NUMERICAL STUDIES ON TWO PHASE FLOW ELECTROPHORESIS

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ABSTRACT

A numerical investigation was conducted to simulate the transport of charged molecules in a flow system of two immiscible fluids and an electrical field orthogonal to the flow direction. A finite element based method was implemented using COMSOL Multiphysics 3.5a to solve the multiphase transport in rectangular type geometries. The Level set method was employed to explicitly describe the location of the moving fluid interface. Solutions to the Navier-Stokes equations coupled with the Level set and electrophoretic transport relations were obtained for typical proteins encountered in two phase extraction systems. System properties were modeled to be consistent with a polyethylene glycol – dextran – water solution at the macroscopic scale.
EXECUTIVE SUMMARY

All novel separation techniques born in the experimentalist’s laboratory must be tested for mathematical and physical continuity with the governing theory. It is true that the scientific experiment serves as the ultimate proof of a particular concept. However, there must also exist methods to interpret the said concept in terms of the current science. History has demonstrated that in the instances where current science fails to fully explain the physically observable phenomena, science and the understanding of the universe are both greatly expanded. The use of numerical methods has been shown to be an effective discipline for modeling physical systems. One of the more popular approaches to test the aforementioned continuity in flow systems is to utilize the tools of computational fluid dynamics.

The separation technique known as aqueous two-phase electrophoresis provides a unique modeling endeavor. Aqueous two-phase electrophoresis is an attractive separation technique as it combines multiphase and electrophoretic mass transport in a continuous flow device. Additionally, aqueous two-phase electrophoresis has been shown to be effective at the macroscopic level, which permits a high volumetric throughput to be separated in suitable flow arrangements.

The present work provides a typical review of prior experimental and theoretical works regarding the components of aqueous two-phase electrophoresis. A small discussion on the proper term to describe the simultaneous two-phase and electrophoretic transport of charged particles in a flow system is included in an attempt to standardize the name of the actual process as multiple terms were encountered in the literature. The mathematical equations utilized to describe the two phase transport are robust and generally well behaved with very little modification. The multiphysics engine utilized to solve the governing transport equations, COMSOL 3.5a, is a powerful and user friendly solver.

Ultimately, the present work is a representative of the initial two-phase electrophoresis modeling effort. The modeling results show agreement with experimental investigations with properly adjusted physical properties.
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INTRODUCTION

Aqueous two-phase systems (ATPSs) are naturally occurring phenomena where the chemical components of a solution spontaneously separate into two distinct fluid phases. ATPSs are not esoteric or pure academic constructs. The classic example of a two-phase system is the oil and water solution (O-W), which is physically observable in most households. Two-phase systems are typically employed in industrial separation techniques, most notably in liquid-liquid extraction (LLE). Aqueous two-phase systems are a special subset of two-phase systems where both phases are comprised of primarily water, usually 80 – 95%. Another of the widely employed separation techniques is electrophoresis. Electrophoresis is defined as the motion of a charged surface or particle relative to the motion of the bulk fluid that is influenced by a uniform electrical field. Essentially, electrokinetic or alternatively termed electrophoretic transport, is the additional motion of a charged particle in an electrical field that results from repulsive forces produced by the interaction of layers or shells of electrical charge. Typically, electrophoresis is a laboratory scale technique used to separate biological materials such as proteins, amino acids, and other cellular components. When electrophoresis is integrated into aqueous two-phase extraction systems, a novel separation technique known as aqueous two-phase electrophoresis (ATPE) emerges.

The combination of electrophoresis with other separation and purification techniques is not unique to ATPE. Protein purification techniques commonly include chromatography. Protein fingerprinting or peptide mapping is a two-step process consisting of a chromatography step and then a sequential electrophoresis assay. Additionally, electrophoresis is carried out twice at right angles on the same support medium in a process known as two-dimensional electrophoresis. The level of purification is undoubtedly linked to the purpose of the protein. For example, in the production of a monoclonal antibody, a 50% pure protein may be adequate. However, in the raising of a monospecific polyclonal antibody, a greater than 95% pure protein stock is often required.

Electroextraction and aqueous two-phase electrophoresis have both been utilized by researchers to describe the observed mass transport of charged molecules in a system of immiscible fluids and an applied electrical field. The evidence from the literature suggests strongly that Stichlmair and coworkers at the University of Essen were at the forefront of integrating electrophoretic transport with liquid-liquid
extraction techniques in a process they termed as electroextraction. A few years later Clark and coworkers began to use both electroextraction and aqueous two-phase electrophoresis in the titles of their published experimental work. At present, it appears as if electroextraction has become more synonymous with an electrochemical metal deposition process known as electrowinning than with a multiphase separation technique. Therefore, the more prudent nomenclature to use to describe the aforementioned combined separation technique would be aqueous two-phase electrophoresis.

The use of ATPE in a separation scheme provides several benefits. Firstly, ATPE significantly reduces the convective mixing\textsuperscript{46,48,49} that results from a phenomenon known as Joule heating in electrophoresis. The interface between each phase effectively confines convective currents to the phase in which they originated. This feature prevents the undesired convective transport of particles from one electrode to the other as shown in Figure 0.1 as illustrated by the open circular currents.

![Figure 0.1: Aqueous Two-Phase Extraction: Convection Currents and Interface Delineation](image)

Mass transport across the interface in ATPSs is largely controlled by the surface tension. ATPE systems employ suitable liquids with extremely low surface tensions, which permits relatively easy mass transport across the interface. Typically, polyethylene glycol (PEG)-dextran (DEX) - water systems are utilized with proper pH balancing agents in the ATPE of biological materials\textsuperscript{5,33}. However, certain systems of a polymer and a high concentration of a salt also demonstrate a similar phase splitting phenomenon as PEG and dextran systems. PEG and dextran systems are also capable of achieving high overall yields in extractive purification steps, while still maintaining the specific activity of the protein. PEG and dextran are nonionic-phase forming organic polymers with high molecular weights, typically in the vicinity of 8,000 and 500,000 Daltons respectively. The base units of PEG and dextran have been reproduced in
Figure 0.2 and Figure 0.3 respectively. Published phase-diagrams report that the PEG and dextran rich phases contain between 85% and 99% water.

Figure 0.2: Polyethyene glycol Diagram

Figure 0.3: Dextran Diagram

Preferential partitioning of proteins and other charged particles is another integral phenomenon of ATPE systems. In the absence of the electrical field, it is well known that certain proteins and particles will partition into the more favorable phase. The partition coefficient and electrophoretic mobility of the particle are influenced by the phase pH and other physio-chemical properties. The preferential partitioning of the particles in the two-phase system can be overcome with a suitable electrical field in both static and flow systems.

Experimentally, ATPE has been demonstrated to be an intelligent biomaterial separation technique. Currently, ATPE systems have been designed on the micro and macro scales up to 200 cm in total length and 5 cm in total width. The scale up of ATPE devices is thought to be limited by the available cooling ability of the apparatus\textsuperscript{18}. Theoretically, ATPE has received some attention in the literature\textsuperscript{43}, though the prime focus has been on systems without flow. In the present investigation, we have utilized COMSOL Multiphysics 3.5a to analytically model ATPE in a continuous flow device under laminar conditions.
SUMMARY OF PRIOR WORKS

Aqueous Two-Phase Electrophoresis: Origins

The origins of what has come to be known as aqueous two-phase electrophoresis may be traced as far back to 1809. Reuss\textsuperscript{11} is credited as being the first to describe the migration of clay particles in the presence of an electric field. Hittorf further investigated the effect of an applied electric field on ions in a solution by measuring concentration changes in a small tube\textsuperscript{12}. Hittorf observed a distinct deviation from the constant concentration profile when an electric current was passed through the silver electrodes immersed in the silver nitrate solution. Additionally, Hittorf documented the nullifying effect that Joule heating has on the ion segregation at high current densities. A schematic of the Hittorf experiment has been reproduced in Figure 1.1

![Figure 1.1: The Hittorf Experiment](image)

Kohlrausch\textsuperscript{13} is credited as being one of the first to determine the velocities of several ions in an electric field. The first investigations to utilize the electrophoretic migration as a specific means of separation were performed by Picton and Lindner\textsuperscript{14,15} using simple colloidal systems. The extension of electrophoretic migration as an analytical tool is often credited to the work of Tiselius\textsuperscript{16} in his doctoral
thesis. Tiselius’ innovative U-tube electrophoresis apparatus has been diagrammed in **Figure 1.2**. Typical applications of Tiselius’ U-tube feature a mixture containing proteins in solution loaded into the U-bend of the apparatus and appropriate electrolytic solutions of different densities being loaded on top of the protein solution. The U-tube design with different density feed and electrolytic solutions facilitate the stabilization of the system against convective disturbances and re-mixing, yet only for relatively small power inputs of $0.115 \text{ W cm}^{-3}$ despite provisions for system cooling\textsuperscript{16}.

![Figure 1.2: Tiselius’ Experiment](image)

Electrophoretic separation was expanded to configurations beyond the batch system with the work of Hanning and Grassmann\textsuperscript{17} in 1949. Hanning and Grassmann introduced a mixture to be separated into a continuous flow of an electrolytic solution passing through an electric field orthogonal to the aqueous flow channel. Separated species were collected continuously at distinct points at the end of the flow chamber. The latter technique has come to be known as continuous free-flow electrophoresis. The principle of free-flow electrophoresis has been illustrated in **Figure 1.3** with four hypothetical species with varying electrophoretic mobilities.
Free-flow electrophoresis is nevertheless subject to Joule heating, which induces convection currents in the flow system that may destroy the desired separation of components. Laminar-flow, henceforth becomes imperative in such flow systems to reduce to the convective mixing and preserve the overall separation. Electrophoresis has been investigated under weightless conditions in space missions from Apollo 5 in 1971 to the Spacelab in 1985 as well as in many of the Space Shuttle missions thereafter. Electrodialysis is a successful large-scale separation technique similar to electrophoresis. The large-scale success of electrodialysis principally comes from the presence of a membrane across which the separation occurs that eliminate the convective disruptions associated with electrophoresis. Unfortunately, electrodialysis requires significant capital investment to implement and is restricted to a narrow set of specialized separation conditions.

Liquid-Liquid Extraction and Aqueous Two-Phase Separation

The chemical process industry is known for using liquid extraction techniques to achieve large-scale separations. Briefly, the liquid-liquid extraction (LLE) technique utilizes a multiphase aqueous separation approach based upon the preferential partitioning of the desired product, the extract, in specific phases. Typically the desired product exists in a feed mixture of other non-desirable species confined to one distinct phase. Another, strategically selected liquid phase is combined with the mixture.
containing the desired product to form a multi-phase system. The latter phase is said to act as a solvent to remove the desired product from the feed phase and to the easily separable extract phase. A detailed review of liquid-liquid extraction is beyond the scope of this investigation. However, in depth reviews on advanced LLE techniques may be found elsewhere in the available literature\textsuperscript{19-21}.

Liquid extraction techniques have been applied to biological product recovery. Beijerinck\textsuperscript{30} is credited with first discovering aqueous two-phase systems, but their application to the purification of cell organelles and macromolecules is first attributed to the work of Albersson\textsuperscript{31-33}. The chemical components of the multiphase system may be selected to be compatible with the biological products of interest. The extension of aqueous two-phase systems to biological systems on the industrial scale has been previously established\textsuperscript{22-24, 34-40}. In the late 1980s and early 1990s, an interest in improving liquid extraction with applied electric fields emerged. Scott and Wham utilized an applied electric field to stabilize a high-surface-area emulsion against the upward transport of the continuous phase in a novel countercurrent solvent extraction system\textsuperscript{26}. Brooks and Bamberger investigated droplet coalescence and phase separation in aqueous two-phase systems influenced by an applied electric field at low gravity\textsuperscript{27}. Rao et al. have investigated the performance of what they term “electrokinetic demixing” in conjunction with reducing the phase separation time of a multiphase system\textsuperscript{28, 29}.

Electroextraction, as termed by the researchers, was developed at the Institute of Chemical Engineering at the University of Essen\textsuperscript{41}. The separation technique integrates the principles of LLE and electrophoresis into a single device. Stichlmair et al. present the application of electroextraction to a batch U–tube system separating fuchsin acid from a simple two-phase water and n-butanol system, as well as the application to a continuous countercurrent two-phase electrophoresis device separating citric acid from a water and water saturated n-butanol system\textsuperscript{18}. Stichlmair et al. also review the principles of mass transfer under the action of an electric field. Luo et al. have investigated the separation of dyestuffs from dye effluents using a similar continuous flow two-phase electrophoresis system\textsuperscript{46}.

More frequently observed in the literature is the application of electroextraction to that of biological molecules. Levine and Bier\textsuperscript{42} have investigated the electrophoretic transport of various solutes, primarily human hemoglobin, in aqueous two-phase systems of dextran and polyethylene glycol. Experiments were conducted using a U-tube moving boundary electrophoresis device and a capillary electrophoresis
device to measure the migration velocity more accurately. Levine and Bier report that the electrophoretic transport of proteins across the two-phase interface is greatly impeded in one direction. The authors report that a protein will be readily transported from the non-preferred to the preferred phase, but will not migrate from the preferred to the non-preferred phase. Levine et al. also model the protein transport across the interface in a mathematical simulation\(^{43}\).

Clark and coworkers have also investigated protein separation through means of two-phase electrophoresis in dextran and polyethylene glycol systems\(^{25, 44}\). The authors did not report an insurmountable barrier to protein partitioning that prevents migration from the preferred phase to the non-preferred phase as reported by Levine et al. Experiments were conducted from novel batch and continuous flow two-phase electrophoresis systems as reproduced in Figure 1.4. Clark et al. also demonstrated the utility of two-phase electrophoresis in recovering active $\beta$-Lactamase from *Escherichia coli* cell lysate\(^{44}\).

**Figure 1.4: Two-Phase Electrophoresis Unit**

Zhai et al. have examined the separation of amino acids in dextran-polyethylene glycol-water systems using a batch U-tube two-phase electrophoresis device\(^{48, 49}\). The authors have demonstrated excellent
separation of glutamic acid from phenylalanine and tryptophan using their electrophoretic apparatus. They also have documented the increase in amino acid concentration and the decrease in the mass flux near the upper interface as a function of increasing time. Zhai et al. however, did not record the presence of an insurmountable resistance to interface transport from the preferred to non-preferred phase.

One of the other electrokinetic processing studies available in the literature is from Horvath et al. The authors have reported efficient means of separating hemoglobin from bovine serum albumin, the purification of phycoerythrin from an extract, and the fractionation of serum proteins based upon their sizes while using the Gradiflow™ apparatus. The apparatus consists of an inner series of selective membranes encompassed by typical electrophoresis electrodes. While the authors do not utilize a two-phase system, they report efficient control of Joule heating within the Gradiflow™ device and additionally no apparent limitation to the further scale up.

Continuous Flow Separation in Microfluidic Devices

In more recent studies, there has been significant interest in investigating aqueous separation in microfluidic devices. The scale of the separation requires unique modifications to the macroscopic fluid flow equations. Many of the microfluidic investigations explore subtle changes in the geometry of the system in order to refine the separation. A typical sketch and the associated scale of a microfluidic device utilized for aqueous two-phase separation has been reproduced in Figure 1.5.
Pamme\textsuperscript{57} has assembled an excellent review of the continuous flow separation methods using microfluidic devices available in the literature. Zhao et al. have reported the flow patterns obtained from immiscible two-phase flow in a rectangular microchannel\textsuperscript{50}. Sundaram et al. have attempted to optimize the geometry of a microchamber in order to promote electrokinetically driven mixing. Additionally, several studies\textsuperscript{51-53} have been conducted to investigate the separation of cell material in two-phase systems. Wang et al. have proposed a model for Joule heating-induced dispersion in microscale electrophoresis\textsuperscript{54}. Dimaki et al. have recently conducted a COMSOL multiphysics simulation of a novel microfluidic device for biomedical applications\textsuperscript{55}.

**Numerical Simulation**

The classical evolution of single fluid flow is governed by the Navier-Stokes (NS) equations. In order to properly capture the two-phase flow in ATPE and other multiphase systems, the NS equations must be supplemented by a numerical technique to track the fluid-fluid interface. Historically, the earliest numerical interface-tracking methods were derived from the pioneering work of Harlow and Welch\textsuperscript{79}. The Harlow and Welch based methods track interfaces that are permitted to freely evolve on fixed meshes. Additionally, the latter family of methods may be divided into two principle groups that depend on the manner in which the interface is described. The “Lagrangian techniques” make use of markers to track the interface location and curvature. Front–Tracking\textsuperscript{80,81}, Marker\textsuperscript{82}, and Particle-in-cell\textsuperscript{79,83} methods all comprise the traditional Lagrangian type techniques. Alternatively, the “Eulerian techniques” utilize a type of scalar function to define the interface location. The Level set\textsuperscript{84,85,86} (LS) and Volume of Fluid\textsuperscript{87,88,89} (VOF) methods are common techniques belonging to the latter fixed grid group.

Hybrid interface-capturing techniques utilizing components of the Level set method have received much attention in the recent literature. Tanguy et al.\textsuperscript{90} have applied combined level set methods with ghost-fluid and continuum surface force formulation techniques to simulate droplet collisions. Bonometti et al.\textsuperscript{1} have proposed a level set hybrid method with VOF in order to investigate bubble dynamics. Menard et al.\textsuperscript{91} have extended a combined LS-VOF-ghost fluid method to model the primary breakup of a jet. Wang et al.\textsuperscript{92} have applied a LS-VOF technique the sharp interface simulation of plunging breaking waves. Thommes et al.\textsuperscript{93} have proposed a novel Lattice-Boltzmann (LB) and level set hybrid interface-tracking method.
Numerical simulations of aqueous two-phase electrophoresis related processes in COMSOL Multiphysics are extremely limited. Currently, many of the electrophoresis modeling simulations have been conducted at the micro level and do not investigate the effect of phase-forming polymers on protein or other charged particle partitioning. The modeling of two-phase flow in COMSOL has been conducted by some authors in conjunction with applications other than ATPE. Lund\textsuperscript{2} has conducted a modeling study for two-phase flow in a spouted, fluidized-bed type geometry. Additionally, there has been interest in modeling the two-phase flow from an inkjet printer head.
NUMERICAL METHODOLOGY

Protein Partitioning

In ATP systems, the partitioning of a protein is influenced by the type of polymer used, the concentration of the polymer, the pH of the system, and the ionic strength. Much of the early work regarding the effect of various solution properties on the partitioning of proteins has been conducted by Albertsson. The first theoretical protein partitioning models were proposed separately by Brooks et al. and Albertsson et al. and were based on the lattice model of Flory and Huggins for polymer solution thermodynamics. Diamond and Hsu have developed linear and nonlinear semilogarithmic protein partition coefficient relationships for PEG – dextran – water systems.

Protein partitioning is controlled by the chemical potential. Proteins and other similar charged particles in ATPE systems, will redistribute until the following equilibrium relationship is achieved:

\[ \mu_i^\alpha = \mu_i^\beta \]  

(1)

where \( \alpha, \beta \) represent the top and bottom phases in ATP systems. In many ATPE systems, salts are added to the system in order to balance the environment for the biological components. It has been shown that the addition of salts to the ATP system will induce an electrostatic potential difference between \( \alpha \) and \( \beta \) phases. The latter evidence requires a modification in the chemical potential to account for the electrostatic interactions in applicable systems:

\[ \mu_i^j = \mu_i^{0,j} + N_A \left( \frac{\delta \Delta G_m}{\delta n_i} \right)_{n_j,T,P} + z_i F \psi \]  

(2)

The species partition coefficient, written here as \( K_i \), is defined as the ratio of equilibrium species concentrations in the top and bottom phases in the following relation:

\[ K_i = \frac{C_{i,\alpha}}{C_{i,\beta}} \]  

(3)
where $C_\alpha$ and $C_\beta$ represent the concentrations of the partitioned species in the top and bottom phases, respectfully. Albertsson$^{32,33}$ originally expressed the species partition coefficient in a manner more consistent with the equilibrium distribution governed by the chemical potential:

$$\ln K_i = \ln K_i^0 + \frac{Fz_i \Delta \psi}{RT}$$

where, $\Delta \psi$ is the electrostatic potential difference between each phase and $K_i^0$ is the fundamental species partition coefficient in the absence of electrostatic interactions. Modifications to Albertsson’s partition coefficient have been proposed due to the difficult nature of predicting the electrostatic potential difference in the biphasic system. Furthermore, the species partition coefficient is unique to a particular biphasic system at a given temperature and pressure. Lastly, the partition coefficient is independent of species concentration$^{68}$ and independent of the total volume of the two-phase system$^{33}$.

**Electrophoretic Mobility**

Particle and molecular motion in ATPE systems is composed of advection and electrokinetic transport. The advective transport is the result of the bulk fluid flow. However, the electrokinetic transport is a result of the electrostatic interactions in the system, namely the interactions surrounding the electrical double layer (EDL). In ionic systems such as in electrophoresis, the charged particles are surrounded by a narrow shell where the concentration of counterions in the system is exceedingly high when compared to the bulk phase. The narrow shell of oppositely charged ions in the vicinity of the particle surface is referred to as the Stern layer and is typically on the order of one ionic diameter in width. The coions that are repelled from the particle surface and additional counterions from the solution form a weakly associated shell that is not electrically neutral around the Stern layer and particle. Together, the Stern and diffuse layers form a double layer of electrical charge around each particle. At a certain distance away from the particle surface within the diffuse layer there exists a boundary that corresponds to the slipping plane of the particle as shown in Figure 2.1, within which all ions act as a single entity. The electrical potential difference at the surface of shear is known as the zeta ($\zeta$) potential.
Figure 2.1: Electric Double Layer Diagram

The movement of the charged particle through the solution is opposed by the hydrodynamic friction force and the electrophoretic friction force that is caused by the movement of the oppositely charged ions in the EDL. For a uniformly charged, nonconducting particle, the resultant electrophoretic velocity is often described by the Smoluchowski equation\(^7\) given in the following form,

\[ \nu_e = \frac{\varepsilon_0 \varepsilon_r \zeta E_\infty}{\eta} \]  \hspace{1cm} (5)

\[ \nu_e = \mu_e E_\infty \]  \hspace{1cm} (6)
where \( \varepsilon_0 \) is the dielectric permittivity of a vacuum, \( \varepsilon_r \) the relative permittivity of the fluid, \( \zeta \) the zeta potential, \( \eta \) the fluid viscosity, and \( E_\infty \) the value for the applied electric field strength.

The Smoluchowski equation holds for the particles where the local radii of curvature are much larger than the thickness of the double layer\(^8,9\). Corrections to the Smoluchowski equation are included when particles possess thick or distorted double layers. By definition, the electrophoretic mobility of particles is governed by their zeta potential and is independent of the shape and size of the particle.

As previously stated, the pH of the ATPE solution markedly affects the mobility of the protein. For systems with a low pH, the nitrogen terminus and other amine groups of the protein will be protonated, which gives the protein a net positive charge. Alternatively, for systems with a high pH, the carboxylic acid groups will be ionized, hence giving the protein a net negative charge. Additionally, amino acids, polypeptides, and proteins may take on a zero net charge when the pH is balanced to the proper isoelectric point (pI) of the molecule. In systems where the pH is lower than the pI, the protein will have an overall positive charge\(^4\).

Under certain, yet common conditions, the diffuse EDL is known to be more impeded in its electrophoretic motion through the system than the related particle. The lagging diffuse layer surrounding the particle under such conditions is no longer symmetrically distributed around the particle and is said to exhibit relaxation. Electrophoretic mobility can be appreciatively reduced by relaxation-linked forces\(^9\). For systems where the product of the Debye length (\( \lambda_D \) or \( \kappa \)) and the radius of the particle (\( R \) or \( a \)) takes on an intermediate value, hence \( \lambda_D R \) or \( \kappa a \), such relaxation forces cannot be neglected. Unfortunately, virtually all electrophoresis systems with biological or biochemical particles possess \( \kappa a \) values well within the intermediate zone \( 0.1 < \kappa a < 300^9 \).

The dimensions of the modeling geometry in this investigation are 5-6 orders of magnitude larger than the typical Debye lengths for the electrolytes in ATPE of biological materials. It can therefore be assumed that the \( \zeta \)-potential does not significantly alter the external electrical field lines\(^56\).
Phase Separation

Aqueous phase separation arises from the inability of the polymer coils to penetrate into each other, often due to steric exclusion\(^6\). Thermodynamically, it is the chemical potential that governs the phase separation process. The spontaneous phase separation of incompatible polymers is often represented experimentally by the appropriate binodial phase diagram. Phase diagrams for two mixtures of PEG and dextran have been reproduced in Figure 2.2 and Figure 2.3.

**Figure 2.2**: PEG -6000 / DEX D24 Phase Diagram\(^{33}\)

**Figure 2.3**: PEG -8000 / DEX T500 Phase Diagram\(^{63}\)
Typically, lower concentrations of phase splitting polymers are required for higher molecular weight polymers. Additionally, the larger the molecular weight of the PEG, the lower the experimentally observed value of $K_0$.

Computationally, there are several methods that may be employed to model and track the fluid-fluid interface in two-phase flow systems. Valid two-phase methods must be able to capture the physically realizable steep changes in the system density and viscosity, particularly in the vicinity of the interface, and must also be computationally efficient. Such computational methods include the front tracking method, the boundary integral (BI) method, the volume of fluid (VOF) method, the Lattice Boltzmann method (LBM), the diffuse interface (DI) method, and the level set (LS) method. A full review of each computational method will not be included.

The level set method is a fairly robust numerical method utilized to solve incompressible two-phase flow with terms for surface tension as well as gravity. In the LS method, the interface is represented by the zero or in some instances the 0.5 level curve (isocontour) of a smooth global level set function ($\phi$). As previously mentioned, sharp gradients in density and viscosity exist in the vicinity of the interface and may lead to difficulties in solving the advection equations. The level set method effectively replaces the steep property gradients of the system with a single smooth function to model the interface. The LS function is designed to return certain values depending on which phase domain the method is solving for. Typically, the pure phase values of the LS function are ±1 or 0 and 1, depending on the computational scheme. The level set method is frequently employed to model moving interfaces and is useful for instances where there are two distinct fluid subdomains in the system. The popularity of the LS method is derived from the lack of regridding algorithms as utilized in other multiphase methods as well as the ability to easily calculate surface curvature. However, the unmodified level set method is known to be non-conservative in regards to the observed “phase leakage” as a result of “numerical artifacts introduced by smoothing operators at a finite grid resolution”. Phase-reinjection schemes have been developed to increase the conservation of mass within the system.

Early LS methods employed the following advection equation to describe the motion of the interface:

$$\frac{\partial \phi}{\partial t} = -\nabla (\phi \cdot \mathbf{u})$$  \hspace{1cm} (7)
where, more modern LS methods utilize the following equation with provisions for numerical stability and phase-reinjection scheme known as re-initialization\textsuperscript{77,78}:

\[
\frac{\delta \phi}{\delta t} = -\nabla (\phi \cdot \mathbf{u}) + \gamma_{ls} \nabla \cdot (\epsilon_{ls} \nabla \phi - \phi (1 - \phi) \frac{\nabla \phi}{|\nabla \phi|})
\] (8)

where \(\gamma_{ls}\) is a parameter controlling the amount of re-initialization or stabilization and \(\epsilon_{ls}\) is a parameter controlling the thickness of the interface. Both parameters \(\gamma_{ls}\) and \(\epsilon_{ls}\) require specific tuning for each two-phase investigation. Frequently, \(\epsilon_{ls}\) is linked to the largest value of the mesh size (\(h\)) and \(\gamma_{ls}\) is on the order of the maximum velocity appearing the velocity field.

In this investigation, the 0.5 isocontour represents the interface. The geometric properties of the interface that are of frequent importance are the unit normal and the local curvature. The unit normal to the interface is given by the following relation:

\[
\mathbf{n} = \frac{\nabla \phi}{|\nabla \phi|}\bigg|_{\phi = 0.5}
\] (9)

Additionally, the local curvature (\(K\)) at the interface is defined in the following manner:

\[
K = -\nabla \cdot \mathbf{n}\bigg|_{\phi = 0.5}
\] (10)

Species Balance

The equation for continuity in molar units can be easily derived by conducting a time dependant mass balance around a nonspecific fluid element. For species \(i\) in the fluid system, the corresponding equation of continuity is given by the following,

\[
\frac{\delta c_i}{\delta t} = -(\nabla \cdot \mathbf{N}_i) + R_i
\] (11)

where \(c_i\) is the molar concentration of species \(i\), \(\mathbf{N}_i\) the total molar flux of species \(i\), and \(R_i\) the allotted generation term governed by appropriate mass action kinetics. The total molar flux of each species is governed by electrokinetic, diffusive, and convective transport in all regions except across the phase boundary, where it is assumed that the convective transport is reduced to a zero value.
transport in ATPE systems, excluding the convective contribution, is governed by the electro-chemical potential. The relations describing the chemical potential are modified for systems where the electrical potential ($\Phi_{sys}$) affects species transport.

$$\mu_i = f(a_i, \Phi_{sys})$$  \hspace{1cm} (12)

The activity ($a_i$) of the chemical species is related to the concentration of species $i$ by the activity coefficient ($\gamma_i$) in the following relation:

$$a_i = \gamma_i c_i$$  \hspace{1cm} (13)

Furthermore, the fundamental motion of species $i$ is due to the differences in the electro-chemical potential, such that:

$$v_i = -\nabla \zeta \mu_i$$  \hspace{1cm} (14)

where $\zeta$ is a lumped parameter, which will be considered a constant. The derivative of the electro-chemical potential in respect to the spatial coordinate system can be related to the derivative of the concentration using the chain rule and the following partial derivative relations:

$$\nabla \mu_i = \left( \frac{\delta \mu_i}{\delta c_i} \right)_{\Phi_{sys}, \gamma_i} \nabla c_i + \left( \frac{\delta \mu_i}{\delta \gamma_i} \right)_{c_i, \Phi_{sys}} \nabla \gamma_i + \left( \frac{\delta \mu_i}{\delta \Phi_{sys}} \right)_{c_i, \gamma_i} \nabla \Phi_{sys}$$  \hspace{1cm} (15)

where,

$$\left( \frac{\delta \mu_i}{\delta \Phi_{sys}} \right)_{c_i, \gamma_i} = z_i F$$  \hspace{1cm} (16)

$$\left( \frac{\delta \mu_i}{\delta c_i} \right)_{\Phi_{sys}, \gamma_i} = \frac{RT}{c_i}$$  \hspace{1cm} (17)
and

\[
\left(\frac{\delta \mu_i}{\delta y_i}\right)_{c_i, \Phi_{sys}} = \frac{RT}{\gamma_i} \tag{18}
\]

Subsequent substitution leads to the following expression for \(v_i\):

\[
v_i = -\zeta \left(\frac{RT}{c_i} \nabla c_i + \frac{RT}{\gamma_i} \nabla \gamma_i + z_i F \nabla \Phi_{sys}\right) \tag{19}
\]

Molar flux is related to the velocity of species \(i\) in the following relation:

\[
N_i = c_i v_i + c_i v \tag{20}
\]

where \(v\) is the bulk fluid velocity.

Hence:

\[
N_i = -\zeta RT \left(\nabla c_i + \frac{c_i}{\gamma_i} \nabla \gamma_i\right) - c_i z_i F \nabla \Phi_{sys} + c_i v \tag{21}
\]

In order to transform the latter equation into a more recognizable form, the term involving the gradient of \(\gamma_i\) must be multiplied by a form of one, which in this case is \(\frac{\delta c_i}{\delta c_i}\). The benefit of performing the latter operation is the ability to make the following substitution in the resultant terms:

\[
\frac{c_i \delta y_i}{\gamma_i \delta c_i} = \frac{\delta \ln \gamma_i}{\delta \ln c_i} \tag{22}
\]

Hence:

\[
N_i = -\zeta RT \left(1 + \frac{\delta \ln \gamma_i}{\delta \ln c_i}\right) \nabla c_i - c_i z_i F \nabla \Phi_{sys} + c_i v \tag{23}
\]

The distributed term, resulting naturally in the derivation, is frequently utilized as the thermodynamic correction factor (\(\beta_i\)) to the diffusivity of a chemical species. Following the definition of the diffusion coefficient of Stichlmair et al.\textsuperscript{18},
\[ D_i = \zeta RT \beta_A \] (24)

the advection-electrokinetic migration equation for ATPE systems becomes the final expression:

\[ \frac{\delta c_i}{\delta t} = -\nabla \cdot (-D_i \nabla c_i - z_i \zeta F c_i \nabla \Phi_{sys} + c_i \nu) + R_i \] (25)

\[ \frac{\delta c_i}{\delta t} = D_i \nabla^2 c_i + z_i \zeta F (\nabla \cdot c_i \nabla \Phi_{sys}) - \nu \cdot \nabla c_i + R_i \] (26)

The term \( z_i \zeta F \) must have units of \( \frac{m^2}{s} \) in order to be consistent with the other terms appearing in the mass balance as the general electrokinetic constant \( \zeta \) possesses units of \( \frac{m^2}{V_s} \). In some instances \( \zeta \) is termed the electrophoretic mobility, denoted \( u_{mig} \) for a given species \( i \). However, as developed here, the electrophoretic mobility will refer to the \( z_i \zeta F \) term and will also possess the following equality:

\[ \mu_e = z_i \zeta F \] (27)

And thus,

\[ u_{mig} = \frac{\mu_e}{F z_i} \] (28)

In instances where the diffusivity of the charged species is unknown, the Nernst-Einstein relation is often employed:

\[ D_i = \frac{RT \mu_e}{zF} \] (29)

The latter equation is more appropriate for describing the diffusion of a charged species in the direction of the applied electrical field. The orthogonal convective flow in continuous ATPE does not give rise to the same unidirectional “drift velocity” and hence diffusivity as derived in the case of pure electrophoresis.

**Flow System**

In all investigations, fluid flow was considered to take place in the traditional control volume \( \Omega \) possessing the bounding control surface \( \delta \Omega \) and the unit outward normal defined as \( n \). The three
dimensional and time dependant velocity field was defined in the standard way as \( \mathbf{v} \) such that \( \mathbf{v}(x,t) = (u,v,w) \). System pressure and density were denoted \( p \) and \( \rho \) respectively. All fluids encountered in the investigation were considered to be Newtonian, thereby permitting the stress tensor and viscous stress tensor to become the following in terms of the velocity gradient,

\[
\sigma_s = -p + \tau_s
\]  

(30)

\[
\tau_s = \eta[\nabla \mathbf{v} + (\nabla \mathbf{v})^T] + A(\nabla \cdot \mathbf{v})
\]  

(31)

with \( \eta \) and \( A \) representing the first and second coefficients of viscosity. In this investigation, the second coefficient of viscosity will be neglected. The governing fluid dynamics were represented by the Navier-Stokes equations and have been reproduced in vector-tensor notation in the following two equations:

\[
\frac{\delta \rho}{\delta t} + \nabla \cdot (\rho \mathbf{v}) = 0
\]  

(32)

\[
\rho \frac{\delta \mathbf{v}}{\delta t} + \rho (\mathbf{v} \cdot \nabla) \mathbf{v} = -\nabla p + \nabla \cdot \tau + \mathbf{F}
\]  

(33)

where, \( \mathbf{F} \) accounts for all additional external forces. \( \mathbf{F} \) may be decomposed into the following relation:

\[
\mathbf{F} = F_s + F_g + F_b
\]  

(34)

where the subscripts \( s \), \( g \), and \( b \) correspond to forces attributed to surface tension, gravity, and additional body forces, respectively. The gravity term \( F_g \), is included only in the appropriate coordinate directions as dictated by the modeling situation. Typically, the force of gravity acts in the negative \( y \) direction for two-dimensional and in the negative \( z \) direction for three-dimensional modeling simulations. Hence, the gravity force is given by the following:

\[
F_g = \rho g
\]  

(35)

where, \( \mathbf{g} \) is the gravity vector. The interfacial surface tension force is given by the following:

\[
F_s = \sigma \mathbf{n} \delta n
\]  

(36)
Equation 36 is consistent with the mechanical definition of surface tension and the derivation of the law of Laplace\textsuperscript{98}. It is known that the latter construction for the surface tension force can lead to numerical inaccuracy since $K$ is dependent on the derivatives of $\phi$. Hence, the surface tension force may be alternatively expressed as the following:

$$F_s = \nabla \cdot (\sigma (I - (nn^T)) \delta)$$  \hspace{1cm} (37)

Lastly, the benefit of using a level set type function to describe the two-phase flow is ability to solve one set of Navier-Stokes relations. The density and viscosity terms appearing in the Navier-Stokes relations must be coupled with the level set function $\phi$ in order to model the two-phase flow. One of the methods to accomplish the latter is to make the local density and viscosity an average of pure phase values that have been weighted by the value of the local level set function. The density and dynamic viscosity therefore become the following functions of the level set function:

$$\rho = \rho_x + (\rho_y - \rho_x)\phi$$  \hspace{1cm} (38)

$$\eta = \eta_x + (\eta_y - \eta_x)\phi$$  \hspace{1cm} (39)

The equation sets utilized to describe the fluid motion in the various subsequent cases consist of the single fluid Navier-Stokes relations and the two-phase level set (LS) equations coupled with the Navier-Stokes (NS) relations. Both equation sets have been included into various modeling packages within the 3.5a version of COMSOL Multiphysics. Additionally, COMSOL has also included the Phase Field Application that utilizes the Cahn-Hilliard equation in conjunction with the Navier-Stokes relations in order to simulate two-phase flow. The COMSOL notations for the latter equation sets take the following forms:

(Incompressible) Navier-Stokes (NS)

$$\nabla \cdot \mathbf{u} = 0$$  \hspace{1cm} (40)

$$\rho (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot [ - p \mathbf{I} + \eta (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)] + \mathbf{F}$$  \hspace{1cm} (41)
**Level Set (LS) Method**

\[
\nabla \cdot \mathbf{u} = 0 \\
\frac{\rho \delta \mathbf{u}}{\delta t} + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot \left[ -p \mathbf{I} + \eta \left( \nabla \mathbf{u} + (\nabla \mathbf{u})^T \right) \right] + \mathbf{F} + \rho \mathbf{g} + \sigma \kappa \delta \mathbf{n}
\]

(40)

\[
\frac{\delta \phi}{\delta t} + \mathbf{u} \cdot \nabla \phi = \gamma \nabla \cdot \left( \varepsilon \nabla \phi - \phi (1 - \phi) \frac{\nabla \phi}{|\nabla \phi|} \right)
\]

(42)

**Phase Field Application**

\[
\nabla \cdot \mathbf{u} = 0 \\
\rho (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot \left[ -p \mathbf{I} + \eta \left( \nabla \mathbf{u} + (\nabla \mathbf{u})^T \right) \right] + \mathbf{F}
\]

(41)

\[
\psi = -\nabla^2 \cdot \varepsilon^2 \nabla \phi + (\phi^2 - 1) \phi + \frac{\varepsilon^2}{\lambda} \frac{\delta f}{\delta \phi}
\]

(44)

\[
\frac{\delta \phi}{\delta t} + \mathbf{u} \cdot \nabla \phi = \nabla \cdot \left( \frac{\gamma \lambda}{\varepsilon^2} \right) \nabla \psi
\]

(45)

where \( \frac{\delta f}{\delta \phi} \) is the \( \phi \) - derivative of the system free-energy, \( \varepsilon \) is an interface thickness parameter, \( \lambda \) is equal to \( 3 \frac{\varepsilon^2}{\sqrt{15}} \), \( \gamma \) is equal to \( X \varepsilon^2 \), and \( X \) is a mobility tuning parameter.
RESULTS AND DISCUSSION

In order to properly simulate aqueous two-phase electrophoresis, the performance of each component in the process was modeled separately before being combined in the last simulation case. The electrokinetic transport, Navier-Stokes single fluid, and level set two-phase flow equations were assessed prior to their final combination to simulate the single complete ATPE system. Many of the modeling geometries are based on the scale flow systems of Clark and coworkers. As a suitable convention the modeling geometries are orientated such that the inlets are located near the origin, the force of gravity acts purely in the negative y direction, and the principle flow channel extends lengthwise along the x coordinate.

Case 0: Electrophoretic Transport Equations

The preliminary case was designed to demonstrate a reliable and functional simulation of the electrokinetic transport equations for a single fluid. A two-dimensional rectangular geometry, measuring 0.015 m by 0.35 m, was selected as the modeling region. By convention, the inlet was located at the origin and the flow was permitted to develop in the positive x direction. Two charged species with different physical properties were analyzed simultaneously. In order to account for the electrophoretic transport, a general PDE mode was selected in COMSOL Multiphysics. Present versions of COMSOL feature equation modes designed for electrophoretic transport. However, some difficulties were encountered when utilizing the preloaded electrokinetic modes in conjunction with the conductive media mode. The general PDE mode in COMSOL solves the following equation:

$$\nabla \cdot \Gamma = F \quad (46)$$

For charged species with molar concentrations $Y_1$ and $Y_2$, the generalized PDEs possess the following respectful substitutions:

$$\Gamma_1 = -DY_{1x} - z_{elec1} C_{elec1} Y_1 \Phi_{sysx} - DY_{1y} - z_{elec1} C_{elec1} Y_1 \Phi_{sysy} \quad (47)$$

$$\Gamma_2 = -DY_{2x} - z_{elec2} C_{elec2} Y_2 \Phi_{sysx} - DY_{2y} - z_{elec2} C_{elec2} Y_2 \Phi_{sysy} \quad (48)$$
\[ F_1 = -uY_{1x} - vY_{1y} \]  \hspace{1cm} (49)

\[ F_1 = -uY_{2x} - vY_{2y} \]  \hspace{1cm} (50)

where, \( C_{\text{elec}} \) is equivalent to \( c_{\text{elec}} F \) from the earlier development. The electrical field was simulated between the upper and lower \( y \) coordinate boundaries of the modeling region by an electrical field of \( 0.05 \frac{V}{m} \). In the COMSOL generalized PDE mode notation, the electrical field is described in the following manner:

\[ \Gamma = -\Phi_{\text{sys}x} - \Phi_{\text{sys}y} \]  \hspace{1cm} (51)

\[ F = 0 \]  \hspace{1cm} (52)

For the purposes of this simulation, \( C_{\text{elec}} \) was equivalent for species \( Y_1 \) and \( Y_2 \). A summary of the COMSOL input parameters has been provided in Table 1.

<table>
<thead>
<tr>
<th>Case: Electrophoretic Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geometry</strong></td>
</tr>
<tr>
<td>Dimension</td>
</tr>
<tr>
<td>Rectangular</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Physics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation Set</td>
</tr>
<tr>
<td>Init. Cond.</td>
</tr>
<tr>
<td>Boundary Cond.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
The results from the test of the electrokinetic transport equations are well behaved and logical. For the non-interacting species, $Y_1$, the simulation correctly predicts a uniform molar concentration throughout the modeling geometry as shown in **Figure 3.01**.

![Figure 3.01: Non-interacting Particle Under Electrokinetic Transport Equations](image1)

Species $Y_2$, possessing a negative electrical charge, is correctly drawn to the simulated upper positive electrode as shown in **Figure 3.02**. The electrokinetic parameters utilized undoubtedly suggest a much quicker confinement of species $Y_2$ to the upper wall than observed in standard protein electrophoresis. Nevertheless, the results presented in this case are easily reproducible and demonstrate clearly the correct functionality of the electrokinetic equations.

**Case 1: Pressure Boundary Test I**

The first flow system test case was designed to demonstrate the functionality of the pressure boundary at the inlet. In the ATPE system of Clark, the outlet ports are directly connected to individual pumps, while the inlet is a pressure boundary dictated by the fluid height in an open, continuously stirred tank. The modeling geometry is similar to the preliminary electrokinetic equation test case with the following...
modifications to the system summarized in Table 2. The outlet velocity was selected to simulate a $6 \frac{mL}{min}$ pumping rate.

<table>
<thead>
<tr>
<th>Case: Pressure Boundary Test I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geometry</strong></td>
</tr>
<tr>
<td>Dimension</td>
</tr>
<tr>
<td>Rectangular</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Properties</strong></td>
</tr>
<tr>
<td>Density = 998.2 kg/m$^3$</td>
</tr>
<tr>
<td><strong>Physics</strong></td>
</tr>
<tr>
<td>Equation Set</td>
</tr>
<tr>
<td>Init. Cond.</td>
</tr>
<tr>
<td>Boundary Cond.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Table 2: Pressure Boundary Test Case Simulation Summary

The results for this test case are also well behaved and well anticipated as suggested by the velocity surface plot in Figure 3.10. The parabolic velocity profile that is shown is predicted from classical fluid mechanics.

Figure 3.10: Velocity Profile For First Pressure Boundary Test Case
Case 2: Pressure Boundary Test II

The second pressure boundary test case was provided in an effort to simulate the tri-exit port ATPE system of Clark as an extension of the first pressure boundary case. The modeling geometry of the second case is identical to the first, with the exception of the three, properly scaled outlet ports. Table 3 summarizes the COMSOL input parameters for the second pressure boundary test. Additionally, the exit velocity of each port was set to reflect a $2 \text{ mL/min}$ flow rate.

<table>
<thead>
<tr>
<th>Case: Pressure Boundary Test II</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geometry</strong></td>
<td><strong>Density</strong> = 998.2 kg/m$^3$</td>
</tr>
<tr>
<td>Dimension</td>
<td>Viscosity = 0.001 Pa*s</td>
</tr>
<tr>
<td>Rectangular w/ tri exit ports</td>
<td></td>
</tr>
<tr>
<td>Mesh</td>
<td>Triangular</td>
</tr>
<tr>
<td>1116</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Physics</strong></th>
<th><strong>Equation Set</strong></th>
<th>Incompress. NS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Init. Cond.</strong></td>
<td>u, v, P = 0</td>
<td></td>
</tr>
<tr>
<td><strong>Boundary Cond.</strong></td>
<td>Inlet: $P_0 = 101325$ Pa</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Each Outlet: $U_0 = 0.004716$ m/s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Walls: No slip</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Pressure Boundary Test Case 2 Simulation Summary

Once again, the results presented in Figure 3.20 demonstrate a properly functioning set of NS equations that give rise to a parabolic velocity profile for each section of the modeling region. One of the important features to note is the existence of a potential mass accumulation trap in the corners near the outlet ports. For charged species held tightly near the upper and lower walls by the electrical field, the configuration of the outlet ports may prevent such species from exiting the device.
Case 3: Pressure Boundary Test III

A scale simulation of a single fluid flow through the ATPE device of Clark was carried out in the third pressure boundary test case. The physical properties of the simulate fluid are representative of the PEG-rich phase despite the fluid density being similar to that of pure water. The modeling region for the third test case maintains the same $x$ length coordinate as the second test case. However, in the third case the height dimension is mapped to the $z$ coordinate, while the width dimension is mapped to the $y$ coordinate. The flow device features a circular inlet cut into the rectangular face and a tapering in the width coordinate near the tri-port outlet. A summary of the simulation parameters and the flow system results may be found in Table 4 and Figure 3.30 respectively.

![Figure 3.20: Velocity Profile for Second Pressure Boundary Test Case](image)

| Case: Pressure Boundary Test III |
|---|---|
| **Geometry** | **Properties** |
| Dimension | Mesh | Density = 998.2 kg/m$^3$ | Viscosity = 0.001 Pa*s |
| Rectangular Tapered Solid w/ tri exit ports | Tetrahedral |  |
|  |  | 1681 |

**Physics**

<table>
<thead>
<tr>
<th>Init. Cond.</th>
<th>Boundary Cond.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation Set</td>
<td>Incompress. NS</td>
</tr>
<tr>
<td>$u, v, P = 0$</td>
<td>Inlet: $P_0 = 101325$ Pa</td>
</tr>
<tr>
<td></td>
<td>Each Outlet: $U_0 = 0.000513$ m/s</td>
</tr>
<tr>
<td></td>
<td>Walls: No slip</td>
</tr>
</tbody>
</table>

Table 4: Pressure Boundary Test Case 3 Simulation Summary
Figure 3.30: Velocity Profile for Third Pressure Boundary Test Case

Analysis of the flow system solution reveals no erroneous finding, despite the low number of tetrahedral elements. The speed and stability of the solution for this test case provide cause to extend the two-phase flow modeling regimes to the third dimension. The effect of the tapering scheme on the single fluid flow is most pronounced in the latter third of the tapered region as the velocity of the core flow begins to double in a relatively short length.

Case 4: Level Set Test I

The predominant flow system geometry from the latter cases has featured a single inlet stream. In order to provide the theoretical mass required for a two-phase system in a single inlet port device, the initial inlet boundary would have to be of the well-mixed condition. Assuming that the difficulties of utilizing such a mixed boundary condition in COMSOL were elucidated, the two-phase flow regimes in these instances would require long lengths of pipe to spontaneously separate. Well-mixed solutions of dextran, PEG, and water at rest are known to require several minutes, potentially several hours, to completely separate into the $\alpha$ and $\beta$ phases. Thus, as an adequate test of the functionality of the LS equations, the inverse problem, the interaction of the two pure phases in the mixing zone of a pipe junction, was investigated.
Correct implementation of the LS equations is critical for accurate results. Unfortunately, some difficulty was encountered getting the LS equations coupled with the NS relations to converge to a final solution. Firstly, the size of the mesh elements contributes greatly to the accuracy and solution time of the simulation. The LS method employed in COMSOL was based on a fixed-mesh grid. As such, in areas where the number of mesh elements is lower, the fine details of the two-phase interface may be lost or incorrectly distorted. The accumulation of such errors from each time-step to the next is likely to advance the LS equations to erroneous or failed results. Secondly, the LS equations must be initialized or perhaps “seeded” in order to solve time dependant problems. Initialization permits the LS equations to develop the first attempt at the two-phase system without the need to satisfy the NS relations. The time step selected to initialize the LS equations is typically on the order of $10^{-4}$ seconds and nonzero for systems where the average core velocity is less than $0.001 \frac{m}{s}$. For some systems encountered, the initialization time had to be decreased to $10^{-5}$ seconds. Thirdly, the LS tuning parameter $\gamma$, requires a bit of attention. The parameter $\gamma$ possesses units of $\frac{m}{s}$ and is related to the local interfacial speed. Typically, converging and time efficient solutions are obtained when the $\gamma$ parameter is set to slightly less than the maximum core velocity of the system. Fourthly, the interfacial thickness parameter $\varepsilon$ is another adjustable quantity requiring attention. The default setting in COMSOL for the $\varepsilon$ parameter, $h_{max} chns * 0.5$, relates the local mesh height, $h$, to the local interfacial thickness. For many of the simulations carried out in this investigation, this default setting has worked well. Lastly, the LS equations tend to perform better when the initial pure phase regions in the modeling geometry are larger. The LS application mode in COMSOL requires that the initial conditions on the interior of the modeling geometry be of a pure phase. Simulations tend to fail or have great difficulty with boundaries or regions that are specified as the interface. As a recommendation, modeling geometries implementing the LS equations should be constructed with regions for the initial pure phase material to occupy that possess length and width approximately one forth the diameter of the modeling system. The latter provision attempts to keep such initial pure phase regions from becoming thin slices that tend to lack the adequate “seed mass” that the LS equations require to initialize and converge.

In the first test case of the LS equations a “Y” type junction was investigated. The main flow channel was rectangular and initially consisted of a pure PEG-like phase. The dextran-like inlet port was trapezoidal and located on the top, angling downward into the main body of the flow channel. An additional smaller rectangular space was added to the lower left of the main channel in order to provide an adequate amount of “seed mass” for the PEG-like inlet port. The physical properties of the simulated system are
not true to PEG, dextran, and water systems. The viscosity values were modeled accurately. However, the density values are only five percent of the actual values. The surface tension for similar PEG, dextran, and water systems is known to be on the order of $10^{-6} \frac{N}{m}$. As a first test case investigation, it was deemed acceptable to model the surface tension coefficient with a zero value. Inlet velocities were set to higher values than in previous cases in an effort to test the LS formulation for irregularities in the interface solution. A summary of the initial simulation setup has been reproduced in Table 5.

<table>
<thead>
<tr>
<th>Case : Level Set Test I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geometry</strong></td>
</tr>
<tr>
<td>Dimension</td>
</tr>
<tr>
<td>Modified Y Junction</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Physics**

<table>
<thead>
<tr>
<th>Equation Set</th>
<th>Level Set</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Init. Cond.</strong></td>
<td>u, v, P = 0</td>
</tr>
<tr>
<td><strong>Boundary Cond.</strong></td>
<td>Lower Inlet: $u_0 =$ 0.75 m/s</td>
</tr>
<tr>
<td></td>
<td>Upper Inlet: $U_0 =$ 1.0 m/s</td>
</tr>
<tr>
<td></td>
<td>Walls: No slip</td>
</tr>
<tr>
<td></td>
<td>Outlet: $P_0 =$ 101325 Pa</td>
</tr>
<tr>
<td><strong>Additional</strong></td>
<td>$g_y =$ -9.81</td>
</tr>
</tbody>
</table>

*Table 5:* Level Set Test Case 1 Simulation Summary
The results for the first LS test case, shown in Figure 3.40, feature a velocity arrow plot superimposed upon a gradient plot of the LS function variable $\phi$. Pure regions are indicated by $\phi$ values of 1 for the PEG-like phase and 0 for the dextran-like phase, while the interface is represented by the 0.5 functional value. The prediction of the velocity profile beyond the mixing region is consistent with the velocity profile derivation of Bird, Stewart, and Lightfoot from fundamental fluid mechanical principles as shown in Figure 3.41.

**Figure 3.40:** Velocity Profile and Phase Distribution in Level Set Test Case 1

**Figure 3.41:** Theoretical Adjacent Immiscible Two Fluid Flow Velocity Profile

It has been shown that in the case of a pressure-induced, two-phase flow with a planar interface, the velocity profiles for the $\alpha$ and $\beta$ phases, $v^\alpha$ and $v^\beta$, take on the following theoretical relations:

\[
v^\alpha = \frac{(p_0 - p_L)b^2}{2\mu^\alpha L} \left( \frac{2\mu^\alpha}{\mu^\alpha + \mu^\beta} b \left[ x - \frac{x^2}{b} \right] - \frac{x^2}{b^2} \right) \tag{53}
\]

\[
v^\beta = \frac{(p_0 - p_L)b^2}{2\mu^\beta L} \left( \frac{2\mu^\beta}{\mu^\alpha + \mu^\beta} b \left[ x - \frac{x^2}{b} \right] - \frac{x^2}{b^2} \right) \tag{54}
\]
Perturbations in the interface located in the mixing zone of the flow device appear to be inconsistent with experimental flow system results. Perturbations and phase-boundary instabilities have been shown to become more prominent in micro-devices with large aspect ratios and high electric fields\textsuperscript{56}. However, the latter simulation was conducted in the absence of an electric field and carried out in a device several orders of magnitude larger than a typical micro-device. Additionally, the densities and inlet velocities are not representative of the experimental PEG, dextran, and water system.

Case 5: Level Set Test II

The second test of the level set equations was carried out in a rectangular channel of length 0.3 m and height of 0.03 m with a lower rectangular inlet port. Initially, the channel was filled with the heavier, more viscous $\beta$ phase before it was pushed out by the lighter $\alpha$ phase entering into the system through the lower inlet port. The objective for this case was the shape determination and behavior of the advancing interface as the lighter phase replaced the static and denser phase. The physical properties once again have been selected in order to obtain a properly converged solution, though the target property set still remains to be consistent with a PEG, dextran, and water system. A summary of the input parameters for the second level set test has been reproduced in Table 6.
Contrary to the latter level set case, the surface plot for the second case, reproduced in Figure 3.50, represents the pure viscous phase by the 1.0 functional value of $\phi$. The advancing interface front is smooth and parabolic. Additionally, the results do not suggest any visible instability along the two-phase boundary.

![Figure 3.50: Lighter Phase Displacement in Level Set Test Case 2](image)

**Table 6: Level Set Test Case 2 Simulation Summary**

<table>
<thead>
<tr>
<th>Geometry</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimension</td>
<td>Mesh</td>
</tr>
<tr>
<td>Rectangular w/</td>
<td>Triangular</td>
</tr>
<tr>
<td>lower rect. Inlet</td>
<td></td>
</tr>
<tr>
<td>Density$_1$ = 1 kg/m$^3$</td>
<td>Viscosity$_1$ = 0.5 Pa*s</td>
</tr>
<tr>
<td>Density$_2$ = 2 kg/m$^3$</td>
<td>Viscosity$_2$ = 1.5 Pa*s</td>
</tr>
<tr>
<td>Surface Tension</td>
<td></td>
</tr>
<tr>
<td>= 0.05</td>
<td></td>
</tr>
</tbody>
</table>

---

**Physics**

<table>
<thead>
<tr>
<th>Equation Set</th>
<th>Level Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Init. Cond.</td>
<td></td>
</tr>
<tr>
<td>$u, v, P = 0$</td>
<td>For main tube: Phi (t=0) = 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Boundary Cond.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Inlet: $u_0 = 1.0$ m/s</td>
</tr>
<tr>
<td>Phi$_0 = 0$</td>
</tr>
<tr>
<td>Walls: No slip</td>
</tr>
<tr>
<td>Outlet: $P_0 = 101325$ Pa</td>
</tr>
<tr>
<td>Additional</td>
</tr>
<tr>
<td>$Y = 1$ m/s</td>
</tr>
<tr>
<td>$e = hmax_chns*0.5$</td>
</tr>
</tbody>
</table>
The force of gravity, acting in the positive y direction in this case, appears to functioning correctly. In early solution times, the velocity profile centerline of entering lighter phase does migrate upward to the center of the flow channel. In later solution times, the mass of the denser phase near the horizontal walls is greater for the upper boundary than the lower boundary. The latter is consistent with the described sense of gravity. At infinite time, the simulation does predict the complete expulsion of the denser phase from the flow channel.

**Experimental Two-Phase Flow System**

In order to validate the functionality and accuracy of the level set method as implemented in COMSOL, a series of scale flow devices were constructed in the Bio-Separations Lab in Goddard Hall. Two similar flow systems were constructed from “T” and “Y” glass junctions measuring one-half inch in diameter. Clear acrylic one-half inch diameter tubing was joined to the outlet of each junction using Tygon connectors. In both systems the two-phase flow was allowed to develop in a minimum of eight inches of straight tubing. Each flow system was oriented such that the PEG and two-phase flows were parallel to the ground and hence orthogonal to the force of gravity. A photograph of the experimental setup has been provided in Figure 3.60.
The inlet flows were maintained by two Micropumps, one for each of the separated phases. Each Micropump was calibrated using the PEG – rich phase as the test fluid. Volumetric flow rates were measured as a function of the maximum power supplied to the pump. The respectful calibration curves have been reproduced in Figure 3.61 and Figure 3.62.
In order to be consistent with the COMSOL simulations, the dextran-rich phase was introduced into the flow devices through the top inlet using the “B” Micropump.

The two-system was prepared using dextran and PEG of molecular weights 282,000 and 8,000, respectively. A solution of 1000 mL of distilled water, 74.01 g of dextran, and 39.02 g of PEG was well
mixed and permitted to separate for 12 hours while refrigerated. The top and bottom phases were then collected using pipettes and stored in separate flasks.

For the case of the “Y”-junction, the inlet dextran-rich phase flow was set at 18 mL/min, while the PEG-rich phase was set at 10.4 mL/min. The PEG-rich flow was permitted to develop and fill the entire flow device before the introduction of the dextran-rich phase. Direct photographic measurement of the shape and location of the interface in the mixing zone was not reliable with the available digital camera. Ideally, a dye or stained protein that favors one phase over the other or perhaps Schlieren-type photography should have been used to demarcate the two phases. The initial qualitative experimental fluid interface in the mixing zone of the “Y” device has been reproduced in **Figure 3.63**.

![Figure 3.63: Experimental Y-Junction Interface](image)

One of the unique features of the initial interface is the distinct “heel-shape” that arises in the mixing zone of the “Y” and “T” junctions. A similar initial interfacial curve is noted in the “T” junction when the dextran and PEG inlet flow rates are 21 mL/min and 12 mL/min respectively:
As shown in Figure 3.64, the dextran phase constricts the flow of the PEG phase significantly both inside and outside the mixing zone than in the first case.

**Simulated Two-Phase Flow System**

Two COMSOL simulations were conducted to model the experimental flow systems. Experimental geometries were reproduced faithfully in COMSOL with the exception of a deviation in the total length of tubing after the mixing region and a deviation in the system densities. The length of the channel following the mixing region was shortened to 0.1524m in an effort to reduce the computation time required to have both phases exiting the flow device. In the experimental system, the PEG-rich flow was allowed to develop before the dextran-rich phase was introduced into the flow device. A similar effect was designed into the COMSOL simulation. Secondly, the system densities were thirty percent the experimental values, though the simulated viscosities were accurate. The surface tension component was once again reduced to a zero value. Additionally, the modeling geometry features a smaller inlet to reflect the 0.003175m diameter barbed adapter fitting at each inlet and outlet.

The input parameters to each “Y” and “T” junction simulation have been summarized in Table 7 and Table 8 respectively. In an effort to assist the numerical solver, new initial conditions specifying the velocities in the interior of the flow device were included. In prior simulations all interior initial velocities and pressures were set to zero. Furthermore, the number of mesh elements was greatly increased in each simulation to reduce irregularities along the phase boundary. As a consequence to the increased
number of mesh elements, the computational time required to solve for 10 second of simulated flow was greatly increased to 22.4 hours for the “Y” junction and 7.8 hours for the “T” junction.

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Mesh Density</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300 kg/m³</td>
<td>0.007 Pa*s</td>
</tr>
<tr>
<td>Modified Y Junction</td>
<td>Triangular Density</td>
<td>Viscosity</td>
</tr>
<tr>
<td>2</td>
<td>400 kg/m³</td>
<td>0.332 Pa*s</td>
</tr>
</tbody>
</table>

Table 7: Experimental Y-Junction Simulation Summary

<table>
<thead>
<tr>
<th>Physics</th>
<th>Level Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation Set</td>
<td>Init. Cond.</td>
</tr>
<tr>
<td>Upper ( U_0 ), ( P = 0 )</td>
<td>For lower tube: ( \Phi(t=0) = 0 )</td>
</tr>
<tr>
<td>Lower ( u_0 = 0.03 ) m/s</td>
<td>For upper tube: ( \Phi(t=0) = 1 )</td>
</tr>
<tr>
<td>Boundary Cond.</td>
<td></td>
</tr>
<tr>
<td>Lower Inlet: ( u_0 = 0.03 ) m/s</td>
<td>( \Phi_0 = 0 )</td>
</tr>
<tr>
<td>Upper Inlet: ( U_0 = 0.05 ) m/s</td>
<td>( \Phi_0 = 1 )</td>
</tr>
<tr>
<td>Walls: No slip</td>
<td></td>
</tr>
<tr>
<td>Outlet: ( P_0 = 101325 ) Pa</td>
<td></td>
</tr>
</tbody>
</table>

The development of the two-phase flow in the “Y” device has been illustrated in Figure 3.70 through Figure 3.77. Figure 3.71 and Figure 3.72 suggest that the formation of the “heel” shaped interface is more a feature of the early two-phase flow development than a steady-state interface. The simulated interface also extends very deep into the \( \alpha \) - phase flow, significantly confining it to the bottom of the main flow channel at several time instances.
Figure 3.70: Simulated Time Evolution of Y-Junction Interface 0.67 s

Figure 3.71: Simulated Time Evolution of Y-Junction Interface 0.95 s

Figure 3.72: Simulated Time Evolution of Y-Junction Interface 1.4 s

Figure 3.73: Simulated Time Evolution of Y-Junction Interface 1.8 s
Figure 3.74: Simulated Time Evolution of Y-Junction Interface 2.4 s

Figure 3.75: Simulated Time Evolution of Y-Junction Interface 3 s

Figure 3.76: Simulated Time Evolution of Y-Junction Interface 6 s

Figure 3.77: Simulated Time Evolution of Y-Junction Interface 12 s
The breakup of the $\beta$ - phase in the intermediate Figure 3.74 and Figure 3.75 is only postulated here and was not substantiated in an actual experiment. However, the latter time instants correctly suggest the development of a two-phase flow dominated by the $\beta$ - phase across the diameter of the flow device. The results from the “T” junction simulation, displayed in Figure 3.78, are more consistent with the experimental observations. The simulated flow system, similar to the experimental, features a $\beta$ – phase that nearly constricts the $\alpha$ - phase to a thin region on the bottom of the channel. The extent of the constriction of the $\alpha$ – phase is correctly predicted to be greater in the “T” junction than in the “Y” junction. Intermediate solution times suggest that the interfacial boundary breaks up as the two-phase flow develops. However, the extent of the simulated interfacial boundary break-up is not as severe as predicted in the “Y” junction. The boundary break-up in the “Y” junction gives rise to the development of a lagging “tail” of the $\beta$ – phase near the bottom of the flow channel.
Table 8: Experimental T-Junction Simulation Summary

The simulated interface in the mixing zone for both the “Y” and “T” junction simulations is predicted to partially climb the curved wall near the joint of the β – phase inlet stem with the α – phase inlet channel. The latter behavior was difficult to clearly capture experimentally with the curvature of the glass near the junction of the inlet stems.
Nevertheless, the latter simulations of the experimental flow systems greatly suggest the validity of image captured for the “T” junction in the laboratory. Originally it was thought that the bubbles collecting on the inner glass wall in the mixing zone of the experimental “T” junction were not deposited along the exact interface. The latter simulations suggest that the curve formed by the inner gas bubbles may have been a much more reliable steady-state reference of the interface.
Figure 3.79 is the raw image captured in the laboratory for the two-phase flow in the “T” junction. The gas bubbles on the inside of the glass tubing lie very closely to the observed interface, developed after several minutes of fluid flow. An additional image appears in Appendix V with the red level increased to better depict the two-phase interface.

Complete ATPE Simulation

In the concluding set of COMSOL simulations, the electrokinetic transport equations were linked with the Navier-Stokes and level set equations to simulate a complete ATPE system. One of the difficulties in directly coupling the mass transport equations with the flow relations is the fact that certain charged species exhibit a preference for one phase over the other. The unequal partitioning of proteins and other charged species requires an additional condition, which ultimately leads to a jump-discontinuity in the concentration profile across the interface. In order to remedy the discontinuity in the species concentration, the assumption of an instantaneous equilibrium across the interfacial boundary or the use of a mass transfer expression have been suggested as plausible boundary conditions\textsuperscript{94,95}. In the context of the level set method, the additional species boundary condition would have to be applied at
the $\phi = 0.5$ level set. Imposing such a condition requires a system of $\phi$–dependant mass transport equations. The latter methodology is complex and may lead to deleterious numerical instability, especially with the viscous fluids utilized in the system.

An alternative methodology was carried out in this investigation. First, the level set equations were solved in order to obtain the steady-state location of the fluid-fluid interface. Next, the interface was redrawn in the flow geometry as an interior boundary to create two subdomains, one for each distinct phase, and the level set equations turned off. The unmodified Navier-Stokes relations were then applied to each subdomain with physical properties representative of the PEG–rich and dextran–rich phases. Finally, electrokinetic equations were coupled to the NS relations in the modified flow geometry and subsequently solved. The sequential solving methodology was implemented to reduce computational time. It was also perceived logical to solve for the fluid-fluid interface geometry at a steady-state since it would most reflect a continuously running ATPE system with fully developed fluid flow.

The rectangular flow channel with three equally sized outlet ports from Case 2 was selected as the modeling geometry. Two rectangular inlet ports measuring 0.003 m in length were added to the main flow channel geometry in an effort to provide the “seed mass” for both phases in the implementation of the level set equation. The flow channel possessed a principle length of 0.23 m, excluding the length of the inlets and outlets, and a principle height of 0.015 m. Initially, pure $\alpha$-phase was introduced into the system by the upper inlet at $0.05 \text{ m/s}$ and conversely pure $\beta$-phase was introduced via the lower inlet at $0.06 \text{ m/s}$. The pressure at each of the three exit ports was set at 101325 Pa. Due to some numerical difficulties, the densities of the $\alpha$–phase and $\beta$–phase were 100 $\text{kg m}^{-3}$ and 200 $\text{kg m}^{-3}$ respectively. Phase viscosities were correctly modeled as 0.007 Pa*s and 0.332 Pa*s respectively. A 9,968 element mesh was utilized to solve for the first 20 seconds of simulated fluid flow. The level set function was initialized at $5*10^{-5}$ seconds using a relative tolerance of 0.001 and an absolute tolerance of 0.0001. The stable solution of the level set equations for the given geometry is depicted in Figure 3.81 with the interface denoted by the black line.
Predominately, the results suggest the formation of a planar interface that is established in the upper third of the flow channel. The latter observation is a bit surprising given the presence of the gravitational force acting in the negative $y$ direction and nearly equal pure phase inlet cross sections and velocities. Ultimately, it is the location of the two-phase mixture on its respectful phase diagram that determines the phase volumes. Theoretically, it is plausible to prepare a two-phase mixture that separates into two equal volumes in the flow system. The curvature depicted in the interface near the inlet and outlet ports in Figure 3.81 appears to be the consequence of the particular design of the flow system and not common to all two-phase systems with similar physical properties.

Ideally, the next component of the complete ATPE model would be the simulation of the electrokinetic transport equations in a flow system divided into two subdomains determined by the level set location of the interface. Several simulations were conducted utilizing the exact shape of the interface provided by the level set equations as the interior boundary. However, many of the simulations failed before reaching 1.5 seconds of total simulation time. It was then decided to utilize a completely linear interface down the length of the flow channel in order to avoid failed solutions or solutions requiring time steps on the order of $10^{-8}$ seconds. A sample COMSOL solver log for simulations where the solution to the electrokinetic transport equations using the curved interface was attempted has been included in Appendix VI.

The implementation of the linear interface in subsequent complete ATPE simulations removed the convergence difficulties encountered in the aforementioned initial attempts. In the final complete ATPE simulations, four equation sets were required to model the transport of a single charged species. Additional species would require two generalized PDE modes each, with one mode for the concentration of the species in the $\alpha$ – phase and another mode for the concentration in the $\beta$ – phase.
A subdomain expression was included in the simulation to account for the total concentration of a charged species, $c_t$, in either phase. $c_t$ was assigned to be equal to $c_a$ in the upper subdomain and to be equal to $c_b$ in the lower subdomain. A single PDE mode was deemed sufficient to describe the electrical field encountered by all species. A single Navier-Stokes mode was employed to provide the velocity field in the subdomains created by the redrawn interface. The physical properties of the $\alpha$ – phase and $\beta$ – phase subdomains were consistent with actual PEG – rich and dextran-rich phases encountered in aqueous two-phase systems. The electrophoretic mobility values were modeled after the values reported by Levine and Bier$^{42}$ for human hemoglobin in a 10 mM ammonium acetate buffer. Species diffusivity values were estimated from the mobility values using the Nernst-Einstein relation. The implemented partition coefficient is representative of partition coefficients for hemoglobin, but is not exact for the given type of hemoglobin and given two-phase system. The partition coefficient was however selected to demonstrate a distinct preference of the charged species for the $\beta$ – phase. The 150 V potential utilized is representative of a 100 $\frac{V}{cm}$ electrical field in the modeling geometry. Generally, an electrical field of 100 $\frac{V}{cm}$ is the upper limit in experimental ATPE systems. A summary of the last, full ATPE simulation is presented in Table 9.
The total solve time was reduced when the NS equations were solved simultaneously with the electric field relations before the mass transfer equations were activated. For the complete ATPE simulation represented in Table 9, the simultaneous solution of the NS equations and electrical field relations for 20 total seconds of simulation time required 4,574 seconds to solve. Similarly, the numerical solver required 5,728 seconds to solve the pair of mass transport equations for a single charged species. Plots of the electrical potential and velocity field for the complete ATPE simulation have been reproduced in Figure 3.82 and Figure 3.83 respectively.
The electrical potential steadily decreases across the main cross section of the height, $y$, coordinate. Figure 3.82 suggests the penetration of equipotential lines into the inlet and outlet ports, despite the potential difference being applied only between the upper and lower horizontal surfaces of the principle flow channel. The velocity field for the complete ATPE simulation in Figure 3.83 demonstrates the effect of imposing the same velocity at each of the three exit ports in the two-phase system assuming that the phase volumes remain constant in the main flow channel for all times. Despite the active viscous and dense properties of the $\beta$ – phase subdomain near the lower two exit ports, the solution to the NS relations suggests that the local $\beta$ – phase fluid velocity rapidly increases to meet the boundary conditions. Streamline data suggest that charged species present in the $\alpha$ – phase may exit the flow device via any one of the exit ports by convection alone unless the supplied electrical potential is sufficient to hold the species near the upper horizontal wall of the channel.

Velocity cross sections were plotted from the inlet, center, and exit of the flow channel at a total simulation time of 20.0 seconds. Figure 3.84 represents the developing velocity cross section near the entry ports of the channel, where the maximum velocity in the $\alpha$ – phase is fifty percent greater than
the maximum velocity in the center of the flow device. The velocity cross section from the center of the flow device in Figure 3.85 exhibits excellent agreement, less than seven percent error, with the theoretical velocity profiles provided by (53) and (54). The theoretical profile for fully developed immiscible adjacent fluid flow in Figure 3.86 was generated using the phase viscosities and flow device length from Table 9 and the pressure differential calculated between the inlet and exit ports provided by the COMSOL simulation. The velocity profile near the exit ports in Figure 3.87 suggests that the bulk fluid flow takes on a fluid velocity that is the mean of the two pure phases.

![Figure 3.84: Entry Velocity Profile in Triple Outlet Device](image-url)
**Figure 3.85:** Mid-channel Velocity Profile in Triple Outlet Device

**Figure 3.86:** Theoretical Two-Phase PEG-DEX Velocity Profile
One of the motivations to utilize two PDE modes to describe the mass transport of a single charged species was the need to implement the “stiff-spring” boundary condition at the interface. As previously discussed, the unequal partitioning of charged species at the fluid-fluid interface creates a discontinuity in the concentration profile. In COMSOL, one of the easiest ways to specify the flux on each side of an interior boundary is to deactivate a PDE mode in a neighboring subdomain. In such instances, the interior boundary is actually treated in each of the PDE modes as an interior-exterior boundary. The latter permits the specification of the single-sided flux directly. The interior boundary conditions for single PDE modes are specified by the difference in the fluxes from the adjacent subdomains. The “stiff-spring” boundary condition also forces the flux across the interface to be continuous. Several attempts were made in a single PDE mass transport mode to mimic the results obtained with a pair of PDE modes. Unfortunately, the proper flux difference in the single PDE mode was not found. A representative concentration profile taken across the interface in the center of the flow channel is provided in Figure 3.88, where the jump-discontinuity is accurately depicted.
The migration of the charged species through the flow device was simulated for twenty seconds of total time in two separate cases. In the first case, the applied electrical potential was 60 V in order to simulate an electrical field of $40 \, \frac{V}{cm}$, whereas in the second case a 150 V potential was applied to simulate a $100 \, \frac{V}{cm}$ electrical field. Additionally, the first case was meshed with 25,000 elements, whereas 52,000 elements were implemented in the second case. The resolution of the concentration profile is greatly enhanced in the second case predominately due to the greater number of mesh elements, especially near the interface. Figure 4.0 through Figure 4.5 and Figure 5.0 through Figure 5.5 depict the time evolution of the theoretical species concentration for the two complete ATPE simulation cases.
Figure 4.0: Complete ATPE Simulation Concentration Profile at 60 V, 0.05 s

Figure 5.0: Complete ATPE Simulation Concentration Profile at 150 V, 0.05 s

Figure 4.1: Complete ATPE Simulation Concentration Profile at 60 V, 0.3 s

Figure 5.1: Complete ATPE Simulation Concentration Profile at 150 V, 0.3 s
Figure 4.2: Complete ATPE Simulation Concentration Profile at 60 V, 1.2 s

Figure 5.2: Complete ATPE Simulation Concentration Profile at 150 V, 1.2 s

Figure 4.3: Complete ATPE Simulation Concentration Profile at 60 V, 2 s

Figure 5.3: Complete ATPE Simulation Concentration Profile at 150 V, 2 s

Figure 4.4: Complete ATPE Simulation Concentration Profile at 60 V, 3 s

Figure 5.4: Complete ATPE Simulation Concentration Profile at 150 V, 3 s
After five seconds of total simulation time, the negative effects of the sharp corner at the $\alpha$ – phase inlet begin to cause localized regions of negative concentration to appear. Neither the 60 V, nor the 150 V electrical potential was sufficient to hold the charged species to the upper horizontal wall of the flow device as demonstrated in Case 0. Similarly, at twenty seconds of total simulation time the sharp corners near the exit ports appear to contribute to the appearance of localized regions of extremely high species concentration. The remedies to the latter complications may be additionally linked to the number of mesh elements and the numerical tolerance.

The simulation results presented at 7.0 seconds in Figure 5.5 suggest that the first particles of the charged species that reach the end of the flow device will exit through the middle port, mostly as a result of the velocity profile and the preference of the species for the $\beta$ – phase. The charged species initially in the $\beta$ – phase appear to exhibit an upward velocity as a result of the similar convective and electrokinetic transport magnitudes.

The assumption to utilize the pure phase properties in separate subdomains delineated by the solution to the level set equations was evaluated in the last set of simulations. The same triple outlet port geometry previously described was utilized in a series of level set and electrokinetic transport simulations. The interface location was found in the same manner required to generate Figure 3.81. The fundamental difference in the implementation of the mass transport equations in the evaluation of the latter assumption is the specification of the velocity boundary condition at both inlet ports instead of the pressure. The COMSOL simulation summary for the evaluation of the latter assumption is similar to
the details presented in Table 9, except that the densities of the pure phases were reduced to $300 \frac{kg}{m^3}$ and $400 \frac{kg}{m^3}$ in order to be more consistent with the parameters utilized to solve for the interface in the implementation of the level set equations. The details of the evaluation of the fundamental assumption for the complete ATPE simulation have been reproduced in Appendix IX. There is strong evidence to suggest that the specification of two adjacent immiscible fluids using a single NS equation set modeled in two subdomains with pure phase physical properties in each does not make the velocity field similar to that of the level set derived case. The results suggest that the parameter controlling the interfacial thickness in the level set formulation may be too large when left to the default settings.
CONCLUSION

A comprehensive review of prior works and development of transport equations pertinent to aqueous two-phase electrophoresis was conducted. Additionally, COMSOL Multiphysics was implemented in some of the first macroscale simulations of ATPE systems in two dimensions. Furthermore, the utility and performance of the level set method application to ATPE systems were also assessed.

The complete simulation of ATPE systems requires the validation of several components, ranging from the mechanism selected to describe immiscible two-phase flow to the description of the interior boundary flux conditions that give rise to a discontinuous species concentration across the interface. The intent of this investigation was to demonstrate the performance of all the components of ATPE systems in COMSOL before they were combined in a single simulation. The majority of the ATPE experiments at present have been carried out in microdevices. Very few modeling investigations of ATPE systems have been conducted. The most probable reason for the latter is linked to the difficulty of modeling time dependent viscous, two-phase flow.

It is evident from this investigation that the ATPE flow system geometry and inlet velocities greatly affect the separation efficacy. Certain orientations of outlets may actually lead to the unwanted convective mixing of separated particles. Additionally, the inlet flow rates to the ATPE system must be properly adjusted to ensure that the convective motion does not negate the electrokinetic motion. The use of exceedingly powerful electrical fields in ATPE systems with fluid velocities may not be economically or chemically prudent.

The level set method was shown to be a useful means of modeling two-phase flow in COMSOL Multiphysics. Success was achieved in modeling viscous, two-phase flow using the level set equations. However, modeling fluids with densities greater than water with extremely low, yet non-zero surface tensions, proved to be laden with simulation errors. It has still yet to be seen if the error was caused by an inherent program or user problem. The initial specification of a modeling system implementing the level set method was found to be most important. Setting the re-initialization parameter to a value just below the slowest fluid velocity, ensuring that each pure phase entry into the system possesses the proper “seed mass”, and limiting the system to as few subdomains as possible were all found to be most important when using the level set method in COMSOL.
Finally, the success of the complete ATPE simulations is evident in the early time steps. The generalized PDE modes utilized to describe the advective and electrokinetic transport functioned properly when coupled to the velocity field relations. Additionally, the build-up of charged particles on the preferred side of the fluid-fluid interface was clearly demonstrated with the implementation of the “stiff-spring” boundary condition. Ultimately, the future simulations of ATPE systems will need to resolve the inconsistencies in the latter time steps that give rise to erroneous concentrations.
FUTURE RECOMMENDATIONS

Traditional simulations of electrophoretic experiments include the transport of all ions in the system. In systems where electrodes and the analyte are not separated, the concentration of the analyte and other ions near the electrodes can affect the potential difference. By imposing the electroneutrality condition, the solution maintains no net charge and thus does not affect the potential established by the electrodes. In the present investigation, it was assumed that a membrane separated the analyte from the electrodes and that the positively charged analyte was supplied to the system in equal proportion to similarly charged negative species. Future simulations of ATPE systems should attempt to formally satisfy and demonstrate their adherence to the electroneutrality condition.

Electrophoresis is accompanied by a resistive heating of the buffer solution of the analyte molecule known as Joule heating (JH). Many of the buffer solutions used in electrophoresis will conduct an electrical current. Nevertheless, the electrical field used in electrophoresis is often sufficient to induce a current within the buffer solution and consequently, contributes to a resistive heating of the system. The temperature rise in the system due to Joule heating is not usually uniform and hence creates regions where the electrophoretic mobility is different from the bulk or neighboring phase. It has been shown experimentally that the zeta potential ($\zeta$) of the charged species is independent of temperature. Ultimately, it is the temperature dependence of the phase viscosity that causes the local changes in the electrophoretic mobility. The convective transport in ATPE systems may be sufficient to limit mixing attributed to Joule heating, but such a claim was not substantiated in this investigation. Future COMSOL investigations simulating Joule heating would also need to include a complete energy balance on the system.

With the computing capabilities continuing to rise, it is logical for future simulations of ATPE systems to be expanded to the third dimension. It is possible that the error message that arises when densities greater than 400 kg m$^{-3}$ are utilized for level set calculations is linked to the need for fluid motion in all three dimensions. The error message, reproduced in Appendix VII, may also be linked to the manner in which COMSOL handles the initialization of the level set functions. The elucidation of the common error message encountered in this investigation should be at the heart of future studies.
One of the other level set related complications to be resolved is the actual location of the interface in the triple outlet port flow device when the inlet boundaries are specified using a pressure condition and the outlet boundaries specified using a velocity condition. A few concluding attempts using properties similar to the experimental junction simulations were made. The solution after two hundred seconds of simulation time, reproduced in Appendix VIII, is not congruent with the interface location established in the complete ATPE simulations.

After working with the level set formulation in COMSOL and running several attempts using the same model, it is highly recommended that the entire model be reset between every trial. The restart command does not clear enough of the local history to remove all numerical artifacts. The reset command is found under the FILE dropdown menu on the COMSOL graphical user interface.

Weak boundary conditions may prove to be more useful in resolving some of the problems encountered in the level set and complete mass transport simulations. A few level set simulations were conducted with weak constraints, but no significant improvements to the solution were obtained. Additionally, changing the shape function type may prove to be reasonable.

Lastly, geometry optimization studies have become quite popular in recent years. COMSOL Multiphysics should prove to be very useful for optimizing the geometry of future ATPE systems. A few proposed geometries have been included in Appendix IV. Additionally, there still remains a few complex, three dimensional geometries to investigate. A sample of such geometries is available in Appendix III.
NOMENCLATURE

\( \lambda_D \) (or \( \kappa \))  Debye length. The quantities \( \frac{1}{\lambda_D} \) and \( \frac{1}{\kappa} \) both are utilized to reference the thickness of the diffuse ionic double layer and are equal to the following expression:

\[
\frac{1}{\kappa} = \left[ \frac{\epsilon k T}{4 \pi e^2 \sum z_i^2 n_i} \right]^{1/2}
\]

\( K \)  Major component to the local curvature

\( \beta_A \)  Thermodynamic correction factor to diffusion. Equal to the follow expression:

\[
1 + \frac{\delta \ln \gamma_i}{\delta \ln c_i}
\]

\( \mu_i \)  Electro-chemical potential for species \( i \) in \( \frac{kJ}{kmol} \). Classically, \( \mu \) is reserved for the chemical potential and is expressed in the following manner:

\[
\mu_i = \mu_i^0 + RT \ln \gamma_i c_i
\]

The electro-chemical potential may also be expressed in the following classical thermodynamic construction:

\[
\mu_i = \mu_i^0 + N_A \frac{\delta \Delta G_m}{\delta n_i} n_i T P + z_i F \Phi_{sys}
\]

\( \rho_i \)  Density of species \( i \) in \( \frac{kg}{m^3} \)

\( \sigma \)  Surface tension coefficient in \( \frac{N}{m} \)

\( \delta(\phi) \)  The smoothed delta function with dependence upon the level set function consistent with the following:

\[
\delta(\phi) = 6|\nabla \phi| |\phi(1 - \phi)|
\]

\( \Phi_{sys} \)  Electrical potential of system in \( V \)

\( \phi \)  Level set global function

\( \psi_{ab} \)  Electrostatic potential between top and bottom phases

\( \eta_i \)  Dynamic viscosity of species \( i \) in \( \text{Pa*s} \)

\( \gamma_i \)  Activity coefficient of species \( i \)

\( \gamma_{ls} \)  Level set re-initialization / stability parameter

\( a_i \)  Activity of species \( i \)

\( c_i \)  Concentration of species \( i \) in \( \frac{mol}{m^3} \)

\( D_i \)  Diffusion coefficient of species \( i \)

\( F \)  Faraday’s constant, \( 96.49 \times 10^{-6} A \frac{s}{kmol} \)

\( N_i \)  Total molecular flux of species \( i \)
\( n \)          Unit outward normal
\( n_i \)       Number of moles of species \( i \)
\( R \)         Universal gas constant in \( \frac{kJ}{kmol \ K} \)
\( T \)         Temperature in Kelvin
\( x, y, z \)   Cartesian coordinate directions

Superscript

\( \alpha \)     Top phase in an ATP system
\( \beta \)      Bottom phase in an ATP system
\( i \)          The \( i \)th species, \( i \)th component in an ATP system
\( j \)          The \( j \)th phase of an \( n \)-phase system
\( 0 \)          Reference to the standard state

Subscript

\( \alpha \)     Top phase in an ATP system, usually the lighter and the least viscous phase
\( \beta \)      Bottom phase in an ATP system, usually the heavier and the most viscous phase
\( i \)          The \( i \)th species, \( i \)th component in an ATP system
REFERENCES


APPENDICES

Appendix I
Analytical Methods: Flow of Two Adjacent Immiscible Fluids:

Assumptions
- Fluids I and II are immiscible, incompressible
- Flow in single direction in a thin slit of length \( L \), width \( W \)
- Influence of horizontal pressure gradient
- Fluid I is the more dense phase
- Flow rates have been adjusted such that half of the slit is filled with each fluid
- Flow is sufficiently slow such that no instabilities are present, hence interface remains exactly planar

Derivation

Analytical Flow System

\[
\frac{d \tau_{xz}}{dx} = \frac{p_0 - p_L}{L}
\]

\[
\tau_{xz}^I = \left(p_0 - p_L\right) \frac{x}{L} + c_1
\]

\[
\tau_{xz}^{II} = \left(p_0 - p_L\right) \frac{x}{L} + c_2
\]

Boundary Conditions

Continuity of momentum flux at fluid – fluid interface

At \( x = 0 \), \( \tau_{xz}^I = \tau_{xz}^{II} \)

This implies that \( c_1 = c_2 \)

Application of Newton’s Law of Viscosity
\[- \frac{\mu^I d v_z^I}{dx} = \frac{[p_0 - p_L]x}{L} + c_1 \]
\[- \frac{\mu^{II} d v_z^{II}}{dx} = \frac{[p_0 - p_L]x}{L} + c_1 \]

**Application of Remaining Boundary Conditions**

No slip condition at each wall, for distance \( b \) from interface centerline

\[ v_z^{I(II)} = 0 \text{ at } x = \pm b \]

Continuity of velocity at interface

\[ v_z^I = v_z^{II} \text{ at } x = 0 \]

**Solution of Analytical Velocity Profiles**

\[ v_z^I = \frac{[p_0 - p_L]b^2}{2\mu^I L} \left( \left[ \frac{2\mu^I}{\mu^I + \mu^{II}} \right] + \left[ \frac{\mu^I - \mu^{II}}{\mu^I + \mu^{II}} \right] \frac{x}{b} - \left( \frac{x}{b} \right)^2 \right) \]

\[ v_z^{II} = \frac{[p_0 - p_L]b^2}{2\mu^{II} L} \left( \left[ \frac{2\mu^{II}}{\mu^I + \mu^{II}} \right] + \left[ \frac{\mu^I - \mu^{II}}{\mu^I + \mu^{II}} \right] \frac{x}{b} - \left( \frac{x}{b} \right)^2 \right) \]

**Appendix II**

*Supplemental Cases: Phase Field Test 1*
Appendix III

Sample Modeling Geometries:

Levine U Tube
Batch Type Geometry
Appendix IV

Optimization Study:

Upper and Lower Ribbon Set Geometry
T-Needle Junction Geometry

Venturi 5 -1- 10 Geometry
Appendix V

Experimental Flow System:
Even with the color scheme manipulated for the “T” junction case, the two-phase flow is difficult to record. The blue circle has been drawn to direct one’s attention to the two-phase interface. However, it was noted that the black-red transition does not exactly capture the exact location of the interface during the early time steps. Glare and small gas bubbles on the glass add to the deception.

Appendix VI
Sample Time Step Logs From Abandoned and Failed Complete ATPE Simulations Using a Curved Interface
Appendix VII
Reoccurring Level Set Error Message For Systems With Large Densities

Error: 6199

Attempt to evaluate real square root of negative number.
- Function: sqrt

Failed to evaluate expression.
- Expression: nojac((min((gijgij\_chns2^0.25),((rho\_chns2*U\_ref\_chns2)/(48*eta\_chns2)))*(min((sqrt((((((tauc\_chns2*res\_p2\_chns2)/(nojac((rho\_chns2*U\_ref\_chns2)/(vol\_g1\_chns2))))^2+((taum\_chns2*res\_u2\_chns2))^2)+((taum\_chns2*res\_v2\_chns2)^2)))*ck\_chns2),U\_ref\_chns2)*0.5)))

Failed to evaluate temporary symbolic derivative variable
- Variable: shock\_capt\_chns2@VDN$(test@8)@VDN$\_v2x
- Defined as: ((rho\_chns2*nojac((min((gijgij\_chns2^0.25),((rho\_chns2*U\_ref\_chns2)/(48*eta\_chns2)))*(min((sqrt((((((tauc\_chns2*res\_p2\_chns2)/(nojac((rho\_chns2*U\_ref\_chns2)/(vol\_g1\_chns2))))^2+((taum\_chns2*res\_u2\_chns2))^2)+((taum\_chns2*res\_v2\_chns2)^2)))*ck\_chns2),U\_ref\_chns2)*0.5)))))*g21\_chns2)

Failed to evaluate expression.
- Expression: d(d(((\(-2*eta\_chns2*u2x+p2)*test(u2x)-eta\_chns2*(u2y+v2x)*test(u2y)-rho\_chns2*\(u2^2*u2x+v2*u2y)\)*test(u2)+gls\_chns2+shock\_capt\_chns2)-(rho\_chns2*u2t*test(u2)))*(dvol),(test@8)),\_v2x)
Appendix VIII

Triple Exit Port Level Set Interface Re-Simulation:

Appendix IX

Principle Assumption Evaluation of Complete ATPE Simulations

As expected, the interface location at twenty seconds of total simulation time is identical to the results presented in Figure 3.81. The velocity field and streamline data that are provided by the solution to the level set equations are shown in Figure 6.1 and Figure 6.2 respectively.

The representative velocity profile taken from the center of the flow device at twenty seconds of total simulation time using the level set equations is shown in Figure 6.3. The velocity profile greatly contradicts the velocity profiles obtained in Figure 3.84, Figure 3.85, Figure 3.86, and Figure 6.4.
The center velocity profile and streamline data from the simulation utilizing pure-phase properties in each subdomain have been reproduced in the following diagrams:
The following graphics depict the time evolution of the solution to the electrokinetic transport equations using the velocity field generated with the velocity specified at the inlets.