{dt} \) is the rate of change of the concentration of \( i \) with respect to time. This rate can also be described as a mathematical function of the concentrations of the species involved, given in a general form by (23) below [14]

\[
r = k[A]^m[B]^n
\]  

(23)

Where \( k \) is the rate constant, \( m \) is the rate exponent of species \( A \), and \( n \) is the rate exponent of species \( B \). The order of the reaction is equal to \( m + n \).

There are a few things that are important to note. First, the units of the rate constant \( k \) change as the order of the reaction changes to make sure the units of the reaction rate are \( \text{mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1} \). Second, the values of the constants \( k \), \( m \) and \( n \) cannot be determined from stoichiometry and must be determined experimentally, unless the reaction is an elementary reaction [14]. For elementary reactions, the rate exponents are equal to unity and the reaction proceeds as it is written. Therefore, at equilibrium, the forward and reverse reaction rates are equal, as shown in (24)

\[
k_f[A][B] = k_b[C]
\]  

(24)

Where \( k_f \) is the forward reaction rate constant and \( k_b \) is the reverse reaction rate constant. (24) can be rearranged to yield

\[
\frac{k_f}{k_b} = \frac{[C]}{[A][B]} = K_{eq}
\]  

(25)

Therefore, if the forward rate constant and the equilibrium constant of a reaction are known, the backward rate constant can be computed with (25).

Even the most complex reactions can be decomposed into a set of simpler, one step reactions without intermediaries, known as elementary reactions [14]. A set of elementary reactions that describe a more complicated reaction is known as a reaction mechanism. Proposed reaction mechanisms must be verified through comparison with experimental data on the overall reaction. The rate of an overall reaction is controlled by the slowest elementary reaction in its mechanism, as faster reactions must wait for the slower reaction before they are able to progress [14]. This slowest step is also known as the rate-determining step.

In this problem there are nine reactions occurring simultaneously. The literature shows that protonation/ionization reactions are very fast compared with reactions of complex formation [22]. Therefore, any such reactions can be considered at equilibrium and knowledge of the rate constant is not required. This eliminates the need for knowledge of kinetics for reactions (7a)-(7f) and (15c). The complexation reactions (15a)
and (15b) are considered kinetically controlled [23] and kinetic information must be determined.

The complexation of the calcium ion and EDTA is actually a ligand exchange reaction with EDTA as the incoming ligand and water as the exiting ligand. The accepted reaction mechanism for this type of exchange reaction in aqueous solution is known as the Eigen mechanism [19]. This mechanism consists of the rapid formation of an outer-sphere complex between the hydrated metal and the incoming ligand, followed by a slow, rate-limiting dehydration step [23]. The overall rate of complexation is therefore determined by a combination of the rate constant for the removal of water molecules from the inner coordination sphere of the metal and the stability constant of the intermediate outer-sphere complex. The reaction rate for the dehydration of the outer-sphere complex can be written as

\[ \frac{d[ML]}{dt} = k_w [M(H_2O)_6L] \]  \hspace{1cm} (26)

Where \([ML]\) is the concentration of the metal-ligand inner-sphere complex and 
\([M(H_2O)_6L]\) is the concentration of the outer-sphere complex. These two quantities are related by the stability constant of the outer-sphere complex

\[ K_{os} = \frac{[M(H_2O)_6L]}{[M][L]} \]  \hspace{1cm} (27)

Where \(K_{os}\) is the stability constant for the outer-sphere complex, \([M]\) is the concentration of the free metal ion and \([L]\) is the concentration of the free ligand ion. Combining (26) and (27) gives (28), which gives the overall rate of complex formation

\[ \frac{d[ML]}{dt} = k_{eff} [M][L] \]  \hspace{1cm} (28)

Where \(k_{eff}\) is the effective rate constant and is equal to

\[ k_{eff} = K_{os} k_w \]  \hspace{1cm} (29)

The water removal rate constant is a characteristic of the metal ion and has a value of \(6 \times 10^8\) for calcium [19]. The value of the outer-sphere stability constant is dependent on the ionic strength of the solution and the charges of the metal and incoming ligand. As a result, the value of \(K_{os}\) is different for each of the two forms of EDTA that form complexes with calcium. These values can be estimated using statistical thermodynamic theory as described in detail in [22]. For simplicity, the values obtained for cadmium ions and EDTA in [23] were used in the present investigation as the metal ion dependent factors for the calculation should be of the same order of magnitude for calcium and cadmium. The order of magnitude for the value of \(K_{os}\) is more important than the exact
value. The values for $K_{os}$, the resulting effective forward rate constants, and the backward rate constants computed using the appropriate equilibrium constants and (25). The values are given below in Table 3.

Table 3: Outer sphere stability constants and effective formation rate constants

<table>
<thead>
<tr>
<th>EDTA Form</th>
<th>$K_{os}$ [23]</th>
<th>$k_{eff}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>249</td>
<td>1.49E+11</td>
</tr>
<tr>
<td>HEDTA</td>
<td>25</td>
<td>1.50E+10</td>
</tr>
</tbody>
</table>

The reaction system is now fully defined, with rate constants for the kinetically controlled reactions and equilibrium constants for all reactions.

With these rate constants in hand, the characteristic time scale of the reaction can be compared with that of the mass transport. The Damkohler number is a dimensionless number which gauges the relative importance of reaction rates and mass transport rates in determining the steady-state response of a chemical system over given a given length scale.

$$Da = \frac{\text{reaction rate}}{\text{mass transport rate}} = \frac{\text{mass transport time scale}}{\text{chemical time scale}}$$  \hspace{1cm} (30)

The mass transport time scale can be characterized in terms of the diffusion or convection. In this problem, the z-direction is dominated by convection, while the x direction is dominated by diffusion. Therefore, the Damkohler number should be calculated for both. The diffusion time scale is given by

$$\tau_D = \frac{L^2}{D}$$  \hspace{1cm} (31)

Where $\tau_D$ is the characteristic diffusion time scale, $L$ is a characteristic length scale for the system, and $D$ is a characteristic mass diffusion coefficient. The convection time scale is given by

$$\tau_C = \frac{L}{V}$$  \hspace{1cm} (32)

Where $\tau_C$ is the characteristic convection time scale and $V$ is a characteristic flow velocity. The equation for the time scale varies with the order of the reaction, as the units of the rate constant change with reaction order. Both of the kinetically controlled reactions in this problem are of second order, and the rate constants have units of $M^{-1}s^{-1}$. Therefore, the reaction time scale is given by

$$\tau_R = \frac{1}{kC}$$  \hspace{1cm} (33)
Where $\tau_R$ is the characteristic reaction time scale, $k$ is a characteristic rate constant, and $C$ is a characteristic concentration. Table 4 below lists the choices of characteristic values.

<table>
<thead>
<tr>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>1.00E+10 1/ Ms</td>
</tr>
<tr>
<td>$D$</td>
<td>5.00E-10 m$^2$/s</td>
</tr>
<tr>
<td>$L$</td>
<td>1.00E-06 m</td>
</tr>
<tr>
<td>$V$</td>
<td>1.00E-02 m/s</td>
</tr>
<tr>
<td>$C$</td>
<td>1.00E-04 M</td>
</tr>
</tbody>
</table>

The characteristic rate constant was chosen to be of the same order of magnitude as the slowest of the two kinetically controlled reactions. The lowest diffusion coefficient, that of EDTA, was selected for the characteristic diffusion coefficient as it will limit the mixing process. The characteristic length of one micron was chosen to be conservative and representative of a very fine mesh for the computational domain. The characteristic velocity was chosen to be the same order of magnitude as the inlet fluid velocity. Finally, the characteristic concentration was chosen to be the concentration of EDTA, as it is the lowest concentration and will limit the reaction rate. Using these values, the diffusion and convection Damkohler numbers for a cell of the computational domain were calculated. These values are displayed in Table 5.

<table>
<thead>
<tr>
<th>$\tau$</th>
<th>$Da$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion</td>
<td>1.67E-03</td>
</tr>
<tr>
<td>Convection</td>
<td>3.03E-05</td>
</tr>
<tr>
<td>Reaction</td>
<td>1.00E-06</td>
</tr>
</tbody>
</table>

Both Damkohler numbers are much greater than unity, indicating that the rates of chemical reactions occurring in each cell are much faster than those of mass transport. Therefore, the reactions within each cell of the computational domain can be considered to be at equilibrium. This will facilitate the construction of a simplified reaction system.

3 Problem Setup

The goal of this study is to determine if the proposed device is capable of determining thermodynamic information of a given reaction. The reaction system, species concentrations, and pH are taken from a test procedure for an isothermal titration
calorimetry (ITC) system, provided by MicroCal [24]. This enables comparison of the current device with current ITC calorimeters and to see if the device can achieve the same results with the same inputs.

3.1 Experimental Parameters

The current simulation involves mixing of CaCl₂ and EDTA at pH=6.0 in the presence of MES buffer, as outlined in the MicroCal Test Kit [24]. The concentrations of each component are listed in Table 6.

Table 6: Chemical species concentration [24]

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂</td>
<td>1</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.1</td>
</tr>
<tr>
<td>MES</td>
<td>10</td>
</tr>
</tbody>
</table>

Since the buffer concentration is much greater than the EDTA concentration, it is assumed that the buffer maintains the pH inside the reaction cell at 6. Although the species concentrations shown in Table 6 are considered the default values, other cases will be performed as well to provide points of comparison. The results of the MicroCal Test Kit calibration are displayed in Table 7.

Table 7: MicroCal Test Kit Results [24]

<table>
<thead>
<tr>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>2.36E+06</td>
</tr>
<tr>
<td>ΔH</td>
<td>-17510</td>
</tr>
<tr>
<td>N</td>
<td>0.976</td>
</tr>
</tbody>
</table>

K is a conditional equilibrium constant in terms of the total EDTA added, given by

\[ K' = \frac{[CaEDTA]}{[Ca][EDTA]_t} \]  

(34)

Where [EDTA]ₜ is the concentration of all uncomplexed forms of EDTA. It is often used due to the difficulty of measuring the concentration of any of the specific EDTA forms. This conditional equilibrium constant differs in value from the equilibrium constant given in Table 1 (log K = 10.28) because it is in terms of the total EDTA concentration rather than the fully deprotonated EDTA. Combining (34) and (14) yields (35) for the conditional equilibrium constant

\[ K' = \alpha_{-4}K_f^{MY} \]  

(35)
For the selected pKa values shown in Table 1, $\alpha_{p-K}$ is equal to 2.247e-5 at pH=6. This results in a value of 4.281e5 for $K'$, which is reasonably close to the reported value of 2.36E+06. Discrepancies between these two values result from the choices of the pK values. It is important to understand $K'$ and the dependence of its value on solution pH, should the pH of the solution be altered during the course of the device development.

$\Delta H$ is the observed enthalpy of formation of the calcium-EDTA complex. This value represents the net effect of the intrinsic enthalpy of binding and the ionization of the buffer, as shown in (20).

$$\Delta H_{obs} = \Delta H_b + n\Delta H_{ion}$$  \hspace{1cm} (20)

For calcium-EDTA, the intrinsic enthalpy is -22 kJ/mol [21] [11], while the total observed heat release at pH=6 is -17.5 kJ/mol, which is consistent with the literature [15] [21]. The number of protons released at pH=6 is 1.73 [21], meaning the net enthalpy of ionization due to ionization of the buffer and ionization of EDTA is -10 kJ/mol. The net observed value of -17.5 kJ/mol is used as the enthalpy of formation in the simulation, as it represents the total heat release associated with complex formation and is what would be observed in the micromixer. To determine the intrinsic enthalpy of binding from experimental measurements, the enthalpy change due to ionization of the buffer and the EDTA must be accounted for. Performing this correction is outside the scope of this work.

$N$ is the number of binding sites and is indicative of the stoichiometry of the reaction. Its value near unity shows that calcium and EDTA form 1:1 complexes.

### 3.2 Reaction System

With the pH fixed at 6, more details can be determined about the reaction system. Three key assumptions are made in the development of the reaction system model:

1. The MES buffer maintains solution pH at a constant value of 6.0.
2. Protonation/deprotonation reactions (7a)-(7f), (16c) are much faster than complexation reactions and can be considered at equilibrium.
3. Concentrations of species governed by equilibrium reactions respond immediately to changes in concentration produced by the kinetically controlled reactions.

Using the procedure described in Section 2.3.2, the distribution of EDTA forms was calculated. This distribution is shown in Table 8 below.
**Table 8: Equilibrium Distribution of EDTA at pH=6**

<table>
<thead>
<tr>
<th>EDTA Form</th>
<th>Fraction of Total EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$EDTA^{-4}$</td>
<td>2.247E-05</td>
</tr>
<tr>
<td>$HEDTA^{-3}$</td>
<td>4.088E-01</td>
</tr>
<tr>
<td>$H_2EDTA^{-2}$</td>
<td>5.909E-01</td>
</tr>
<tr>
<td>$H_3EDTA^{-1}$</td>
<td>2.196E-04</td>
</tr>
<tr>
<td>$H_4EDTA$</td>
<td>2.894E-08</td>
</tr>
<tr>
<td>$H_5EDTA^+1$</td>
<td>4.088E-13</td>
</tr>
<tr>
<td>$H_6EDTA^+2$</td>
<td>5.775E-18</td>
</tr>
</tbody>
</table>

It can be seen that only $HEDTA^{-3}$ (40.88%) and $H_2EDTA^{-2}$ (59.09%) are present in significant amounts. Of these two species, only $HEDTA^{-3}$ forms a complex with calcium, per (15b). $HEDTA^{-3}$ is therefore the dominant species in the reaction system, and a simplified reaction system that is centered around it can be defined.

To justify this choice with numbers, consider the reaction rate equations for (15a) and (15b).

\[
\begin{align*}
    r_1 &= k_{1f} [Ca^{2+}][EDTA^{-4}] \quad (36a) \\
    r_2 &= k_{2f} [Ca^{2+}][HEDTA^{-3}] \quad (36b)
\end{align*}
\]

Expressing $[EDTA^{-4}]$ and $[HEDTA^{-3}]$ as fractions of the total EDTA concentration, $[EDTA_t]$, yields

\[
\begin{align*}
    r_1 &= k_{1f}' [Ca^{2+}][EDTA_t] \quad (37a) \\
    r_2 &= k_{2f}' [Ca^{2+}][EDTA_t] \quad (37b)
\end{align*}
\]

Where $k_{1f}'$ and $k_{2f}'$ are effective rate constants given by

\[
\begin{align*}
    k_{1f}' &= k_1 \alpha_{\gamma^{-4}} \quad (38a) \\
    k_{2f}' &= k_2 \alpha_{\gamma^{-3}} \quad (38b)
\end{align*}
\]

Using the rate constant values from Table 3 and EDTA fraction values from Table 8, these effective rate constants have values of 3.4e6 and 6.1e9, respectively. The rate of reaction (15b) is 1800 times faster than that of (15a).

Reaction (15b) forms $CaHEDTA^{-1}$, which rapidly deprotonates to form $CaEDTA^{-2}$ through reaction (15c). The concentrations of $HCaEDTA^{-1}$ and $CaEDTA^{-2}$ are related through (16c). Evaluation of (16c) for the hydrogen ion concentration at a pH of 6, shown below in (39), reveals that the ratio of $[HCaEDTA^{-1}]$ to $[CaEDTA^{-2}]$ is less than 0.3%.

\[
\frac{[HCaEDTA^{-1}]}{[CaEDTA^{-2}]} = K_{MY}^{HY} [H^+] = 0.00295 \quad (39)
\]
Therefore, a simplifying assumption is made that all \( HCaEDTA^{-1} \) is converted to \( CaEDTA^{-2} \). \( HEDTA^{-3} \) will therefore drive the reaction system even though it does not react with \( Ca^{2+} \) to directly form the \( CaEDTA^{-2} \) complex. Reaction (15b) is the dominant kinetically controlled reaction, and will drive the formation of \( CaEDTA^{-2} \) due to the rapid reaction (15c). This allows construction of a simple reaction system consisting of only three species: \( Ca^{2+} \), \( EDTA_t \) and \( CaEDTA^{-2} \). This reaction is shown below in (40)

\[
Ca^{2+} + EDTA_t \leftrightarrow CaEDTA^{-2}
\]  

(40)

Its rate is given by equation (41)

\[
\frac{d[CaEDTA^{-2}]}{dt} = -\frac{d[Ca^{2+}]}{dt} = -\frac{d[EDTA]_t}{dt} = k'_{2f}[Ca^{2+}][EDTA]_t
\]  

(41)

Where \( k'_{2f} \) is given by (38b).

The backwards rate constant is determined from the forward rate constant and equilibrium constant given by MicroCal as shown in (25). The resulting values of the forward rate constant, backwards rate constant, and equilibrium constant for the simplified reaction scheme are shown below in Table 9.

<table>
<thead>
<tr>
<th>( k )</th>
<th>( k_f )</th>
<th>( k_b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td>2.360E+06</td>
<td>6.132E+09</td>
<td>2.598E+03</td>
</tr>
<tr>
<td>Units</td>
<td>Units</td>
<td>Units</td>
</tr>
<tr>
<td>( M^{-1} )</td>
<td>( M^{-1}s^{-1} )</td>
<td>( s^{-1} )</td>
</tr>
</tbody>
</table>

Table 9: Reaction constants

This approach is consistent with that in [22] in which the authors examined the complexation reaction between cadmium and EDTA. They determined that although the MY complex was most thermodynamically stable, the reaction system was driven by the kinetics associated with the MHY complex.

Rigorously modeling the reaction system and including all of the reactions would involve determining the equilibrium speciation in each cell of the computational domain. This involves solving a nonlinear system of equations and is a computationally intensive process [25]. It also introduces complexities related to the inherently different timescales of the flow and chemical reactions. By simplifying the reaction system, computational cost can be drastically reduced while still resolving the temperature and concentration fields resulting from the reaction. A more detailed model that couples the equilibrium speciation calculations with the fluid flow is a potential subject of future work.
4 Numerical Simulation

4.1 Geometry and Boundary Conditions

The ANSYS Fluent commercial CFD software package was used to perform the numerical simulations. Channel geometry was created in SolidWorks and imported into Fluent for analysis. Figure 12 shows the SolidWorks model, and Table 10 displays the physical dimensions.

The boundary conditions for the simulation are the same as determined in previous work by this research group [2]. These conditions are summarized below in Table 11.
Table 11: Boundary conditions

<table>
<thead>
<tr>
<th></th>
<th>Inlet</th>
<th>Outlet</th>
<th>Glass (y=0)</th>
<th>PDMS (y=0.1 mm)</th>
<th>Sides (x=−0.25, 0.25 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Momentum</strong></td>
<td>$V = V_{in}$</td>
<td>Pressure outlet</td>
<td>$\vec{V} = 0$</td>
<td>$\vec{V} = 0$</td>
<td>$\vec{V} = 0$</td>
</tr>
<tr>
<td><strong>Energy</strong></td>
<td>$T = T_{amb}$</td>
<td>Pressure outlet</td>
<td>$T = T_{set}$</td>
<td>Convection: $h, T_{amb}$</td>
<td>$\frac{\partial T}{\partial x} = 0$</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>$x_{Ca}, x_{EDTA}$</td>
<td>Pressure outlet</td>
<td>$\frac{\partial C}{\partial y} = 0$</td>
<td>$\frac{\partial C}{\partial y} = 0$</td>
<td>$\frac{\partial C}{\partial x} = 0$</td>
</tr>
</tbody>
</table>

The values of the different constants are displayed below in Table 12.

Table 12: Default boundary condition values

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{in}$</td>
<td>1.67E-03</td>
<td>m/s</td>
</tr>
<tr>
<td>$T_{amb}$</td>
<td>298.15</td>
<td>K</td>
</tr>
<tr>
<td>$T_{set}$</td>
<td>298.15</td>
<td>K</td>
</tr>
<tr>
<td>$h$</td>
<td>18</td>
<td>W/m²K</td>
</tr>
<tr>
<td>$x_{Ca}$</td>
<td>1.81E-05</td>
<td>N/A</td>
</tr>
<tr>
<td>$x_{EDTA}$</td>
<td>1.81E-06</td>
<td>N/A</td>
</tr>
</tbody>
</table>

$V_{in}$ was chosen to achieve a desired volumetric flow rate through each inlet of the device. It was calculated using (42) below

$$V_{in} = \frac{Q}{A} \quad (42)$$

Where $Q$ is the volumetric flow rate in m³/s and $A$ is the cross-sectional area of the channel in m². The mass fractions of calcium and EDTA at the inlets are computed from the desired inlet concentrations. It is assumed that the addition of the dissolved calcium and EDTA to the water does not change the density of the water. Concentrations are given in moles per liter of solution, and the number of moles of water in a liter can be calculated using the density and molecular weight of water using (43a) below. The mole fraction can then be calculated using (43b).

$$c_{w} = \frac{\rho_{w}}{M_{w}} \quad (43a)$$

$$y_{i} = \frac{c_{i}}{c_{i} + c_{w}} \quad (43b)$$
Where \(c_w\) is the mole density, or concentration, of water in moles per liter, \(\rho_w\) is the mass density of water in \(\frac{g}{L}\) and \(M_w\) is the molecular weight of water in \(\frac{g}{mol}\). With the number of moles per liter of water and calcium/EDTA known, the mole fraction of calcium/EDTA can be calculated using (43b), where \(y_i\) is the mole fraction of calcium/EDTA and \(c_i\) is the concentration of calcium/EDTA.

The mass density of calcium/EDTA can then be calculated using (44a), and the mass fraction using 44b.

\[
\rho_i = \frac{c_i}{M_i} \quad (44a)
\]

\[
x_i = \frac{\rho_i}{\rho_i + \rho_w} \quad (44b)
\]

Where \(\rho_i\) is the mass density of calcium/EDTA in \(\frac{g}{L}\), \(M_i\) is the molecular weight of calcium/EDTA in \(\frac{g}{mol}\), and \(x_i\) is the mass fraction of calcium/EDTA. The calculations are summarized in Table 13 below.

<table>
<thead>
<tr>
<th></th>
<th>Ca (Inlet 1)</th>
<th>EDTA (Inlet 2)</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mole Density (mol/L)</strong></td>
<td>1.00E-03</td>
<td>1.00E-04</td>
<td>55.39401</td>
</tr>
<tr>
<td><strong>Mass Density (g/L)</strong></td>
<td>4.01E-05</td>
<td>2.93E-05</td>
<td>0.9982</td>
</tr>
<tr>
<td><strong>Mole Fraction</strong></td>
<td>1.81E-05</td>
<td>1.81E-06</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Mass Fraction</strong></td>
<td>4.02E-05</td>
<td>2.94E-05</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### 4.2 Computational Domain

A structured, hexahedral mesh was generated using the built-in ANSYS meshing utility. To reduce computational time, the grid independence study was performed on a 2.5mm long channel instead of a 7mm long channel. This is long enough to capture the initial mixing region, which is the area which requires the highest mesh density. Grid independence was achieved by starting with a coarse mesh and repeatedly refining the mesh by making the cells smaller. Velocity and concentration profiles were monitored at a distance of 1mm down the channel for the x, y and z directions until no changes were observed with increasing mesh resolution. The plots are shown below in Figures x-y.
Figure 13: Plot of CaEDTA Concentration in Z-Direction for Mesh Independence

Figure 14: Plot of CaEDTA Concentration in X-Direction for Mesh Independence
For the x and z directions, mesh independence was achieved once the maximum cell side length was set to 12.5 $\mu$m. 20 cells were required in the y-direction. A cross-sectional image of the mesh in the xy-plane is shown below in Figure 16.

This mesh verification procedure was conducted for the default cause (1mM Ca, 0.1mM EDTA, 0.0033 m/s inlet velocity) and the higher concentration, high velocity case (1mM Ca, 1mM EDTA, 0.033 m/s inlet velocity). It was found that the mesh shown above was suitable to achieve mesh independence for both cases and it was assumed that it would be the same for the other inlet concentration/velocity settings as well.

4.3 Models

The pressure-based, steady-state solver was used to solve the equations of momentum, heat and mass transfer within the flow domain. For the mass transfer, the species transport model with volumetric, laminar finite-rate reactions was used. The diffusion energy source and thermal diffusion options were enabled. A two reaction mechanism was used as described in Section 3.2, with Reaction 1 representing the
forward reaction and Reaction 2 the reverse reaction. FLUENT calculates the rate constant of the forward reaction through the Arrhenius equation (45) [26]

\[
k_f = A_f T^{\beta_f} e^{-E_f / RT}
\]

Where \( A_f \) is the pre-exponential factor, \( \beta_f \) is the temperature exponent, \( E_f \) is the activation energy of the reaction, \( R \) is the universal gas constant and \( T \) is the absolute temperature. The constants \( A_f, \beta_f \) and \( E_f \) are determined empirically for a given reaction. These constants are not typically reported for complexation style reactions in the literature due to their complicated reaction mechanisms. The effective rate constant for this reaction has been determined using the assumptions and simplifications outlined in Section 3.2. By entering this value for the pre-exponential factor, and zero for the temperature exponent and activation energy, (45) will always evaluate to the desired rate constant. The inputs for the forward reaction (Reaction 1) are shown in Figure 17.

FLUENT typically computes the equilibrium constant of a reaction from the thermodynamic quantities standard-state enthalpy and standard-state entropy of each reactant per (46) [26]

\[
K_r = \exp\left(\frac{\Delta S_r}{R} - \frac{\Delta H_r}{RT}\right) \left(\frac{p_{atm}}{RT}\right)^{\sum_{i=1}^{N} (n''_{ir} - n'_{ir})}
\]

Where \( K_r \) is the equilibrium constant, \( \Delta S_r \) is the entropy change of the reaction, \( \Delta H_r \) is the enthalpy change of the reaction, \( p_{atm} \) is atmospheric pressure, \( n''_{ir} \) is the reverse stoichiometric coefficient of species \( i \), and \( n'_{ir} \) is the forward stoichiometric coefficient of species \( i \). Fluent then uses (38b) to calculate the reverse rate constant from the forward rate constant and equilibrium constant. Since the equilibrium constant is known for this reaction, the “Backwards Reactions” option in Fluent is disabled, and (46) is not used. Rather, the reverse reaction is explicitly defined as its own reaction (Reaction 2) as shown in
Figure 18.

Fluent automatically computes the enthalpy of reaction per (47) [26]

\[ \frac{\Delta H_r}{RT} = \sum_{i=1}^{N} (v''_i - v'_{i,r}) \frac{h_i}{RT} \]  

(47)
Where \( h_i \) is the standard-state enthalpy of species \( i \). Values of standard-state enthalpy for EDTA complexes are generally not reported in the literature. Rather, the overall enthalpy of reaction is reported, as it is in [24]. By setting the standard-state enthalpy of \( CaEDTA^{-2} \) to the overall enthalpy of reaction and the standard-state enthalpy of \( Ca^{2+} \) and \( EDTA_e \) to zero, (47) evaluates to the correct value for enthalpy of reaction. In this way the heat release due to the reaction can be modeled using Fluent’s built-in capabilities.

To represent the fluid, a mixture of liquid water, \( Ca^{2+} \), \( EDTA_e \) and \( CaEDTA^{-2} \) was created. The following assumptions were made regarding the material properties of the mixture:

1. Due to small concentrations of dissolved species, the mixture properties are represented by properties of pure water at 25°C.
2. Thermophysical properties of water are constant and unaffected by mixture composition and temperature. This is justified based on the low species concentrations and expected small temperature changes due to heat of reaction.
3. The dilute solution approximation is applied, and the diffusion coefficient of each species is a constant independent of the mixture composition.

\( Ca^{2+} \), \( EDTA_e \) and \( CaEDTA^{-2} \) were created as fluids with properties identical to that of water. This ensures that the properties of the mixture are the same as that of water regardless of the concentration of the aqueous species. The steady-state enthalpy of each component was defined as described previously. The diffusion coefficients were taken from published values in the literature. Table 14 below summarizes all important material property value inputs.

Table 14: Material properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Units</th>
<th>Water</th>
<th>( Ca^{2+} )</th>
<th>( EDTA )</th>
<th>( CaEDTA )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>( \frac{kg}{kmol} )</td>
<td>18.02</td>
<td>40.08</td>
<td>292.24</td>
<td>332.32</td>
</tr>
<tr>
<td>Density</td>
<td>( \frac{kg}{m^3} )</td>
<td>998.2</td>
<td>998.2</td>
<td>998.2</td>
<td>998.2</td>
</tr>
<tr>
<td>Thermal Conductivity</td>
<td>( \frac{W}{m-K} )</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Dynamic Viscosity</td>
<td>( \frac{kg}{m-s} )</td>
<td>0.001003</td>
<td>0.001003</td>
<td>0.001003</td>
<td>0.001003</td>
</tr>
<tr>
<td>Specific Heat</td>
<td>( \frac{J}{kg-K} )</td>
<td>4182</td>
<td>4182</td>
<td>4182</td>
<td>4182</td>
</tr>
<tr>
<td>Mass Diffusivity</td>
<td>( \frac{m^2}{s} )</td>
<td>N/A</td>
<td>1.2e-9 ( [27])</td>
<td>5.5e-10 ( [28])</td>
<td>5.5e-10 ( [28], [29])</td>
</tr>
</tbody>
</table>
4.4 Solution Methods

The following schemes were used by the solver: SIMPLE for pressure-velocity coupling, Least Squares Cell-Based for gradient discretization, and Standard discretization for Pressure. The momentum, energy and all species equations used the Second Order Upwind discretization. Comparison of different numerical schemes was beyond the scope of this study and can be the object of future work once more experimental data is available for comparison.

Convergence was determined by monitoring residuals and values at points of interest within the solution domain. The velocity, concentration, and temperature were monitored at a point in the middle of the channel at a distance of 1mm from the confluence of the two input streams. A mass balance was also computed for the flow domain to ensure continuity was satisfied. An energy balance was also calculated to ensure that the reaction heat source balanced the heat flow through the boundaries. Residuals below 1e-6 for continuity, x-velocity, y-velocity, z-velocity, and energy equations were required. These solutions converged rapidly, as the geometry is simple and the flow field uniform.

Obtaining convergence for the species equations was more difficult. It was necessary to set residual criteria for the species equations to 1e-8, which was never achieved. Rather, the solution was allowed to run until the residuals and monitored points of interest stabilized. A typical residuals plot is shown below in Figure 19.

![Figure 19: Typical residuals plot](image)

The number of iterations and time required for satisfactory convergence varied with the case. Lower flow rates required up to four days, while higher flow rates only needed around one day. Generally, on the order of $10^5$ iterations were required. Runs with lower flow rates converged slower (more mass diffusion), while higher flow rates converged
faster (less mass diffusion). Convergence was accelerated by first solving the problem on a coarser mesh, interpolating the results, and using that interpolation to initialize the solution for the real mesh.

5 Results and Discussion

The results of the simulation are contained in this section. The default case is analyzed first, followed by a selection of cases with alternative flow rate and concentration values. The results are summarized at the end in Table 15.

5.1 Case 1: $Q=5\mu$L/min, $C_{Ca}=1mM$, $C_{EDTA}=0.1mM$

Figure 20: XZ-Midplane Velocity Contours, $Q=5\mu$L/min

Figure 20 shows the velocity contours for the flow field. As expected, the velocity is faster in the inlets regions due to the smaller cross-sectional area. The flow becomes fully developed quickly after entering the main channel, at a distance of around 0.5mm. The velocity plots in Figure 22 and Figure 22 confirm this. This is consistent with the observation that the length for the flow to develop fully is approximately equal to the width of the channel [6]. The fully developed velocity profile is shown in Figure 23 and is consistent with results obtained elsewhere as shown in Figure 3 [5].
Figure 21: X-direction velocity profiles at different distances along channel length, Q=5 ul/min

Figure 22: Y-direction velocity profiles at different distances along channel length, Q=5 ul/min
A series of contour plots of EDTA concentration in the xy-plane at different distances along the channel z are shown in Figure 24. The asymmetry of the profile is immediately noticeable. The degree of this asymmetry is related to the ratios of the diffusion coefficients of the two species, as well as the ratio of their concentrations [6]. Calcium, on the right side, has a diffusion coefficient roughly twice that of EDTA ($D_{Ca}=1.26\times10^{-9}$, $D_{EDTA}=5.5\times10^{-10}$). As a result, it penetrates into the EDTA side of the channel more quickly and shifts the CaEDTA concentration profile towards that side of the channel. Also contributing to the asymmetry of the profile is the fact that the concentration of the calcium is 10 times higher than that of EDTA. This means there is a higher concentration gradient driving the diffusion of the calcium, resulting in more diffusion by the calcium than by the EDTA.
Another noticeable feature of the concentration profiles are their “butterfly” shape. This is a consequence of the velocity profile within the channel, see Figure 23. At the top and bottom of the channel, the flow velocity is much lower. This gives the species in those laminae longer residence time, allowed increased diffusion and mixing and creating the wider diffusion zones at the top and bottom of the channel. Since the reaction is diffusion controlled as described in Section 2.3.4, the reaction will occur first in these areas, and is why the highest CaEDTA concentration occurs at the top and bottom of the channel and not equally throughout. Further down the channel, the concentration profile becomes more uniform, as the CaEDTA at the tops and bottoms of the channel has more time to diffuse down into the higher velocity region in the middle of the channel.

A view of the CaEDTA concentration in the xz-plane is shown in Figure 25. This image was obtained by averaging the concentration over the height of the channel. This was done to be consistent with the physics of the device sensing technology. Incident light from an LED travels through the flow channel before reaching the NHA sensor, and is affected by each “parcel” of fluid that it passes through. Therefore, the signal that reaches the NHA represents the total contribution of the entire volume of fluid above it. This vertical averaging was performed in MATLAB, and the code is contained in Appendix B. Only the cells in the mixing region of the channel were processed (inlets
were neglected). The concentration plume is consistent with the shape shown in Figure 24, with asymmetry towards the EDTA side of the channel.

Figure 25: Averaged CaEDTA concentration, Q=5ul/min, Cca=1mM, Cedta=0.1mM

Figure 26: Averaged temperature, Q=5 uL/min, Cca=1mM, Cedta=0.1mM

Figure 27: Averaged Heat of Reaction, Q=5ul/tmin, Cca=1mM, Cedta=0.1mM

The averaged CaEDTA concentration, temperature, and heat of reaction are shown in Figure 25, Figure 26 and Figure 27, respectively. The heat of reaction is confined to the inlet when the reactants first come into contact. At this location, enough mixing by diffusion occurs so that the maximum concentration of CaEDTA is produced during the reaction. This limits the major heat release to the inlet, as the amount of Ca and EDTA reacting within the plume is then very small. The CaEDTA then diffuses outward along the flow, creating the concentration plume shown in Figure 25. The temperature plume is confined to the area around the heat release associated with the chemical reaction. The total heat release is 72.5nW, and the maximum temperature rise within the fluid is 30.8μK. This is lower than the sensitivity of the EOT sensor, and is not suitable for calorimetric measurements.

There is limited experimental data from the previous work [1] on the reaction between Ca and EDTA. The raw EOT signals from an experiment with the same flow cell geometry, flow rates, and species concentrations are shown in Figure 28 and Figure 29. The signal does show a peak at the center of the channel, which is consistent with the concentration plume shape shown in Figure 25. However, the author in [1] notes that the small concentrations involved make it unlikely that the EOT signal peak is due to concentration differences and concludes that is due to the heat release of the reaction. The results of this study disagree with that interpretation, as the temperature plume has disappeared by 2.2mm down the channel. It is possible that the presence of the CaEDTA
does produce more of a change in EOT signal than anticipated, or that the peak can be attributed to noise.

Figure 28: EOT signal data from EDTA-CaCl₂ experiment, run 1 [1]; Z₁=0.9mm, Z₂=2.2mm.

Figure 29: EOT signal data from EDTA-CaCl₂ experiment, run 2 [1]; Z₁=0.9mm, Z₂=2.2mm.

5.2 Case 2: \( Q=5\mu L/min, \; C_{Ca}=100mM, \; C_{EDTA}=100mM \)

The second case used an identical flow rate of 5 uL/min, but increased the concentration of the calcium by 100x to 100mM, and the concentration of the EDTA by 1000x to 100mM. In Case 1, the lower concentration of EDTA limited how much CaEDTA could be formed. For this case, the reactants were increased to equal concentrations, as they combine to form 1:1 complexes. As a result, an increase in the heat release by a factor of approximately 1000x is expected.
Figure 30 shows CaEDTA concentration profiles for different distances along the channel. The butterfly shape is still visible, for the same reasons described previously. However, the degree of asymmetry is lower because the concentrations of the two reactants are equal. There is still a slight shift towards the left side of the channel because of calcium’s higher diffusion coefficient.

Figure 31: Averaged CaEDTA concentration, Q=5μL/min, Cca=100mM, Cedta=100mM

Figure 32: Averaged temperature, Q=5μL/min, Cca=100mM, Cedta=100mM

Figure 33: Averaged heat of reaction, Q=5μL/min, Cca=100mM, Cedta=100mM

The averaged CaEDTA concentration, temperature, and heat of reaction for Case 2 are shown in Figure 31, Figure 32 and Figure 33, respectively. There is greater symmetry in the CaEDTA plume than in Case 1 because the reactant concentrations are
equal. As in Case 1, the majority of the heat of reaction is confined to the inlet when the reactants first come into contact. However, due to the higher reactant concentrations, the maximum CaEDTA concentration is not reached right away. As a result, the heat of reaction plume reaches farther down the channel. As a result, the thermal plume reaches further down the channel than in Case 1. The magnitude of the temperature rise is also greater than in Case 1, with a peak of 6.2mK. The larger temperature rise can be attributed to the larger total heat release of 29.1 μW, which is due to the higher reactant concentration. This is about three orders of magnitude higher than in Case 1, as expected. A temperature rise on the order of mK is suitable for use with the EOT sensor in the microcalorimeter design.

5.3 Case 3: $Q=2.5\mu L/min, Cca=1mM, Cedta=0.1mM$

Figure 34: Contour Plots of CaEDTA Concentration at different $z$ locations. From top to bottom, $z=0.25mm$, 0.5mm, 1mm, 2.5mm, 5mm, 7mm. $Q=2.5\mu L/min, Cca=1mM, Cedta=0.1mM$

Figure 34 shows CaEDTA concentration profiles for different distances along the channel. The butterfly shape is still visible, for the same reasons described previously. The degree of asymmetry and the width of the diffusion region are greater than in Case 1, due to the lower flow rate and longer residence time of the reactants.
The averaged CaEDTA concentration, temperature, and heat of reaction for Case 3 are shown in Figure 35, Figure 36 and Figure 37, respectively. As in Case 1 and Case 2, the majority of the heat of reaction is confined to the inlet when the reactants first come into contact. Since the flow rate is lower, the mixing of reactants is greater than in Case 1, and the maximum CaEDTA concentration is reached earlier. This is why the heat of reaction plume is shorter than in Case 1. Due to the lower flow rate, the plume is also wider than in Case 1. Despite greater mixing, there is less heat release and associated temperature rise, with values of 51.2nW and 22.2μK, respectively. This is due to the lower flow rate, which limits the speed at which the reactants are brought together to react, limiting the heat release. The temperature rise on the order of μK is lower than the sensitivity of the EOT sensor, and is not suitable for calorimetric measurements.
5.4 Case 4: $Q=500\mu$L/min, $C_{ca}=1\text{mM}$, $C_{edta}=1\text{mM}$

Figure 38: Contour Plots of CaEDTA Concentration at different $z$ locations. From top to bottom, $z=0.25\text{mm}$, $0.5\text{mm}$, $1\text{mm}$, $2.5\text{mm}$, $5\text{mm}$, $7\text{mm}$. $Q=500\mu$L/min, $C_{ca}=1\text{mM}$, $C_{edta}=1\text{mM}$

Figure 38 shows CaEDTA concentration profiles for different distances along the channel. The butterfly shape is still visible, for the same reasons described previously. The degree of asymmetry is less pronounced than before, due both to the equal concentrations of reactants and the high flow rate. Since the flow rate is high, there is very short residence time and consequently little time for asymmetry to develop.

Figure 39: Averaged CaEDTA concentration, $Q=500\mu$L/min, $C_{ca}=1\text{mM}$, $C_{edta}=1\text{mM}$

Figure 40: Averaged temperature, $Q=500\mu$L/min, $C_{ca}=1\text{mM}$, $C_{edta}=1\text{mM}$

Figure 41: Averaged heat of reaction, $Q=500\mu$L/min, $C_{ca}=1\text{mM}$, $C_{edta}=1\text{mM}$
The averaged CaEDTA concentration, temperature, and heat of reaction for Case 3 are shown in Figure 39, Figure 40 and Figure 41, respectively. Unlike the previous cases, the heat of reaction is not confined to the channel inlet. This is due to the extremely high flow rate, which makes the residence time and resulting diffusion very small. As a result, the full concentration of CaEDTA is not reached until well down the channel. This allows the reaction to occur throughout the length of the channel, and the heat of reaction plume crosses the entire length of the channel. The associated thermal plume does so as well, and is actually still increasing in width at the channel outlet. This case best shows the implication of having a lower thermal Peclet number than mass diffusion Peclet number. As a result, the thermal plume is wider than the mass diffusion plume. Due to the higher flow rate bringing more reactants together, the heat release is greater than in Case 1 with a total of 1510 nJ. The peak temperature rise, however, is only 64.4μK. This is due to the fact that the heat release is not as concentrated as in the previous cases, but is distributed over the entire length of the channel. The temperature rise on the order of μK is lower than the sensitivity of the EOT sensor, and is not suitable for calorimetric measurements.

Table 15: Summary of Results

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>Units</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>ul/min</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>500</td>
</tr>
<tr>
<td>[Ca]</td>
<td>mol/L</td>
<td>1.00E-03</td>
<td>1.00E-01</td>
<td>1.00E-03</td>
<td>1.00E-03</td>
</tr>
<tr>
<td>[EDTA]</td>
<td>mol/L</td>
<td>1.00E-04</td>
<td>1.00E-01</td>
<td>1.00E-04</td>
<td>1.00E-03</td>
</tr>
<tr>
<td>Re</td>
<td>unitless</td>
<td>0.269</td>
<td>0.269</td>
<td>0.135</td>
<td>26.920</td>
</tr>
<tr>
<td>$Pe_{mass}$</td>
<td>unitless</td>
<td>555.56</td>
<td>555.56</td>
<td>277.78</td>
<td>55555.56</td>
</tr>
<tr>
<td>$Pe_{th}$</td>
<td>unitless</td>
<td>1.93</td>
<td>1.93</td>
<td>0.97</td>
<td>193.26</td>
</tr>
<tr>
<td>$Q_{rxn}$</td>
<td>W</td>
<td>7.25E-08</td>
<td>2.91E-05</td>
<td>5.12E-08</td>
<td>1.51E-06</td>
</tr>
<tr>
<td>Peak ΔT</td>
<td>K</td>
<td>3.08E-05</td>
<td>6.20E-03</td>
<td>2.22E-05</td>
<td>6.44E-05</td>
</tr>
</tbody>
</table>

6 Conclusions

Using a simplified model of the reaction between CaCl$_2$ and EDTA, three different combinations of flow rates and concentrations were examined. It was found that the default values of 5 uL/min for flow rate and concentrations of 1mM Ca and 0.1mM EDTA will not produce a suitable heat of reaction and associated temperature rise to be useful for calorimetry. A number of other cases were simulated to gauge the effect of the flow rates and concentrations of reactants. Decreasing the flow rate increases the mixing
and the width of the diffusion region, but decreases the overall heat release. Conversely, increasing the flow rate increases the overall heat release, but results in a thin plume which will complicate sensor placement and measurement capability. Increasing the reactant concentrations results in higher heat release and temperature rise, making calorimetric measurements easier. However, increasing the reactant concentration increases the quantity of reactant required for each measurement.

One particularly interesting finding was that for slower flow rates, the degree of mixing at the confluence of the reactant streams allows the bulk of the reaction to occur immediately, creating a shorter, wider thermal plume. For the higher flow rate, the mixing was limited and the reaction was distributed over the channel length, creating a smaller plume. An intermediate flow rate which allows for a larger heat release than in Case 1 and Case 3, but a thermal plume that is wider than in Case 4, may be of interest. This would create a larger temperature rise than in Case 1 and Case 3, and larger region where NHA arrays could be placed to obtain measurements. Reactant concentration could then be increased as necessary to achieve the required temperature rise for measurement.

7 Future Work

There is much opportunity for future work in this area of research, especially as the device fabrication and experimental techniques are refined and more data becomes available. One of the goals of this work was to outline a methodology for predicting the temperature and concentration fields within the device for a reaction. Now that this methodology exists, different reactions between different compounds can be easily simulated provided the reaction kinetics and thermodynamic information is known. It may also be beneficial to empirically measure these quantities moving forward for the most accurate results.

Several simplifications and assumptions were made to develop the reaction model used in this work. Developing a model that incorporates the equilibrium speciation of the calcium-EDTA system with the fluid flow equations would produce more accurate results and allow differentiation of the total observed heat release into the valuable intrinsic heat of binding and the unwanted heats of ionization of the EDTA and buffer. Using a model that can separate these heats would be useful in optimizing the reaction system for obtaining the intrinsic heat of binding. The heat of ionization can also be corrected for by conducting the experiment at different pH’s. Development of this technique would be
valuable for understanding how to correct for unwanted energy releases in observed reactions.

In this study, the device geometry was held constant. Future work could investigate optimizing the geometry of the device in addition to the concentrations and flow rates of the reactants. Another potential area of study includes choice of numerical solution methods such as discretization scheme on the solution accuracy. This requires more experimental data to validate numerical results.

Another limitation on the scope of this work was the time required to perform the simulations. Future work could investigate different ways of improving convergence speed and reducing simulation run time through parallel processing, optimizing solver settings, etc.
% The purpose of this program is to calculate the distribution of EDTA forms in solution for pH range 0-14.

% Solution pH
pH_solution=6;

% EDTA dissociation constants
pK=[1.15 1.15 2.12 2.57 6.16 10.26];
K=10.^-pK;

% Initialize vectors
cumulative_K=zeros(1,6);

% Calculate cumulative K (beta)
mult_summation=1;
for m=1:6
    mult_summation=mult_summation*K(m);
cumulative_K(m)=mult_summation;
end

i=0;
for pH=0:0.1:14
    i=i+1;
    pH_vec(i)=pH;
    C_h=10^-pH;

    % Compute alpha
den=C_h^6;
    for n=1:6
        den=den+cumulative_K(n)*C_h^(6-n);
    end
    alpha=cumulative_K(6)/den;

    % Determine equilibrium speciation
term=alpha;
    HnY(i,7)=alpha;
    for q=1:6
        term=term*C_h/K(7-q);
        HnY(i,7-q)=term;
    end

    if pH==pH_solution
        alpha_vector=HnY(i,:);
    end
end

mol_H_per_EDTA=alpha_vector(6)+2*alpha_vector(5)+3*alpha_vector(4)+4*alpha_vector(3)+5*alpha_vector(2)+6*alpha_vector(1);

% Plot equilibrium speciation
figure1 = figure('Name', 'EDTA Equilibrium Speciation');
axes1 = axes('Parent', figure1);

% xlim(axes1, [5.5 6.5]);
box(axes1, 'on')
hold on
plot(pH_vec, HnY(:, 1), 'Color', [0 0 0], 'DisplayName', 'H6Y');
plot(pH_vec, HnY(:, 2), 'Color', [1 0 0], 'DisplayName', 'H5Y');
plot(pH_vec, HnY(:, 3), 'Color', [0 1 0], 'DisplayName', 'H4Y');
plot(pH_vec, HnY(:, 4), 'Color', [0 0 1], 'DisplayName', 'H3Y');
plot(pH_vec, HnY(:, 5), 'Color', [.75 0 1], 'LineStyle', '.', 'DisplayName', 'H2Y');
plot(pH_vec, HnY(:, 6), 'Color', [0 0.5 0], 'LineStyle', ':', 'DisplayName', 'HY');
plot(pH_vec, HnY(:, 7), 'Color', [0 0 0.5], 'LineStyle', '--', 'DisplayName', 'Y');

% Create title
title('EDTA Equilibrium Speciation');

% Create xlabel
xlabel('pH');

% Create ylabel
ylabel('Alpha');

% Create legend
legend1 = legend(axes1, 'show');
set(legend1, 'Location', 'EastOutside');
Appendix B: MATLAB Code for Averaging Values over Y-Direction

% This function performs vertical averaging over the flow cell domain.

% Constants
x_min=-2.5e-4;
x_max=2.5e-4;
z_min=0;
z_max=7e-3;
grid_divisions=100;
tolerance=0.00175;

% Import solution data
[bw_FileName,bw_PathName] = uigetfile({'*.csv';},'Select bottom wall data file');
[celldata_FileName,celldata_PathName]=uigetfile({'*.csv';},'Select fluid zone data file');
tic;
[x_bw,z_bw,n_bw]=import_bw(bw_FileName);
time_bw=toc;
fprintf('Bottom wall data imported in %f seconds. \n', time_bw);
tic;
[x,z,temp,conc,hor,dcdx,dcdz,gradc,gradt,volume,dtdx,dtdz,n_cells]=import_cells(celldata_FileName);
time_fz=toc;
fprintf('Fluid zone data imported in %f seconds. \n', time_fz);

% Initialize vectors
temp_bw=zeros(n_bw,1);
conc_bw=zeros(n_bw,1);
hor_bw=zeros(n_bw,1);
dcdx_bw=zeros(n_bw,1);
dcdz_bw=zeros(n_bw,1);
gradc_bw=zeros(n_bw,1);
gradt_bw=zeros(n_bw,1);
dtdx_bw=zeros(n_bw,1);
dtdz_bw=zeros(n_bw,1);
volume_bw=zeros(n_bw,1);
y_cells_bw=zeros(n_bw,1);

% Determine step size
x_step=min(abs(x_bw))/10;
z_step=min(z_bw)/10;
tic;

% Loop over fluid zone cells
for i=1:n_bw
    temp_total=0;
    conc_total=0;
    hor_total=0;
    dcdx_total=0;
    dcdz_total=0;
    gradc_total=0;

gradt_total=0;
dtdx_total=0;
dtdz_total=0;
volume_total=0;
y_cells=0;
x_lowerlim=abs(x_bw(i))-x_step;
x_upperlim=abs(x_bw(i))+x_step;
z_lowerlim=z_bw(i)-z_step;
z_upperlim=z_bw(i)+z_step;
for j=1:n_cells
    x_current=abs(x(j));
    z_current=z(j);
    if (x_current>=x_lowerlim && x_current<=x_upperlim) &&
        (z_current>=z_lowerlim && z_current<=z_upperlim)
        if sign(x_bw(i))==sign(x(j))
            temp_total=temp_total+temp(j)*volume(j);
            hor_total=hor_total+hor(j)*volume(j);
            dcdx_total=dcdx_total+dcdx(j)*volume(j);
            dcdz_total=dcdz_total+dcdz(j)*volume(j);
            gradc_total=gradc_total+gradc(j)*volume(j);
            gradt_total=gradt_total+gradt(j)*volume(j);
            dtdx_total=dtdx_total+dtdx(j)*volume(j);
            dtdz_total=dtdz_total+dtdz(j)*volume(j);
            volume_total=volume_total+volume(j);
        end
    end
end

temp_bw(i)=temp_total/volume_total;
conc_bw(i)=conc_total/volume_total;
hor_bw(i)=hor_total/volume_total;
dcdx_bw(i)=dcdx_total/volume_total;
dcdz_bw(i)=dcdz_total/volume_total;
gradc_total=gradc_total/volume_total;
gradt_bw(i)=gradt_total/volume_total;
dtdx_bw(i)=dtdx_total/volume_total;
dtdz_total=dtdz_total/volume_total;
volume_bw(i)=volume_total;
y_cells_bw(i)=y_cells;
y_ave=mean(y_cells_bw(1):y_cells_bw(i));
y_std=std(y_cells_bw(1):y_cells_bw(i));
if rem(i,100)==0
    fprintf('%d cells remaining, y_ave = %f, y_std=%f \n',
        n_bw-i,y_ave, y_std);
end
end
time_ave=toc;
fprintf('Vertical averaging performed in %f seconds. \n',
time_ave);

% Determine utility
[utility_bw]=determine_utility(temp_bw,conc_bw,gradt_bw,gradc_bw,
    n_bw);
x_bw_mm=-x_bw.*1000;
z_bw_mm = z_bw .* 1000;
conc_bw_mM = conc_bw .* 1000;
gradc_bw_mM = gradc_bw .* 1000;

min_x = min(x_bw_mm);
max_x = max(x_bw_mm);
min_z = min(z_bw_mm);
max_z = max(z_bw_mm);

tic;

% Create contour plots
plotcontour(z_bw_mm, x_bw_mm, temp_bw, min_z, max_z, min_x, max_x, grid_divisions, 'Averaged Temperature [K]');
plotcontour(z_bw_mm, x_bw_mm, conc_bw_mM, min_z, max_z, min_x, max_x, grid_divisions, 'Averaged CaEDTA Concentration [mM]');
plotcontour(z_bw_mm, x_bw_mm, gradc_bw_mM, min_z, max_z, min_x, max_x, grid_divisions, 'Averaged CaEDTA Concentration Gradient [mM/m]');
plotcontour(z_bw_mm, x_bw_mm, gradt_bw, min_z, max_z, min_x, max_x, grid_divisions, 'Averaged Temperature Gradient [K/m]');
plotcontour(z_bw_mm, x_bw_mm, hor_bw, min_z, max_z, min_x, max_x, grid_divisions, 'Averaged Heat of Reaction [W]');
plotcontour(z_bw_mm, x_bw_mm, utility_bw, min_z, max_z, min_x, max_x, grid_divisions, 'Utility');
time_plot = toc;
fprintf('Data plotted in %f seconds. 
', time_plot);

writedata(x_bw, z_bw, temp_bw, conc_bw, hor_bw, gradt_bw, gradc_bw, volume_bw);

function [x, z, n_new] = import_bw(FileName)
    data = dlmread(FileName, ',', 1, 0);
x = data(:, 2);
z = data(:, 4);
n = length(x);

    neg_count = 0;
    for i = 1:n
        if z(i) < 0
            neg_count = neg_count + 1;
            delete_vector(neg_count) = i;
        end
    end
    x(delete_vector) = [];
z(delete_vector) = [];
n_new = length(z);

function [x, z, temp, conc, hor, dcdx, dcdz, gradc, gradt, volume, dtdx, dtdz, n_new] = import_cells(FileName)
data = dlmread(FileName, ',', 1, 0);
x = -1 * data(:, 2);
z = data(:, 4);
temp=data(:,5);
conc=data(:,6);
hor=data(:,7);
dcdx=data(:,8);
dcdz=data(:,10);
gradc=data(:,11);
gradt=data(:,12);
volume=data(:,13);
dtx=data(:,14);
dtz=data(:,16);
n_cells=length(x);

neg_count=0;
for i=1:n_cells
    if z(i)<0
        neg_count=neg_count+1;
        delete_vector(neg_count)=i;
    end
end
x(delete_vector)=[ ];
z(delete_vector)=[ ];
temp(delete_vector)=[ ];
conc(delete_vector)=[ ];
hor(delete_vector)=[ ];
dcdx(delete_vector)=[ ];
dcdz(delete_vector)=[ ];
gradc(delete_vector)=[ ];
gradt(delete_vector)=[ ];
volume(delete_vector)=[ ];
dtx(delete_vector)=[ ];
dtz(delete_vector)=[ ];
n_new=length(z);

function plotcontour(xdata1, ydata1, zdata1, x_min, x_max, z_min, 
z_max, grid_divisions,plot_title)
%CREATEFIGURE3(XDATA1, YDATA1, ZDATA1)
% XDATA1:  contour x
% YDATA1:  contour y
% ZDATA1:  contour z

% Auto-generated by MATLAB on 01-Dec-2015 18:34:09

% Create regular grid across data space
[X,Z] = meshgrid(linspace(x_min,x_max,grid_divisions),
linspace(z_min,z_max,grid_divisions));

% Create figure
figure1 = figure('Color',[1 1 1]);
% Create axes
axes1 = axes('Parent',figure1,'Layer','top');
axis equal tight
% Uncomment the following line to preserve the X-limits of the axes
xlim(axes1,[x_min x_max]);
%

% Uncomment the following line to preserve the Y-limits of the axes
ylim(axes1,[z_min z_max]);
box(axes1,'on');
hold(axes1,'all');

% Create contour
contour(X,Z,griddata(xdata1,ydata1,zdata1,X,Z),'Fill','on','Paren
t',axes1);

% Create xlabel
xlabel('x (mm)');

% Create ylabel
ylabel('z (mm)');

% Create title
title(plot_title);

% Create colorbar
colorbar('peer',axes1);

function writedata(x_bw,z_bw,temp_bw,conc_bw,hor_bw,gradt_bw,gradc_bw,volume_bw)
data = [x_bw'; z_bw'; temp_bw'; conc_bw'; hor_bw'; gradt_bw';
gradc_bw'; volume_bw'];
file_title=input('Enter title of data file: 
','s');
file_title_full=strcat(file_title,'.txt');
fileID = fopen(file_title_full,'w');
fprintf(fileID,'%24s %24s %24s %24s %24s %24s %24s
%24s\r\n','x','z','Temperature (K)', 'Concentration [M]', 'Heat of Reaction [W]', 'Temperature Gradient [K/m]', 'Concentration Gradient [M/m]', 'Volume [m^3]');
fprintf(fileID,'%24.8e %24.8e %24.8e %24.8e %24.8e %24.8e %24.8e
%24.8e\r\n',data);
fclose(fileID);
Appendix C: FLUENT User-Defined Function for Exporting Concentration Gradient Values

#include "udf.h"

DEFINE_EXECUTE_ON_LOADING(on_load, libname)
{
    Set_User_Memory_Name(0,"caedta_x_gradient");
    Set_User_Memory_Name(1,"caedta_y_gradient");
    Set_User_Memory_Name(2,"caedta_z_gradient");
    Set_User_Memory_Name(3,"caedta_gradient_magnitude");
    Set_User_Memory_Name(4,"temperature_gradient");
}

DEFINE_ON_DEMAND(mass_fraction_gradients)
{
    Domain *d;
    Thread *t;
    cell_t c;

    d=Get_Domain(1);

    thread_loop_c (t,d)
    {
        begin_c_loop (c,t)
        {
            C_UDMI(c,t,0)=C_YI_G(c,t,1)[0];
            C_UDMI(c,t,1)=C_YI_G(c,t,1)[1];
            C_UDMI(c,t,2)=C_YI_G(c,t,1)[2];
            C_UDMI(c,t,3)=NV_MAG(C_YI_G(c,t,1));
            C_UDMI(c,t,4)=NV_MAG(C_T_G(c,t));
        }
        end_c_loop (c,t)
    }
}
9 References


