Characterization and Application of Vortex Flow Adsorption for Simplification of Biochemical Product Downstream Processing

by

JUNFEN MA

B.S., Chemical Engineering, Tsinghua University, 1995
M.S., Chemical Engineering, Tsinghua University, 1998
M.S., Chemical Engineering Practice, Massachusetts Institute of Technology, 2000

SUBMITTED TO THE DEPARTMENT OF CHEMICAL ENGINEERING IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN CHEMICAL ENGINEERING AT THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

SEPTEMBER 2003

© 2003 Massachusetts Institute of Technology. All rights reserved.

Signature of Author: ___________________________________________________________

Department of Chemical Engineering
July 10, 2003

Certified by: _________________________________________________________________

Charles L. Cooney
Professor of Chemical and Biochemical Engineering
Thesis Supervisor

Accepted by _________________________________________________________________

Daniel Blankschtein
Professor of Chemical Engineering
Chairman, Committee for Graduate Students
One strategy to reduce costs in manufacturing a biochemical product is simplification of downstream processing. Biochemical product recovery often starts from fermentation broth or cell culture. In conventional downstream processing, the initial steps are clarification, concentration, and purification. Simplification of downstream processing may be achieved by reducing the number of unit operations. Integrative technologies seek to combine steps into a new single unit operation, thereby tightening the whole process.

Vortex flow occurs in the annular gap between an inner rotating solid cylinder and an outer stationary cylindrical shell. Above a critical rotation rate, circular Couette flow bifurcates to a series of counter-rotating toroidal vortices. By suspending adsorbent resin in the vortices, a novel unit operation, vortex flow adsorption (VFA), is created. In VFA, the rotation of the inner cylinder facilitates the fluidization of the adsorbent resin. In addition, VFA has high fluid voidage so that it can be used to recover biochemical products directly from fermentation broths or cell homogenates without removing cells or cell debris first.

VFA was characterized through two experimental approaches, tracer residence time distribution (RTD) study and breakthrough capacity measurements, and two modeling approaches, a one-dimensional dispersion convective model and a two-region vortex flow model. It was concluded that the axial dispersion in the vortex flow system is distinct in different vortex flow regimes. The effect of the operating variables, including the rotation rate of the inner cylinder, the axial loading flowrate, and the adsorbent volume fraction, on the performance of VFA was explored.

In this research, recombinant human α1-antitrypsin (α1-AT) was expressed in Escherichia coli as a C-terminal fusion to a modified intein containing a chitin-binding domain. The VFA results indicated that VFA not only captures the fusion protein from crude cell extract containing cell debris but also purifies α1-AT. Therefore, vortex flow adsorption is an integrative technology to combine the primary clarification, concentration, and purification steps to simplify conventional downstream processing.

Thesis Supervisor: Charles L. Cooney
Title: Professor of Chemical and Biochemical Engineering
Acknowledgements

The PhD life is like a long journey. It needs the continuous effort to make it. However, a thesis is actually an accumulation of efforts from a large community. I would like to take this opportunity to acknowledge and thank all those who have helped me along this journey.

First and foremost, I would like to thank my thesis advisor, Professor Charles Cooney, for his continuous supporting and guiding me through a very rewarding stay at MIT. He provided me with a perspective of biochemical issues in the pharmaceuticals and biotech industry. My appreciation is extended to my committee members Professors Bill Deen, Alan Hatton, and Dane Wittrup for their tough questions and useful suggestions at each committee meeting to balance my research on the right track.

I also like to thank Dr. Shaorong Chong at New England Biolabs for leading me into the world of the intein-mediated protein purification and his help in constructing the plasmid pT12AT1, Dr. Myeong-Hee Yu at the Korea Research Institute of Bioscience and Biotechnology for her generous donation of the plasmid pEAT8, Heather Amoroso at the MIT Biopolymer Lab for N-terminal amino acid sequencing, Han Hwa Hung at EECS for allowing me to use the fluorescence microscope, as well as Peter Morley and Andrew Gallant at the MIT Central Machine Shop for helping me design a vortex flow reactor for adsorption.

I am especially grateful for the help and friendship of Bill Perry, from minor English questions to suggestions on my thesis research. He gave me a lot of useful comments and suggestions on paper and thesis manuscripts. I also like to thank each member of the Cooney lab, Bill Perry, Steve Griffiths, Asti Goyal, Reuben Domike, Samuel Ngai, Mike Laska, May Sun, Maria Jose Ibanez, C.K. Lai, Jean-Francois Hamel for their help and friendship.
To my Chemical Engineering and MIT friends Chen Wang, Xueping Jiang, Jin Yin, Lan Chen: my whole stay at MIT is enjoyable with your support and encouragement from the tough first year to the subsequent years.

Finally, I would like to recognize my Mom and Dad for their continuous confidence and encouragement over the past five years and my younger sister Junting always as a best friend for sharing some ideas and experiences. Thanks to my husband Yongxiang, for his love, support, and encouragement over the years. He is always the person who I could count on when I was stuck in the lab.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS .................................................................................................................. 3
LIST OF FIGURES .......................................................................................................................... 10
LIST OF TABLES ............................................................................................................................. 14
1 INTRODUCTION ............................................................................................................................. 15
   1.1 OVERVIEW ................................................................................................................................. 15
   1.2 VORTEX FLOW ............................................................................................................................ 18
   1.3 VORTEX FLOW ADSORPTION (VFA) ......................................................................................... 20
      1.3.1 Operating Variables .............................................................................................................. 21
      1.3.2 Adsorptive Characterization ................................................................................................. 23
      1.3.3 Mass Transfer and Its Mathematical Model ........................................................................... 24
      1.3.4 Application ............................................................................................................................ 25
      1.3.5 Model System ....................................................................................................................... 26
   1.4 SUMMARY ..................................................................................................................................... 29
2 GOALS AND OBJECTIVES ............................................................................................................. 30
3 MATERIALS, METHODS, AND EXPERIMENTAL SETUP ............................................................ 31
   3.1 VORTEX FLOW SYSTEM ............................................................................................................ 31
      3.1.1 Vortex Flow Experimental Setup ......................................................................................... 31
      3.1.2 Old Configuration of Vortex Flow Reactor ......................................................................... 32
      3.1.3 Improving the Outlet of Vortex Flow Reactor .................................................................... 34
      3.1.4 Improving Drive Shaft of Vortex Flow Reactor ................................................................... 36
   3.2 MEDIA ............................................................................................................................................ 37
   3.3 EXPRESSION STRAIN ............................................................................................................... 39
   3.4 PLASMIDS .................................................................................................................................... 39
   3.5 CELL GROWTH AND INDUCTION ............................................................................................ 40
   3.6 DOWNSTREAM PROCESSING ................................................................................................... 41
   3.7 PROTEIN ASSAY ......................................................................................................................... 42
      3.7.1 Total Protein Assay ............................................................................................................... 42
      3.7.2 Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) ......... 42
      3.7.3 Western Blotting .................................................................................................................... 43
4.3.2 Breakthrough Capacity ................................................................. 91
4.3.3 The Effect of Axial Loading Flowrate ......................................... 93
4.3.4 The Effect of Adsorbent Resin Volume Fraction .......................... 94
4.3.5 The Effect of Rotation Rate of Inner Cylinder .............................. 95
4.3.6 Analysis and Simulation of Vortex Flow Adsorption .................. 96
4.3.7 Performance Analysis of Vortex Flow Adsorption ..................... 100
4.3.8 Summary .................................................................................. 102
4.4 Conclusions ................................................................................ 103

5 Application of Vortex Flow Adsorption to Protein Recovery .......... 104

5.1 Production of Intein α1-Antitrypsin Fusion Protein ..................... 104
  5.1.1 Introduction ............................................................................ 104
  5.1.2 Experimental Design ............................................................... 105
  5.1.3 Cell Growth .......................................................................... 106
  5.1.4 Fusion Protein Expression ....................................................... 107
  5.1.5 In vivo Cleavage of Fusion Protein .......................................... 110
  5.1.6 Summary ............................................................................... 111

5.2 Preparation of Chitin Resin .......................................................... 112
  5.2.1 Introduction ............................................................................ 112
  5.2.2 Chitin and Chitosan ................................................................. 112
  5.2.3 Procedure for Preparing Chitin Resin ..................................... 113
  5.2.4 Glutaraldehyde Method ........................................................... 114
  5.2.5 Divinyl Sulphone Method ........................................................ 118
  5.2.6 Adsorption of Intein Fusion Protein by Chitin Resin ................. 120
  5.2.7 Summary ............................................................................... 124

5.3 Adsorbent Resin Fluidization ....................................................... 125
  5.3.1 Introduction ............................................................................ 125
  5.3.2 Measurements of Minimum Fluidization Rotation Rates .......... 126
  5.3.3 Correlation of Minimum Fluidization Rotation Rates ............... 130
  5.3.4 Comparison of Measured and Correlated Minimum Fluidization Rotation Rates ................................................................. 131
8.1 Characterization of Adsorption Mechanism between Fusion Protein and Chitin Resin .......................................................................................................................... 170
8.2 Simulation of Fluid Velocity in Vortex Flow System .................................. 170
8.3 Improvement of Vortex Flow Model ........................................................... 171
8.4 Scaling-up of Vortex Flow Adsorption System ......................................... 172

9 Bibliography .......................................................................................................................... 173
List of Figures

Figure 1.1: Schematic structure of vortex flow ................................................................. 19
Figure 1.2: Chemical mechanism of intein-mediated purification of α1-antitrypsin ................. 28
Figure 3.1: Schematic of vortex flow experimental setup ..................................................... 31
Figure 3.2: Old configuration of vortex flow reactor .......................................................... 33
Figure 3.3: Structure of two bearings holding stainless steel core of inner cylinder ................. 35
Figure 3.4: Multiple-hole area and buffer cylinder of vortex flow reactor ......................... 36
Figure 3.5: Batch adsorption experimental setup ............................................................... 48
Figure 4.1: Two-region model for vortex flow system ....................................................... 55
Figure 4.2: Adsorption equilibrium at low BSA concentrations ........................................... 58
Figure 4.3: Adsorption equilibrium at low to high BSA concentrations .............................. 59
Figure 4.4: Experimental and simulated batch adsorption kinetics data ............................. 67
Figure 4.5: Possible outcomes for a step in the downhill simplex method ......................... 69
Figure 4.6: Tracer RTD results at low rotation rates of inner cylinder ................................. 77
Figure 4.7: Peak time of tracer RTD curves at low rotation rates of inner cylinder ............... 77
Figure 4.8: Tracer RTD results at high rotation rates of inner cylinder ............................... 78
Figure 4.9: Two-region vortex flow modeling results ....................................................... 78
Figure 4.10: The number of CSTRs at different rotation rates of inner cylinder .................. 80
Figure 4.11: Fitted axial dispersion coefficients from one-dimensional dispersion convective model at different rotation rates ................................................................. 81
Figure 4.12: Fitted axial dispersion coefficients from two-region vortex flow model at different rotation rates ................................................................. 81
Figure 4.13: Fitted axial dispersion coefficients from one-dimensional dispersion convective model put in the relative Taylor number coordinate ................................................. 84
Figure 4.14: Simulated axial dispersion coefficients from different models ....................... 85
Figure 4.15: Tracer RTD results at different axial flowrates .......................................................... 87

Figure 4.16: Tracer RTD results at different axial flowrates with the dimensionless time as the horizontal axis .......................................................... 87

Figure 4.17: Fitted axial dispersion coefficients at different flowrates from one-dimensional dispersion convective model ........................................ 88

Figure 4.18: Tracer RTD results at different resin volume fractions ........................................ 89

Figure 4.19: Fitted axial dispersion coefficients at different resin volume fractions from one-dimensional dispersion convective model ........................................ 90

Figure 4.20: Breakthrough profiles of vortex flow adsorption at different axial flowrates ...... 93

Figure 4.21: Breakthrough capacities of BSA by Streamline DEAE at different axial loading flowrates .......................................................... 94

Figure 4.22: Breakthrough capacities of BSA by Streamline DEAE at different adsorbent resin volume fractions .......................................................... 95

Figure 4.23: Breakthrough capacities of BSA by Streamline DEAE at different rotation rates of inner cylinder .......................................................... 96

Figure 4.24: Vortex flow adsorption modeling result .......................................................... 98

Figure 4.25: Vortex flow adsorption performance analysis: breakthrough capacity vs. volumetric throughput .......................................................... 101

Figure 4.26: Vortex flow adsorption performance analysis: recovery yield vs. volumetric throughput .......................................................... 102

Figure 5.1: Induction temperatures and induction times for optimization of the expression of the intein α1-antitrypsin fusion protein ........................................ 106

Figure 5.2: E. coli cell growth profiles under different induction temperatures ..................... 107

Figure 5.3: SDS-PAGE analysis of soluble cell extract from E. coli harboring pT12AT1 before and after induction at 30°C .................................................. 108

Figure 5.4: Intein α1-antitrypsin fusion protein expression at different induction times under different induction temperatures ........................................ 109

Figure 5.5: Intein α1-antitrypsin fusion protein productivity at different induction times under different induction temperatures ........................................ 110
Figure 5.6: Western blotting analysis for soluble cell extract from *E. coli* harboring pT12AT1 before and after induction at 30°C.................................111

Figure 5.7: Structure of polysaccharides chitin and chitosan........................................113

Figure 5.8: Glutaraldehyde polymer and its activation of amine-containing matrix...........115

Figure 5.9: Chemical mechanism of glutaraldehyde method for preparing chitin beads......117

Figure 5.10: Chemical mechanism of divinyl sulphone method for preparing chitin beads....119

Figure 5.11: Schematic structure of MBP intein fusion protein....................................120

Figure 5.12: SDS-PAGE assay for the adsorption of MBP intein fusion protein by chitin resin prepared with glutaraldehyde method ........................................121

Figure 5.13: SDS-PAGE assay for the adsorption of MBP intein fusion protein by chitin resin prepared with DVS method.........................................................122

Figure 5.14: Viscosity of sucrose solution vs. concentration in wt % at 20°C......................128

Figure 5.15: Measured and correlated minimum fluidization rotation rates for adsorbent resins........................................................................................................131

Figure 5.16: Measured and correlated minimum fluidization rotation rates in sucrose solutions with different concentrations .............................................132

Figure 5.17: Schematic structure of intein α1-AT fusion protein....................................135

Figure 5.18: Cell debris pulse-response experiment results in vortex flow reactor ..........137

Figure 5.19: *E. coli* cell debris recovery in the pulse-response experiments for both Streamline DEAE anion exchange resin and chitin resin.................................137

Figure 5.20: Outlet stream measurements during loading/adsorption and washing steps in vortex flow adsorption process for recovery of intein α1-antitrypsin fusion protein .............................................................................................................140

Figure 5.21: SDS-PAGE and Western blotting assays for vortex flow adsorption..............141

Figure 5.22: Capture efficiency and cleavage efficiency from SDS-PAGE and Western blotting assays ......................................................................................142

Figure 5.23: Interaction network between components in vortex flow adsorption system.....143
Figure 5.24: Capture efficiency of vortex flow adsorption process for non-clarified and clarified cell extract

Figure 5.25: Activity assay for vortex flow adsorption process

Figure 5.26: Capture efficiency at different adsorbent capacities

Figure 5.27: Capture efficiency at different adsorption kinetics

Figure 5.28: Capture efficiency at different rotation rates of inner cylinder

Figure 6.1: Contour plot of minimum fluidization rotation rate with particle density and particle diameter

Figure 6.2: Critical Taylor number Ta_c for vortex flow reactor with different geometric dimensions

Figure 6.3: Network in the vortex flow adsorption system
List of Tables

Table 3-1: Geometric Dimensions of Vortex Flow Reactor .......................................................... 32
Table 3-2: Geometric Dimensions of Bearings ........................................................................ 37
Table 3-3: Media Stock Solutions .............................................................................................. 38
Table 3-4: SDS-PAGE Sample Buffer Solution ......................................................................... 42
Table 3-5: SDS-PAGE Running Buffer Solution ....................................................................... 43
Table 3-6: Coomassie Blue Staining and Destaining Solutions ............................................... 43
Table 4-1: Modeling Parameters for Adsorption Equilibrium .................................................. 57
Table 4-2: Modeling Parameters for Adsorption Kinetics ......................................................... 67
Table 4-3: Flow Regimes in Vortex Flow Reactor ..................................................................... 84
Table 4-4: Vortex Flow Adsorption Modeling Parameters ........................................................ 99
Table 5-1: Adsorption of MBP Intein Fusion Protein by Chitin Resins .................................... 123
Table 5-2: Parameters in Correlation of Sucrose Density .......................................................... 127
Table 5-3: Minimum Fluidization Rotation Rates for Adsorbent Resins .................................. 129
Table 5-4: Minimum Fluidization Rotation Rates in Sucrose Solutions with Different
Concentrations .......................................................................................................................... 129
Table 5-5: Parameters in Correlation of Minimum Fluidization Rotation Rate ....................... 130
Table 5-6: Parameters for Adsorption Mechanism .................................................................... 153
Table 5-7: Modeling Parameters for the Baseline Case ............................................................ 153
Table 5-8: Three Cases with Different Adsorption Kinetics ..................................................... 155
Table 5-9: Axial Dispersion Coefficient $D_L$ and Vortex Core-Bypass Mass Transfer
Coefficient $K_{bc}$ at Different Rotation Rates ........................................................................ 157
1 Introduction

1.1 Overview

One strategy to reduce costs in manufacturing a biochemical product is simplification of downstream processing (Spalding, 1991). This may be achieved by reducing the number of unit operations. Integrative technologies seek to combine steps into a new single unit operation and tighten the whole process (Thömmes et al., 1995a). The aim is to increase process compactness and yield, to maintain product integrity and purity, and to reduce processing time, equipment number, and labor cost (Beck et al., 1999).

Primary recovery of a biochemical product often starts from feedstock that contains particulates, e.g., cells and cell debris for extracellular products or cell homogenate for intracellular products (Frej et al., 1994). In conventional downstream processing, the initial steps are clarification, concentration, and purification. Disadvantages associated with such processes are long cycle time and low product yield due to cumulative product loss through the sequence of unit operations (Thömmes et al., 1995b). The concept of integration of the early steps can address this problem and thus is economically promising.

Conventional packed column chromatography is constrained in dealing with crude cell extract due to high viscosity and the presence of colloidal materials (Chang and Chase, 1996b). A packed bed acts as a dead-end depth filter, which causes cells and cell debris to build up in the column, flow rate to decrease, and eventually the column to totally block (Frej et al., 1994). However, a packed bed has plug flow behavior, which is critical to high-resolution separation. The recovery of biochemical products from fermentation broths and cell homogenates usually
commences with centrifugation and filtration to remove cells and cell debris. Only the clarified feedstock is applied to packed column chromatography (Frej et al., 1994).

One method to integrate multiple primary steps in a downstream process is to use a batch procedure in which the adsorbent is added directly to the feedstock in a stirred tank. The advantage of employing a stirred tank is that the product is captured directly from non-clarified feedstock. The disadvantage is that the stirred tank acts as one contact stage in a separation process, leading to long processing time (Frej et al., 1994). Another problem of the stirred tank is that an additional step is needed to separate the adsorbent from the feedstock after the adsorption step.

Expanded bed adsorption has emerged as a method where a possibility of high chromatographic resolution is coupled with the advantage of working with a particulate-containing feedstock. In expanded bed adsorption, adsorbent particles with high density are used in an upflow fluidized bed (Gailliot et al., 1990) and the fluid void is sufficient to allow for the free passage of particulate debris. As with a stirred tank procedure, a fluidized bed has back mixing (i.e., axial dispersion), thereby behaving like a column with a low number of contact stages (Frej et al., 1994).

Generally, the adsorption performance in chromatographic processes is influenced by mass transport, adsorption kinetics, and dispersion, which depend on both adsorbent properties and operating parameters (Karau et al., 1997; Li et al., 1995). In order to make expanded bed adsorption an effective process, adsorbent particles with the following criteria are needed (Karau et al., 1997; Thömmes et al., 1995a; Thömmes et al., 1995b):
(1) The distribution of particle size and density should allow for development of a classified inhomogeneous fluidized bed at reduced local mobility of individual adsorbent particles with the aim to preserve the packed-bed hydrodynamics.

(2) Size and density of adsorbents should result in a significant difference in the terminal settling velocity of adsorbent particles and biomass in the feedstock to allow for clarification of the feedstock by solid-solid separation during fluidization.

(3) The adsorbents used for expanded beds should have a relatively high density to allow for using higher flow rates and thereby shorten the processing time.

(4) The adsorbents should be resistant to nonspecific adsorption and fouling by biomass, nucleic acids, lipids, and other materials contained in the feedstock.

Therefore, the success of expanded bed adsorption is closely related to the tight control of fluidization of the adsorbents. A stable bed expansion at reduced mixing within the bed is crucial to achieve capacity and resolution similar to standard chromatography in a packed-bed mode (Thömmes et al., 1995b). To be effective, an expanded bed should guarantee homogeneous particle fluidization, i.e., the absence of flow channels, stagnant zones, as well as aggregates from fluidized particles (Fernández-Lahore et al., 1999).

In addition to expanded bed adsorption, another novel and promising technology, vortex flow adsorption (VFA), also can be applied to dealing with fermentation broths or cell homogenates directly due to a high fluid void fraction and a possible high number of contact stages that result from distinct vortices.
1.2 Vortex Flow

Vortex flow occurs in the annular gap between two concentric cylinders, an inner rotating solid cylinder and an outer stationary cylindrical shell, and it is generated by the rotation of the inner cylinder. Above a critical rotation rate, circular Couette flow bifurcates to a series of counter-rotating toroidal vortices; the schematic structure of vortices is shown in Figure 1.1. G. I. Taylor first determined the critical rotation speed for the vortex formation without axial flow (Taylor, 1923). Therefore, vortex flow is also called Taylor vortex flow. The vortices are regularly spaced along the cylinder axis, each with an axial height approximately equal to the annulus gap width. As is shown in Figure 1.1, there are two boundaries for each circular vortex, one is the inflow boundary toward the inner cylinder and the other is the outflow boundary toward the outer cylinder. Normally the velocity of the inflow boundary is slower than that of the outflow boundary.

In a vortex flow system, the ratio of the inertial centrifugal force to the viscous force is represented by the Taylor number, \( Ta \):

\[
Ta = \frac{\omega \cdot d \cdot R_i}{\nu}
\]  

(1-1)

where \( \omega \) is the angular velocity of the inner cylinder, \( d \) is the annular gap width, \( R_i \) is the radius of the inner cylinder, and \( \nu \) is the kinematic viscosity of the fluid. When \( Ta \) exceeds the critical value, \( Ta_c \), a transition occurs from Couette flow to a flow regime that is marked by a series of toroidal vortices. When an axial flow is superimposed to this system, the resulting flow is so-called Taylor-Couette-Poiseuille flow. For the axial flow, the ratio of the inertial force to the viscous force is described by the axial Reynolds number, \( Re_{ax} \):
\[ \text{Re}_{\text{ax}} = \frac{U_{\text{ax}} \cdot d}{\nu} \]  

where \( U_{\text{ax}} \) is the superficial axial velocity.

**Figure 1.1: Schematic structure of vortex flow**

In the vortex flow system, the rotation rate of the inner cylinder is an important operating variable. By varying the rotation rate, it is possible to span a series of different flow patterns (Wereley and Lueptow, 1999b), ranging from an almost plug flow to an almost perfectly mixed flow (Giordano et al., 1998). The rotation of the inner cylinder may be used to keep the particles suspended at low axial flow, which is an otherwise difficult task in conventional fluidized bed reactors (Giordano et al., 1998). Additionally, the agitation
promoted by the rotation of the inner cylinder is low-shear compared to that obtained with conventional stirrers, which is an important feature especially when sensitive particles and cells are present in the fluid (Giordano et al., 1998).

Vortex flow has been applied to chemical and biochemical processes in several ways. The relatively small inter-vortex mixing, at least in the laminar flow regime, can improve vortex flow reactor performance close to an ideal plug-flow reactor (Haim and Pismen, 1994). In a heterogeneous system, e.g., immobilized enzymes, the rotation of the inner cylinder provides an additional operating variable, or degree of freedom (Giordano et al., 1998), that facilitates particle fluidization. In vortex flow filtration, vortices promoted on the filtration membrane surface by the rotation of the inner cylinder could prevent plugging of the filter pores with particles and thus minimize fouling (Wereley and Lueptow, 1999a).

1.3 Vortex Flow Adsorption (VFA)

Vortex flow adsorption is the application of vortex flow to biochemical downstream processing. In vortex flow adsorption, adsorbent particles are suspended in the annular gap by adjusting the rotation rate of the inner cylinder. Due to high voidage in the vortex flow reactor, crude cell extract with cells or cell debris can be loaded to the reactor directly, and the desired product is adsorbed with cells or cell debris passing through the reactor.

Compared to other integrative technologies, vortex flow adsorption has the following distinct features:

(1) The system can be mixed without stirring. The low-shear mixing is especially beneficial to biological systems (Giordano et al., 1998; Giordano et al., 2000a).
(2) The fluid voidage is increased compared to packed beds, allowing for free passage of entrained particles. Therefore, it can be applied directly to non-clarified cell extracts to reduce the number of steps in downstream processing and improve process efficiency and recovery yield.

(3) Due to the periodical configuration of vortices, multiple contact stages can be formed at least in the flow regime with a low Taylor number, thereby increasing adsorption efficiency.

(4) The rotation rate of the inner cylinder is another operating variable, which makes the operating system flexible and robust (Giordano et al., 1998). Moreover, the rotation of the inner cylinder facilitates the suspension of adsorbent resin and the axial flow may be changed independently to adjust the process throughput.

1.3.1 Operating Variables

As mentioned previously, the rotation of the inner cylinder is very important in the vortex flow adsorption system. The lower limit of the rotation rate should be above the critical rotation rate to induce the vortex flow. The critical rotation rate can be improved if there is axial flow because the axial flow stabilizes the primary Couette flow and delays the appearance of the secondary vortex flow (Moore, 1994a). Additionally, when the Taylor number becomes significantly larger than $Ta_c$, there are several different vortex flow regimes: Taylor vortex flow ($Ta_c < Ta < 15Ta_c$), wavy vortex flow ($15Ta_c < Ta < 30Ta_c$), turbulent vortex flow ($30Ta_c < Ta < 160Ta_c$), etc. (Ohmura et al., 1997; Sczechowski et al., 1995). The operating rotation rate should cover these vortex flow regions so that the mass transfer, the axial dispersion, and the vortex flow adsorption performance can be compared at different vortex flow regions. In
addition, since the rotation of the inner cylinder facilitates fluidization or suspension of adsorbent resin, the operating rotation rate also should be above the minimal fluidization rotation rate above which adsorbent resin can be fluidized completely.

In addition, the axial flowrate is also an important operating parameter. Unlike expanded bed adsorption, there is no fluid distributor at the bottom of the vortex flow reactor and the axial flow cannot facilitate the fluidization of the adsorbent resin. Therefore, the main function of the axial flowrate is to adjust the throughput of the vortex flow adsorption process. This is different from expanded bed adsorption, where the axial flowrate is used to fluidize the adsorbent resin and simultaneously the throughput of the adsorption process is determined.

In packed column chromatography, back mixing or axial dispersion is low compared to expanded bed adsorption. Therefore, it is very natural to postulate that, if the adsorbent resin volume fraction is increased in the vortex flow system, the axial dispersion could be decreased. Therefore, in this research, the effect of the adsorbent resin volume fraction on the vortex flow adsorption performance is investigated.

The effect of the operating variables on the performance of vortex flow adsorption can be investigated through an experimental approach. However, if the performance of vortex flow adsorption can be understood from its intrinsic mechanism, it becomes clear how to adjust the operating variables to optimize the performance of vortex flow adsorption. The usual chemical engineering approach can be applied here: first identify the main characteristics of vortex flow adsorption with tracer RTD study or other experimental approaches; then build a model capable of simulating vortex flow adsorption; finally validate the model against the experimental data.
after a careful assessment of the intrinsic and inherent kinetics and the extra- and intra-particle mass transfer resistances (Giordano et al., 1998).

1.3.2 Adsorptive Characterization

The adsorptive behavior in the vortex flow system can be characterized by measuring the dynamic or breakthrough capacity, which is the capacity of an adsorption column or reactor under a defined actual operating condition instead of an equilibrium condition.

In measuring the dynamic capacity, a protein solution is continuously fed into a vortex flow reactor containing adsorbent resin to generate a breakthrough curve. The protein solution is fed into the reactor until the adsorbent resin is saturated with the adsorbed protein and the concentration of the protein in the outlet stream approaches the inlet concentration or some percent of the inlet concentration. In an ideal operation, all the protein introduced is bound to the resin until saturation is reached; at this point the outlet concentration changes immediately from zero to the inlet concentration. In a real situation, the protein begins to break through the reactor before saturation. The resulting concentration versus time curve is indicative of the protein adsorption capacity of the adsorbent resin and also the non-idealities of the dynamic adsorption behavior. The time axis here is equivalent to the total amount of protein introduced to the reactor.

Therefore, by measuring the dynamic capacities under different operating conditions, e.g., the rotation rate of the inner cylinder, the axial loading flowrate, and the adsorbent resin volume fraction, the vortex flow adsorption performance and its non-idealities can be explored.
1.3.3 Mass Transfer and Its Mathematical Model

Vortex flow is a complex fluid phenomenon and a secondary flow pattern that only occurs above a critical rotation rate. A deep understanding of vortex flow comes from tracer and visualization study. In these experiments, color, fluorescent, or magnetic tracer is always used to facilitate the study of vortex flow.

In the vortex flow system, the mechanical energy, which is transmitted by the rotation of the inner cylinder, produces two rotational flows: a global tangential rotation and a secondary rotation with axial and radial components inside the vortices (Desmet et al., 1996a). Generally, the axial Poiseuille flow is applied to the annular gap in the applications of vortex flow. All these flows are interwoven with each other. In addition, the structure of vortices varies greatly with the rotation rate of the inner cylinder. All these factors make mass transfer occurring in the annular gap very complicated.

Although there are several mathematical models reported to describe the mass transfer in the vortex flow system, they do not agree with each other. The main reason is that each model is based on different assumptions and evaluated by limited experiments (Campero and Vigil, 1997; Desmet et al., 1996a; Desmet et al., 1996b; Desmet et al., 1997a; Desmet et al., 1997b; Moore and Cooney, 1995). As is known, vortex structure and mass transfer are different in different flow regimes. Therefore, it is difficult to develop a general model to describe the mass transfer in the vortex flow system under a wide operational range. It is very likely that some assumptions may work well for one or more flow regimes, but are ineffective in other flow regions. Therefore, to determine an appropriate mass transfer model in the vortex flow system is within the scope of this thesis.
In general, due to the natural periodical configuration of vortices, the annular gap can be split into stages, each consisting of one vortex core and its respective bypass region (Giordano et al., 2000b). Therefore, mass transfer occurs inside vortices (intra-vortex), between different vortices (inter-vortex), and between vortices and bypass regions. At low rotation rates, mass transfer due to inter-vortex dispersion is low, and the vortex flow reactor is expected to resemble a plug-flow reactor with bad intra-vortex mixing. When the rotation rate increases, all the mass transfer rates increase, and the vortex flow reactor can be regarded as a CSTR series with very good inter-vortex mass transfer. If the rotation rate increases further, mass transfer between vortices and bypass regions becomes dominant and the whole vortex flow reactor behaves like a CSTR. Therefore, two or three adjustable parameters are usually needed in a model to simulate the mass transfer in the vortex flow system. In the model, not only mass transfer coefficients are needed, but also the volume ratio between vortices and bypass regions. A simplified model can be obtained with some assumptions that capture the main mass transfer features under a specific situation.

1.3.4 Application

Vortex flow adsorption was put forward in order to recover biochemical products directly from fermentation broths or homogenized cell cultures. Vortex flow adsorption is supposed to be a simplified unit operation that integrates clarification, concentration, and purification, three independent unit operations in conventional downstream processing.

The actual biochemical system is complex in that it is highly viscous and non-Newtonian with particulates and colloidal materials (Chang et al., 1993). All these define higher requirements on vortex flow adsorption. A qualitative analysis of the constituents in a
fermentation broth or cell homogenate is necessary to choose the functional adsorbent to recover and separate the target protein very efficiently. An adsorbent with high specificity and affinity to the target protein is always desired.

As in the expanded bed adsorption process, there are specific requirements for the adsorbent resin used in vortex flow adsorption when it deals with complex biochemical systems. High-density adsorbent particles are needed to create a sufficient difference in the terminal settling velocity of adsorbent particles and biomass in the feedstock to allow for clarification of cells and cell debris by solid-solid separation. However, the adsorbent resin density cannot be too high because the resin should be easily fluidized at moderate rotation rates. In addition, the adsorbent should be resistant to the fouling by biomass, nucleic acids, and lipids in the fermentation broth.

In addition, high viscosity of fermentation broths or cell homogenates is likely to lead to flow channels, stagnant zones, as well as aggregates from fluidized particles (Fernández-Lahore et al., 1999), which produce inhomogeneous particle-containing vortices. All these affect mass transfer and thereby recovery and purification efficiency of vortex flow adsorption. Therefore, an upper limit is expected on initial cell mass concentration in the feedstock loaded to the vortex flow adsorption system (Frej et al., 1994).

1.3.5 Model System

In this research, a recombinant form of human α1-antitrypsin (α1-AT) expressed in Escherichia coli was used as a model system for exploring the application of vortex flow adsorption to directly recovering biochemical products from fermentation broths or cell homogenates. α1-AT is a 394 amino acid (44 kDa) serine protease inhibitor (serpin) in human
plasma. α1-AT is produced in the liver and released into the blood. α1-AT has a primary physiological role in protecting the lungs from neutrophil elastase, which normally digests the elastin of the damaged or aging cells in order to provide for healing. However, once neutrophil elastase is done digesting these cells, it attacks the lung tissue. α1-AT in sufficient amounts traps and destroys neutrophil elastase before it can damage the delicate lung tissue. Insufficient levels of α1-AT in the lungs can cause widespread tissue degradation by elastase, ultimately resulting in the lung damage associated with clinical pulmonary emphysema. Therefore, the production of recombinant human α1-antitrypsin in a genetically engineered microorganism is of particular economic interest.

Recombinant α1-antitrypsin was expressed as a fusion protein with an intein, a protein segment for protein splicing. Protein splicing is a posttranslational processing event in which an internal protein segment, the intein, can catalyze its own excision from a precursor protein and concomitantly ligate the flanking regions, the exteins, to form a mature protein (Perler et al., 1994). As is shown in Figure 1.2, α1-AT was fused as a target protein to the C-terminus of a modified intein (Sce VMA intein, 55 kDa) containing a Bacillus circulans chitin-binding domain (CBD) (Chong et al., 1996). The intein is from the vacuolar membrane ATPase subunit (VMA) of Saccharomyces cerevisiae. The CBD allows the binding of the fusion protein to a chitin resin. The intein is capable of undergoing in vitro peptide bond cleavage at its termini. The intein-mediated cleavage is induced by incubation of the fusion protein bound on the chitin resin with a commonly used thiol, 1,4-dithiothreitol (DTT). DTT cleaves the thiol ester bond formed by an N-S acyl rearrangement at the intein N-terminal cysteine, which subsequently triggers peptide cleavage at the intein C-terminal asparagine (Chong et al., 1998a). The three-
α1-Antitrypsin was fused to the C-terminal asparagine (Asn) of a modified intein containing a chitin-binding domain (CBD) to allow binding to a chitin resin. A thiol ester bond is formed by an N-S acyl rearrangement at the intein N-terminal cysteine (Cys). DTT cleaves the thiol ester bond, which in turn triggers the cleavage at the intein C-terminus with Asn succinimide formed. α1-Antitrypsin and a small N-extein peptide are eluted from the chitin resin whereas the CBD tagged intein remains bound to the chitin resin.

**Figure 1.2: Chemical mechanism of intein-mediated purification of α1-antitrypsin**
dimensional structure of the intein indicates that the terminal residues, Cys and Asn, are in close proximity (Duan et al., 1997). The thiol-induced N-terminal cleavage triggers a conformational change in the intein structure, thereby allowing succinimide formation and the C-terminal cleavage to proceed at basic pH conditions (Chong et al., 1998b). As a result, α1-AT is released from the chitin resin without the use of a protease and is separated from its fusion partner on the chitin resin that purifies the fusion protein. A small N-terminal extein peptide (12 residues, 1.6 kDa), derived from E. coli maltose binding protein (MBP) to provide a favorable translational start, is also purified after the cleavage reaction and can be removed by dialysis (Chong et al., 1998a).

In this research, we use the intein α1-AT fusion protein and the chitin resin system to show that vortex flow adsorption is an integrative technology that can be applied to recovering intracellular recombinant protein directly from homogenized crude E. coli cell extract without first removing cell debris.

1.4 Summary

Vortex flow adsorption is a novel concept in downstream processing of biochemical products. Conceptually, it can directly process particle-containing cell extract without first removing cellular or other particles. It is expected that its implementation would provide a novel perspective about downstream processing to shorten processing time, improve recovery yield, and decrease operational cost. In addition, vortex flow adsorption is a promising application of vortex flow after the success of vortex flow reaction and vortex flow filtration.
2 Goals and Objectives

The goal of this thesis is to characterize the performance of the vortex flow adsorption system using both the experimental and modeling approaches and to apply it as an integrative technology to directly recovering recombinant proteins from crude *E. coli* cell extracts without first removing cellular materials.

Specifically, the objectives of this thesis are to:

- Improve the vortex flow system to be used for the adsorption operation,
- Characterize the performance of the vortex flow adsorption system both as a chemical reactor and as an adsorption column,
- Develop a mathematical model to simulate the mass transfer, the axial dispersion, and the adsorptive behavior in the vortex flow system,
- Define an appropriate operating region for the vortex flow adsorption process based on the experimental and modeling results,
- Apply the vortex flow adsorption technology to a practical biochemical system to explore the recovery efficiency and other relevant problems,
- Put forward some suggestions and considerations for the design and optimization of the vortex flow adsorption system.
3 Materials, Methods, and Experimental Setup

3.1 Vortex Flow System

3.1.1 Vortex Flow Experimental Setup

In this research, most experiments were conducted at room temperature with an experimental setup depicted schematically in Figure 3.1. The core component is a vortex flow reactor, which is composed of two coaxial cylinders. The external stationary cylindrical shell is made of acrylic and the inner rotational solid cylinder of polypropylene. Table 3-1 lists the geometric dimensions of the vortex flow reactor. The details about the vortex flow reactor configuration are described later. A stainless steel shaft was positioned throughout the inner cylinder to increase the system inertia and reduce inner cylinder wobbling.

Figure 3.1: Schematic of vortex flow experimental setup
Table 3-1: Geometric Dimensions of Vortex Flow Reactor

<table>
<thead>
<tr>
<th>Geometric Dimension</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer Cylinder Radius, $R_o$</td>
<td>cm</td>
<td>2.53</td>
</tr>
<tr>
<td>Inner Cylinder Radius, $R_i$</td>
<td>cm</td>
<td>1.71</td>
</tr>
<tr>
<td>Annular Gap Width, $d$</td>
<td>cm</td>
<td>0.82</td>
</tr>
<tr>
<td>Wetted Height, $L$</td>
<td>cm</td>
<td>14.7</td>
</tr>
<tr>
<td>Effective Reactor Volume</td>
<td>ml</td>
<td>160</td>
</tr>
</tbody>
</table>

Rotation was provided by a magnetic drive adapted from Membrex (Garfield, NJ) benchmark filtration equipment, controlled by an Electro-Craft E-652-M unit, and measured with a hand-held digital tachometer (Shimpo Instruments). Two peristaltic pumps (Cole-Parma) were used to control the inlet and outlet flowrate, respectively. A timer and graduated cylinder were used together to measure actual flowrates.

The outlet concentration of the vortex flow reactor was measured by both a continuous flow UV detector with a 280 nm filter (Amersham Bioscience) and a Chem2000-UV-Vis optic fiber spectrometer (Ocean Optics). The voltage signal from the UV detector was collected by a computer and further analyzed with Hyper Terminal software. Chem2000-UV-Vis is an online spectrophotometer that can measure absorbance at multiple wavelengths simultaneously. When necessary, the outlet sample was collected by a Model 2110 fraction collector (Bio-Rad) for further assays.

3.1.2 Old Configuration of Vortex Flow Reactor

A vortex flow reactor with an old configuration, as shown schematically in Figure 3.2, was used at the very beginning of this research work. It was designed by previous colleagues at the Cooney lab and used for chemical or enzymatic reactions (Giordano et al., 2000a; Giordano
et al., 2000b). Inlet and outlet are lateral orifices on the outer cylinder wall with small cross-sectional areas. In addition, a piece of nylon mesh (Sefar America) was positioned on the outlet orifice to retain adsorbent resin.

![Figure 3.2: Old configuration of vortex flow reactor](image)

There are eight lateral ports that can be used as inlets and outlets. The distances of the ports to the bottom of the outer cylinder are labeled. The inlet and outlet used in this work are marked.

When this vortex flow reactor with fluidized adsorbent resin was operated with the axial flow, the resin gradually accumulated on the outlet mesh and partially blocked the outlet orifice. Subsequently, there were a lot of gas bubbles flowing out of the blocked outlet orifice. The appearance of the gas bubbles affected downstream online concentration measurements by both the UV detector and the Chem2000-UV-Vis spectrophotometer. In addition, the outlet fluid flowrate was less than the setting value since the gas bubbles occupied part of the outlet
tubing volume. Therefore, the vortex flow reactor cannot be operated steadily due to the fluid accumulation within the reactor.

In the vortex flow reactor, the rotation of the inner cylinder was provided by a magnetic drive. The smooth transfer of the rotating motion from the magnetic drive to the inner cylinder is mostly determined by the geometric dimensions of two bearings holding the stainless steel core of the inner solid cylinder. The geometric dimensions of two bearings include the inner diameter of the bearings and the distance between the two bearings. The schematic structure of the vortex flow reactor without the outer cylindrical shell is shown in Figure 3.3. For the old configuration of the vortex flow reactor, the two bearings had a small inner diameter and were very close to each other. When the vortex flow reactor was operated at high rotation rates, e.g., 400 rpm, there was serious wobbling at the end of the inner cylinder far from the bearings. The wobbling decreased the annular gap between the inner and outer cylinders. If the inner and outlet cylinders are very close to each other, the fluidized adsorbent resin is very easily broken. In addition, the flow pattern in the vortex flow reactor was abnormal due to the wobbling of the inner cylinder. The abnormal flow pattern could affect the performance of the vortex flow adsorption system.

3.1.3 Improving the Outlet of Vortex Flow Reactor

In order to solve the accumulation and wobbling problems associated with the vortex flow reactor with the old configuration, some modifications were made with the help of the MIT Central Machine Shop. The geometric dimensions listed in Table 3-1 were kept for the new vortex flow reactor.
The sidewall of the outer cylinder was perforated very close to the top with multiple holes that were covered with nylon mesh (Sefar America) to retain the adsorbent resin but allow the passage of the fluid. A short cylindrical shell, called the buffer cylinder, was attached to the outer cylinder to cover the multiple-hole area. By doing this, a so-called fluid buffer region was created. The outlet fluid flowed through the multiple-hole area, where the carried resin particles were separated from the fluid; thus, the fluid with no resin particles flowed out of the buffer cylinder. Some details about the multiple-hole area and buffer cylinder are illustrated in Figure 3.4. Instead of having a very high linear velocity at the outlet with a small
cross-sectional area in the old configuration, the fluid flowed at a very low linear velocity across the multiple-hole area. This is advantageous for the separation between the adsorbent resin and the fluid on the mesh. There were no accumulation of resin particles and appearance of gas bubbles with the new configuration used in the vortex flow adsorption system.

![Figure 3.4: Multiple-hole area and buffer cylinder of vortex flow reactor](image)

3.1.4 Improving Drive Shaft of Vortex Flow Reactor

In the new configuration, the bearings holding the stainless steel shaft positioned through the inner cylinder have a large diameter and are separated by a long distance. The diameter of the bearings and the distance between them in the old and new configurations are compared in Table 3-2. In this way, the rotating motion can be transmitted smoothly from the magnetic drive to the far end of the inner cylinder. Even when the rotation rate goes as high as 500 or 600 rpm, there is no wobbling.
Table 3-2: Geometric Dimensions of Bearings

<table>
<thead>
<tr>
<th></th>
<th>Old Configuration</th>
<th>New Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bearing Diameter (cm)</td>
<td>0.47</td>
<td>0.80</td>
</tr>
<tr>
<td>Bearing Distance (cm)</td>
<td>2.48</td>
<td>4.74</td>
</tr>
</tbody>
</table>

By making all these modifications, the vortex flow reactor with the new configuration can be used to study the vortex flow adsorption process with either clarified protein system or crude *E. coli* cell extract with cellular materials.

3.2 Media

Two types of growth media, complex medium and semi-defined medium, were used in this work. The complex medium was Luria-Bertani (LB) medium prepared at 25 g/L (10 g/L Bacto<sup>TM</sup> tryptone, 5 g/L Bacto<sup>TM</sup> yeast extract, and 10 g/L sodium chloride). LB medium was used to grow overnight cultures and prepare frozen cell stocks. The semi-defined medium was based on the M9ZB medium (Ausubel *et al.*, 1995) with the addition of a trace metals stock solution. The semi-defined medium was used for the majority of the expression experiments as it permitted cell growth to high densities and resulted in significant and consistent yields of recombinant proteins.

Media stock solutions were prepared according to Table 3-3 and autoclaved separately. Semi-defined medium was prepared immediately prior to inoculation by adding the appropriate volume of each stock solution to autoclaved Milli-Q<sup>TM</sup> water in a baffled shake flask (liquid volume = 1/5 total volume). For example, to prepare 100 ml semi-defined medium, add 10 ml M9 stock (10×), 10 ml Tryptone/NaCl stock (10×), 2.5 ml glucose (20%, 40×), 150 µl Trace Metals (667×), 100 µl MgSO<sub>4</sub> (1000×), and appropriate antibiotics to 77 ml water.
Table 3-3: Media Stock Solutions

<table>
<thead>
<tr>
<th>Components</th>
<th>Stock Concentration (g/L)</th>
<th>Concentration in Media</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M9 Stock (10×) Titrated to pH 7.4 with NaOH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>60</td>
<td>42 mM</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>30</td>
<td>22 mM</td>
</tr>
<tr>
<td>NaCl</td>
<td>5</td>
<td>8.6 mM</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>10</td>
<td>18.7 mM</td>
</tr>
<tr>
<td><strong>1 M MgSO₄ Stock (1000×)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgSO₄</td>
<td>120.4</td>
<td>1.0 mM</td>
</tr>
<tr>
<td><strong>Tryptone/NaCl Stock (10×)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptone</td>
<td>100</td>
<td>10.0 g/L</td>
</tr>
<tr>
<td>NaCl</td>
<td>50</td>
<td>86.0 mM</td>
</tr>
<tr>
<td><strong>Glucose Stock (20%, 40×)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>200</td>
<td>5.0 g/L</td>
</tr>
<tr>
<td><strong>Trace Metals Stock (667×)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂EDTA • 2H₂O</td>
<td>20.30</td>
<td>81.80 µM</td>
</tr>
<tr>
<td>CaCl₂ • 2H₂O</td>
<td>0.51</td>
<td>5.20 µM</td>
</tr>
<tr>
<td>FeCl₃ • 6H₂O</td>
<td>16.90</td>
<td>93.80 µM</td>
</tr>
<tr>
<td>CuSO₄ • 5H₂O</td>
<td>0.16</td>
<td>0.96 µM</td>
</tr>
<tr>
<td>MnSO₄ • H₂O</td>
<td>0.13</td>
<td>1.15 µM</td>
</tr>
<tr>
<td>CoCl₂ • 6H₂O</td>
<td>0.18</td>
<td>1.15 µM</td>
</tr>
<tr>
<td>ZnSO₄ • 7H₂O</td>
<td>0.18</td>
<td>0.91 µM</td>
</tr>
</tbody>
</table>

Antibiotics were added to the media as a selective pressure to maintain plasmid-containing cells. Plasmids encoding recombinant proteins included the β-lactamase gene, and ampicillin was added to the media at 50 µg/ml for plasmid maintenance. Ampicillin stock solution was prepared as 100 mg/ml in deionized (DI) water and stocked at –20°C. Recombinant protein expression was induced by addition of an appropriate volume of 1 M
isopropyl β-D-thiogalactopyranoside (IPTG) stock solution to an induction concentration of 0.4 mM.

3.3 Expression Strain

*E. coli* BL21 (DE3) from Novagen (Madison, WI) was used as the expression strain. As a lysogen of bacteriophage DE3, *E. coli* BL21 contains chromosomal copies of *LacI* repressor and T7 RNA polymerase, the latter controlled by an IPTG-inducible *LacUV5* promoter. Addition of IPTG induces transcription of the T7 RNA polymerase. The T7 RNA polymerase binds to the T7 promoter contained on the plasmid vector and transcribes the recombinant protein gene.

3.4 Plasmids

The intein α1-antitrypsin fusion protein is encoded by plasmid pT12AT1. The plasmid pT12AT1 was constructed from the plasmid pTYB12 for the IMPACT™ (Intein Mediated Purification with an Affinity Chitin-binding Tag) protein purification system (New England Biolabs). The expression of the fusion protein was under the control of an IPTG-inducible T7 promoter for T7 RNA polymerase.

In the intein α1-AT fusion protein, human α1-antitrypsin was fused to the C-terminus of a modified *Saccharomyces cerevisiae* intein (Sce VMA intein, 55 kD) containing a *Bacillus circulans* chitin-binding domain (CBD) (Chong et al., 1998a). The N-terminus of the fusion protein is composed of 12 amino acids that are derived from *E. coli* maltose binding protein (MBP) to provide a favorable translational start. Additionally, in cloning human α1-antitrypsin
gene from the plasmid pEAT8-137 (Laska, 2001a) using the *NdeI* site in pTYB12, three extra residues (Ala-Gly-His) were added to the N-terminus of α1-antitrypsin (Xu *et al*., 2000).

In the plasmid pEAT8-137, an N-terminal Glu in the human protein is replaced by Met to permit translation initiation for pEAT8-137 in *E. coli*. Compared to the cDNA sequence reported in the literature (Long *et al*., 1984), the pEAT8-137 α1-antitrypsin sequence contains the following base pair differences: codon 96 [Phe (TTC) → Phe (TTT)], codon 101 [Arg (CGT) → His (CAT)], codon 137 [Leu (UUG) → Leu (CUG)], and Codon 376 [Glu (GAA) → Asp (GAC)]. The initial parental plasmid pEAT8 was a generous gift of Dr. Myeong-Hee Yu of the Korea Research Institute of Bioscience and Biotechnology (Lee *et al*., 1993).

pMYB5 is a control plasmid for the IMPACT™ protein purification system. This plasmid carries the *E. coli malE* gene, encoding the maltose binding protein (MBP, 42 kD), fused to the N-terminus of the *Sce* VMA intein containing a chitin-binding domain. The expression of the fusion gene is also under the control of an IPTG-inducible T7 promoter. In this work, pMYB5 was used as a comparison control since it is the best scenario for the IMPACT™ protein purification system. The detailed information about pMYB5 can be found at the New England Biolabs web site: [http://www.neb.com](http://www.neb.com).

### 3.5 Cell Growth and Induction

Cell growth was monitored by measuring the optical density (OD) at the wavelength of 600 nm. Approximately 1 OD$_{600}$ ml of cells is equal to 0.34 g dry cell weight (Winkler, 1995). Overnight cultures were inoculated with frozen stocks at -85°C and grown in LB medium containing 50 µg/ml of ampicillin at 37°C for a period of ~12 hours. Typically, shake flasks were placed in a temperature-controlled forced air shaker at a rotation rate of 250 rpm for
cell growth. Overnight cultures typically achieved a cell density of ~7.0 and a portion was used to inoculate fresh semi-defined medium containing 50 µg/ml of ampicillin at an OD$_{600}$ of 0.05. When a cell density of 0.6-0.8 was reached, the temperature was lowered to 30°C and IPTG was added to a final concentration of 0.4 mM to induce the expression of the fusion protein for 3 hours. Other induction conditions with different induction temperatures and induction time are to be mentioned where they are used in this work.

3.6 Downstream Processing

The *E. coli* shake flask cultures were harvested and immediately cooled on ice. The cultures were centrifuged using an IEC CRU-5000 centrifuge (International Equipment Company) at 2,000 rpm for 30 minutes, washed with TE3 buffer (100 mM Tris, 5 mM EDTA, pH 8.0), and resuspended in TE3 buffer. The resuspended cells were transferred to 16×100 mm Durex™ borosilicate glass culture tubes and disrupted with a Branson Sonifier 450 with microtip (Branson Ultrasonics) at 50% duty cycle and output control 3 to release intracellular recombinant proteins. Each 10-ml aliquot was sonicated three 60-second cycles. To prevent excessive heating during sonication, glass sample tubes were suspended in an ice water bath and a 30-second rest period was allowed between sonication cycles. A non-clarified cell extract was directly loaded to the vortex flow system to be processed. If necessary, disrupted cell extract was centrifuged using an IEC Centra 4 centrifuge (rotor 820, International Equipment Company) at ~11,000 rpm for 30 minutes in the cold room. The supernatant was decanted and sterile-filtered with a 0.22 µm Millex®-GV syringe filter (Millipore) and stored on ice as soluble protein sample for further assays.
3.7 Protein Assay

3.7.1 Total Protein Assay

The total protein concentration in the samples was determined using the Bradford method with Bio-Rad protein assay reagent (Cat. 500-006). A standard curve was generated with bovine serum albumin (Fraction V, Sigma, A-7906).

3.7.2 Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Tris-HCl gels (Bio-Rad Ready Gels, cat. 161-1119 or 161-1155) were used with reducing sample buffer, shown in Table 3-4, for protein quantification. A Bio-Rad Mini-Protean® 3 Electrophoretic Cell filled with the running buffer listed in Table 3-5 (Sambrook et al., 1989) was used to run the gels. Polyacrylamide gels were stained by immersing in Coomassie blue staining solution with gentle shaking for at least two hours and destained in destaining solution until the background stain was sufficiently removed. The staining and destaining solutions were prepared according to Table 3-6 (Laska, 2001c).

### Table 3-4: SDS-PAGE Sample Buffer Solution

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Reducing Sample Buffer (based on 100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 M Tris • HCl, pH 6.8</td>
<td>12.5 ml</td>
</tr>
<tr>
<td>50% (v/v) Glycerol</td>
<td>20 ml</td>
</tr>
<tr>
<td>10% (w/v) SDS</td>
<td>20 ml</td>
</tr>
<tr>
<td>β-mercaptoethanol</td>
<td>5 ml</td>
</tr>
<tr>
<td>0.5% Bromophenol Blue</td>
<td>20 ml</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>22.5 ml</td>
</tr>
</tbody>
</table>
Table 3-5: SDS-PAGE Running Buffer Solution

<table>
<thead>
<tr>
<th>Reagent</th>
<th>5× (based on 1 Liter)</th>
<th>1× (pH 8.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris Base</td>
<td>15.1 g</td>
<td>25 mM</td>
</tr>
<tr>
<td>Glycine</td>
<td>94.0 g</td>
<td>250 mM</td>
</tr>
<tr>
<td>SDS</td>
<td>5.0 g</td>
<td>0.1 % (w/v)</td>
</tr>
</tbody>
</table>

Table 3-6: Coomassie Blue Staining and Destaining Solutions

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Staining Solution</th>
<th>Destaining Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coomassie Blue R-250</td>
<td>0.003 % (w/v)</td>
<td>-</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>10 % (v/v)</td>
<td>10% (v/v)</td>
</tr>
</tbody>
</table>

3.7.3 Western Blotting

Proteins were transferred from polyacrylamide gels to PVDF membranes (Bio-Rad, 162-0184) using a Bio-Rad Mini Trans-Blot® Electrophoretic Cell. Rabbit anti-human α1-antitrypsin IgG antibody (Sigma, A-0409) was used as the primary antibody and goat anti-rabbit IgG horseradish peroxidase conjugate (Sigma, A-0545) was used as the secondary antibody. ECL Plus detection reagents (Amersham Biosciences, RPN 2132) were used for detection. Membranes were exposed to Kodak X-OMAT AR film or Kodak Biomax MR film and developed.

3.7.4 Densitometry

A Molecular Dynamics Personal Densitometer SI was used with ImageQuant® software (Molecular Dynamics; Sunnyvale CA) to scan Coomassie-stained polyacrylamide gels and imaging films from chemiluminescent Western blotting. Protein bands were quantified
using a local median background correction method and were compared to $\alpha_1$-antitrypsin standards loaded on the gels when necessary.

3.7.5 N-terminal Amino Acid Sequencing

Protein bands separated with SDS-PAGE were transferred from a polyacrylamide gel to a Bio-Rad PVDF membrane with a CAPS (3-cyclohexylamino-1-propanesulfonic acid) transfer buffer (10 mM CAPS, 10% methanol, pH 11). After transfer, the PVDF membrane was stained with Coomassie Blue R-250 acetic acid solution, destained, rinsed with water, and air-dried. N-terminal amino acid sequencing was performed at the MIT Biopolymers Laboratory. Stained protein bands were cut from the membrane and sequenced by Edman degradation using an Applied Biosystems Model 474 Procidie Protein Sequencer, Model 140C Microgradient Delivery System, and Model 785A Programmable Absorbance Detector.

3.7.6 Activity Assay

The activity of $\alpha_1$-antitrypsin can be measured using any protease that is inhibited by antitrypsin. In this work, porcine pancreatic elastase was used to measure the activity of $\alpha_1$-antitrypsin. A major benefit of this assay is that it can be performed in either crude cell extracts containing the intein $\alpha_1$-antitrypsin fusion protein or purified $\alpha_1$-antitrypsin samples.

The elastase inhibitory capacity (EIC) of $\alpha_1$-antitrypsin or the intein $\alpha_1$-antitrypsin fusion protein was determined using a method previously described (Beatty et al., 1982) with slight modifications (Konz, 1998). The antitrypsin activity in a sample is determined by measuring the rate of an elastase-catalyzed reaction in the presence and absence of antitrypsin. The difference in reaction rates is equal to the amount of elastase inhibited by antitrypsin, or EIC of antitrypsin.
A 0.05%(w/v) elastase solution was prepared just before the assay by adding porcine pancreatic elastase (Sigma, E-1250) to 100 mM NaCl. The amount of elastase added was chosen so that the slope of the blank samples free of antitrypsin was approximately 1 mAU/Sec. A substrate working solution of 2 g/L N-succinyl-(Ala)$_3$-nitroanilide (Sigma, S-4760) in Tris buffer (100 mM, pH 8.0) was prepared from a stock of 40 g/L N-succinyl-(Ala)$_3$-nitroanilide in dimethyl sulfoxide stored at –20°C.

α1-Antitrypsin samples were mixed with 50 µl of the elastase working solution and Tris buffer to a final volume of 1.05ml and incubated at room temperature for 30 minutes. Residual free elastase was measured by combining 2 ml of Tris Buffer, 100 µl of substrate working solution, and the incubation mixture into a quartz cuvette. After rapid mixing, the appearance of the N-succinyl-(Ala)$_3$-nitroanilide cleavage product was measured at 410 nm for 30–60 seconds with a Hewlett Packard 8452A Diode Array spectrophotometer. The absorbance data were fit to a straight line and the slope was recorded in mAU/Sec. The EIC was calculated as the difference in slopes between the antitrypsin containing samples and the antitrypsin free controls. The specific activity was typically expressed on a unit volume (ml) or unit soluble protein (mg) basis. The cuvette was washed with dilute HCl (30 mM) between readings to improve the reproducibility of the results. Samples and blanks were incubated and assayed in triplicate.

3.8 Residence Time Distribution (RTD) Study

In residence time distribution (RTD) experiments, Alizarin Red S (Eastman) was used as a pulse tracer with molecular weight 326 and molecular diffusion coefficient, $D_m$, $7.5 \times 10^{-10}$ m$^2$/s at 22°C. A chromatography syringe was used for tracer injection through the sidewall port.
close to the bottom of vortex flow reactor. The injection volume was fixed at 200 µl in all the tracer experiments. The outlet tracer concentration was measured with a continuous flow UV detector with a 280 nm filter (Amersham Biosciences). The output signal in voltage from the UV detector was sent to a computer for further assays with Hyper Terminal software. In order to obtain a linear response from the UV detector, the tracer concentration in the injection sample was fixed at 2 g/L. The UV detector is ~40 cm down the outlet tubing with an inner diameter ~1.5 mm; thus the outlet holdup volume is ~1 ml, which is 0.5% of the whole annular volume of the vortex flow reactor.

3.9 Batch Adsorption Equilibrium Study

In the batch adsorption equilibrium experiments, bovine serum albumin (BSA, Fraction V, Sigma) was used as a model protein with molecular weight 68.5 kDa and pI 4.9. The BSA solution was prepared in Bis-Tris buffer (20 mM, pH 6.2) so that BSA was negatively charged. Streamline diethylaminoethyl (DEAE) anion exchange resin (Amersham Biosciences) was used, with a particle size of 100-300 µm and a particle density of 1.2 g/ml. Streamline is a range of adsorbents based on cross-linked agarose that is modified through the inclusion of a crystalline quartz core to provide the required density and robustness. The starting BSA concentration was in a range of 8 to 40 g/L. 1.5 ml sedimentated Streamline DEAE resin was mixed with 5 ml BSA solution and incubated at room temperature for 3 hours. The supernatant was removed and the residual BSA concentration was analyzed with a Hewlett Packard 8452A Diode Array spectrophotometer at 280 nm.
3.10 Batch Adsorption Kinetics Study

The batch adsorption kinetics experiments were conducted for the BSA and Streamline DEAE system. The preliminary experiments showed that the adsorption process is very fast, i.e., adsorption equilibrium can be reached within 30 minutes. If the initial BSA concentration is low, the BSA concentration must be continuously measured in order to catch concentration changes especially at the beginning of the adsorption process.

A regular glass beaker was modified as an adsorption reactor. As shown in Figure 3.5, there are two lateral ports on the sidewall of the reactor. The top port was used as the inlet port; the bottom one was covered with a piece of mesh and used as the outlet port. The solution, without adsorbent resin, was pumped out of the reactor by a peristaltic pump to a continuous flow UV detector with a 280 nm filter and pumped back to the reactor through the inlet port. 54 ml of 2.0 g/L BSA solution in Bis-Tris buffer (20 mM, pH 6.2) was added to the reactor. A magnetic stirrer was used to mix the solution. Once 35 ml of 50% (v/v) Streamline DEAE resin suspension in Bis-Tris buffer (20 mM, pH 6.2) was added to the beaker, the Hyper Terminal software in the computer connected to the UV detector was started to collect data. For the batch adsorption experiment, the starting BSA concentration was 1.5 g/L and the Streamline DEAE resin volume fraction was 20%. The adsorption experiment was stopped once the Hyper Terminal signal became stable and the adsorption equilibrium was reached.
3.11 Breakthrough Capacity Measurement

The breakthrough capacity was measured for the BSA and Streamline DEAE system. The concentration of BSA in the loading sample was fixed at 1.5 g/L in order to get a linear response from the UV detector. The loading sample was prepared in Bis-Tris buffer (20 mM, pH 6.2) so that BSA was negatively charged. Streamline DEAE resin was used, with a particle size of 100-300 µm and a particle density of 1.2 g/ml. The rotation of the inner cylinder needs to be above 200 rpm in order to fluidize Streamline DEAE adsorbent resin. The breakthrough capacity was obtained by calculating the amount of the loaded protein when the protein was first detected in the effluent of the reactor. In this work, the 10% breakthrough capacity, when the outlet protein concentration went up to 10% of the inlet loading protein concentration, was measured at different operating conditions.

3.12 Vortex Flow Adsorption Process

The operating conditions for the vortex flow adsorption process were as follows: rotation rate of inner cylinder was 330 rpm, which is the minimum rotation rate to fluidize the
chitin resin; axial loading flowrate was 6 ml/min, i.e., 33 cm/hr in superficial linear velocity; and chitin resin volume was 30 ml, i.e., 0.19 in resin volume fraction. 60 ml disrupted crude cell extract, with OD$_{600}$ = 35.0 before disruption and 3.2 g/L total protein after clarification, was loaded to the reactor. After loading, Tris buffer (0.1 M Tris, 5 mM EDTA, pH 8.0) was applied to wash off unbound proteins and cell debris until the readings of the UV detector and the Chem2000-UV-Vis spectrometer went to zero. 20 ml of 100 mM DTT in Hepes buffer (20 mM Hepes, 500 mM NaCl, pH 8.0) was mixed with the chitin resin for ~18 hours to induce in vitro peptide cleavage to release α1-antitrypsin from the chitin resin. Finally, 20 ml of 2% sodium dodecyl sulfate (SDS) aqueous solution was incubated with the chitin resin for ~4 hours to strip all the residual proteins on the chitin resin, which included the intein segment, non-cleaved fusion protein, and other non-specifically adsorbed proteins. Clarified cell extract, cleavage sample, and stripping sample were assayed to quantify the performance of the vortex flow adsorption process.

3.13 Cell Debris Pulse-Response Study

The disrupted crude *E. coli* cell extract was centrifuged and washed with Tris buffer (0.1 M Tris, 5 mM EDTA, pH 8.0) to remove the released proteins, nucleic acids, and other intracellular components. The washed *E. coli* cell debris was diluted with Tris buffer to OD$_{600}$ = 0.5 and used as a pulse tracer to study the possible interaction between *E. coli* cell debris and adsorbent resin (Fernández-Lahore *et al.*, 2000; Feuser *et al.*, 1999; Lin *et al.*, 2001). The cell debris concentration in the outlet stream of the vortex flow reactor was measured by the Chem2000-UV-Vis spectrometer at 600 nm. Two resins were used, Streamline DEAE anion exchange resin (Amersham Bioscience) and chitin resin. The operating conditions for the cell
debris tracer study were as follows: axial loading flowrate was 6 ml/min; rotation rate of inner cylinder was 320 rpm; resin volume in the annular gap of vortex flow reactor was 30 ml.
4 Characterization of Vortex Flow Adsorption System

4.1 Mathematical Model for Vortex Flow System

4.1.1 Introduction

The experiments were conducted extensively to characterize vortex flow adsorption, e.g., the tracer RTD experiments and the breakthrough capacity measurements for a model system of BSA with Streamline DEAE anion exchange resin. More details about the experimental results are discussed later in this chapter. The modeling is necessary to interpret the experimental data and understand the intrinsic mechanism of the vortex flow adsorption system. In this work, two modeling approaches were employed for the simulation of vortex flow adsorption: a one-dimensional dispersion convective model and a two-region vortex flow model.

The one-dimensional dispersion convective model treats the vortex flow reactor as a conventional reactor without considering the distinct features of vortices. It is a classic model that can be used to study the axial dispersion occurring in a reaction system. This model was employed to explore the axial dispersive behavior of the vortex flow reactor under different operating conditions.

Compared to the one-dimensional model, the two-region vortex model was put forward with the unique features of vortices considered. In the two-region model, in addition to the axial dispersion coefficient, a mass transfer coefficient is employed to describe the mass transfer within a natural unit, i.e., vortex. As one might expect, the two-region model can be used to integratively investigate mass transfer, axial dispersion, and adsorptive behavior occurring in the vortex flow adsorption system.
4.1.2 One-Dimensional Dispersion Convective Model

Mass transfer and reaction in a vortex flow reactor are governed by the species conservation equation for an incompressible fluid with concentration $C(r, t)$, fluid velocity $v(r)$, molecular diffusion coefficient $D_m$, reaction or adsorption rate $R(r, C)$, and spatial dimensions $r = (r, \theta, z)$.

$$\frac{\partial C(r,t)}{\partial t} + v(r) \cdot \nabla C(r,t) - D_m \nabla^2 C(r,t) = R(r, C)$$  \hspace{1cm} (4-1)

Since solving this equation directly is complex, simplifying the equation is important. One method of simplifying a complex transport equation is described by macrotransport theory (Brenner, 1980a; Brenner, 1980b). In general, the theory describes how an $n$-dimensional microtransport equation can be reduced into a one-dimensional macrotransport equation with constant coefficients. For the vortex flow reactor, this translates into transforming the three dimensional Equation (4-1) into an equation with just one spatial dimension – the axial direction, $z$ (Moore, 1994b).

Due to the spatial periodicity of vortex flow, Equation (4-1) can be simplified to a one-dimensional dispersion convective equation (Moore and Cooney, 1995) if two criteria are met (Tam and Swinney, 1987): the length of the test section is large compared to the vortices; the species is well mixed within the vortex in the radial and azimuthal directions on a time scale that is short compared to transport in the axial direction. As transport in the axial direction occurs by both convection and dispersion, the corresponding one-dimensional transport equation without reaction is,

$$\frac{\partial C(z,t)}{\partial t} + U \frac{\partial C(z,t)}{\partial z} - D_z \frac{\partial^2 C(z,t)}{\partial z^2} = 0$$  \hspace{1cm} (4-2)
where $C(z, t)$ is the vortex-averaged concentration at axial position $z$, $U$ is the axial superficial fluid velocity, and $D_z$ is the effective axial dispersion coefficient.

In a pulse tracer study, if there is dispersion neither upstream nor downstream of the vortex flow reactor, the closed boundary conditions are satisfied,

$$-D_z \frac{\partial C(z,t)}{\partial z} + UC(z,t) = 0 \quad z = 0 \quad (4-3)$$

$$-D_z \frac{\partial C(z,t)}{\partial z} = 0 \quad z = L \quad (4-4)$$

For a pulse tracer,

$$C(z,0) = C_{in} \delta(z) \quad (4-5)$$

the concentration profile at the outlet of the vortex flow reactor is given by (Yagi and Miyauchi, 1953),

$$\frac{C(L,t)}{C_{in}} = \sum_{i=1}^{\infty} \frac{\lambda_i Pe (Pe \sin \lambda_i + 2 \lambda_i \cos \lambda_i)}{(Pe^2/4 + Pe + \lambda_i^2)(Pe^2/4 + \lambda_i^2)} \exp \left(\frac{Pe}{2} - \left(\frac{Pe + \lambda_i^2}{4Pe}\right) Ut\right) \quad (4-6)$$

where

$$\cot \lambda_i = \frac{\lambda_i}{Pe} \frac{Pe}{4\lambda_i} \quad (4-7)$$

So the analytical solution is an infinite series. $Pe$ is the Peclet number and is defined as follows,

$$Pe = \frac{U \cdot L}{D_z} \quad \text{or} \quad = \frac{U \cdot L}{\varepsilon \cdot D_z} \quad \text{(without or with resin particles)} \quad (4-8)$$

where $\varepsilon$ is the reactor voidage and $L$ is the axial length of the vortex flow reactor.
For the closed boundary conditions in the tracer study, the following correlation
between the Peclet number, \( Pe \), the mean residence time, \( t_m \), and the variance in the residence
time, \( \sigma^2 \), is satisfied (Fogler, 1999).

\[
\frac{\sigma^2}{t_m^2} = \frac{2}{Pe} \frac{2}{Pe^2} (1 - \exp(-Pe))
\]

(4-9)

The mean residence time is equal to the first moment and the variance is the square of the
standard deviation of the tracer RTD curve. Therefore, from the tracer RTD data, the mean
residence time and the variance can be calculated. \( Pe \) and \( D_z \) can be obtained indirectly from
Equations (4-8) and (4-9).

### 4.1.3 Two-Region Vortex Flow Model

It was observed that when a tracer was injected into the outer layer of a vortex during
laminar Taylor vortex flow, narrow bands of dye formed at the interface between vortices
(Desmet et al., 1996a). These bands were most pronounced in vortices close to the injection
vortex. Based on this phenomenon, Desmet et al. proposed a two-zone model that takes into
account two different vortex regions: a vortex core and an external zone. A similar
phenomenon was observed for both laminar Taylor vortex flow and wavy vortex flow regimes
(Campero and Vigil, 1997). A similar approach, a serial network of continuous-stirred tank
reactors (CSTRs) with exchange volumes model, was used to interpret the experimental results.
Giordano et al. also adopted a two-region model with vortex core and bypass regions to
describe mass transfer in a vortex flow system (Giordano et al., 2000b). In all of these two-
region models, there is no mass transfer between two neighboring vortex core regions.
In this research, the two-region modeling approach was also employed to interpret the experimental results from tracer RTD study and dynamic capacity measurements. The modeling framework for the vortex flow reactor is illustrated in Figure 4.1. In this model, the vortex flow reactor is divided into stages along the axial direction and at each stage there are two regions: a vortex core and a bypass. Consequently, there are two coefficients, mass transfer coefficient between vortex core and bypass, $K_{bc}$, and axial dispersion coefficient between bypass regions, $D_L$. The third parameter is the volume ratio between the vortex core and bypass regions. Since we assume each stage to be as high as the annular gap width and the vortex core to be a circular toroid with a diameter equal to the annular gap width as well, this volume ratio is fixed.

![Figure 4.1: Two-region model for vortex flow system](image)

The species conservation equations for the vortex core and bypass regions are as follows:
In the species conservation equation for the bypass region, Equation (4-10), the left side represents the mass change rate of the species, and the right side represents contributions from axial convection, axial dispersion, mass transfer with vortex core, and reaction, respectively. In the species conservation equation for the vortex core region, Equation (4-11), the left side represents the mass change rate of the species, and the right side represents mass transfer with the bypass region and reaction, respectively. The superscripts b, c, and bc represent bypass, vortex core, and bypass-vortex core, respectively. N is the total number of stages, which is 17 for the configuration of the vortex flow reactor used in this work. Other variables are defined in Notation. In the tracer study, the reaction terms disappear.

The ordinary differential equations represented by Equations (4-10) and (4-11) were solved numerically by the backward differentiation formulas, also known as Gear’s method. The numerical integration was implemented by a MATLAB ordinary differential equation solving routine, ode15s. The axial dispersion coefficient $D_L$ and the vortex core-bypass mass transfer coefficient $K_{bc}$ were estimated with the Nelder-Mead simplex nonlinear optimization algorithm (Lagarias et al., 1998; Press et al., 1995). The objective function was the sum of the squares of the errors between the modeled and experimental tracer RTD data. The simplex nonlinear optimization algorithm is discussed later in this chapter.
4.1.4 Adsorption Equilibrium

In order to simulate vortex flow adsorption using the two-region vortex flow model, the adsorption parameters about adsorption equilibrium and kinetics are required.

Batch adsorption equilibrium experiments were conducted for the model system of BSA with Streamline DEAE anion exchange resin at a wide range of initial BSA concentrations. Two adsorption equilibrium mechanisms were employed to fit the experimental data: Langmuir mechanism and Bi-Langmuir mechanism. For Langmuir adsorption mechanism,

\[ C_s = \frac{QK}{1 + KC} \]  
\tag{4-12}

For Bi-Langmuir adsorption mechanism,

\[ C_s = \frac{QK}{1 + KC} + \frac{Q_2K_2C}{1 + K_2C} \]  
\tag{4-13}

In these equations, \( C_s \) is the adsorbed protein concentration, \( C \) is the fluid protein concentration, \( Q \) and \( Q_2 \) are saturated adsorbent capacities, and \( K \) and \( K_2 \) are adsorption affinity constants.

The simulation was implemented with Marquardt nonlinear least square regression in SAS. The modeling parameters and their values are listed in Table 4-1.

<table>
<thead>
<tr>
<th>Model Parameter</th>
<th>Q (g/L)</th>
<th>K (L/g)</th>
<th>Q_2 (g/L)</th>
<th>K_2 (L/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Langmuir</td>
<td>81.78</td>
<td>33.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bi-Langmuir</td>
<td>78.72</td>
<td>36.23</td>
<td>17.58</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The experimental and simulated adsorption equilibrium data are compared in Figures 4.2 and 4.3. It is clearly shown that the Langmuir mechanism works very well at low concentrations, and the error is considerable when it is used at high concentrations. However,
the Bi-Langmuir mechanism has a wide applicable concentration range, from low to high concentration. The possible explanation for the Bi-Langmuir mechanism is that there exist two kinds of adsorption sites on the adsorbent resin surface: one is the specific ion exchange site and the other is the nonspecific site that could adsorb protein molecules only when the protein concentration is high (Miyabe and Guiochon, 2000). In this work, the initial BSA concentration in the dynamic capacity measurements is around 1.5 g/L, and the Langmuir equation is good enough to be used to describe the adsorption equilibrium.

![Figure 4.2: Adsorption equilibrium at low BSA concentrations](image)
4.1.5 Adsorption Kinetics Mechanism

In the modeling of the batch adsorption kinetics, a film resistance and pore diffusion model was used. In this model, three consecutive mass transfer steps are assumed when the solute protein moves from the bulk fluid to the adsorbent resin (Holland and Liapis, 1983): (1) transfer of the solute protein from the bulk fluid to the opening of the pores, characterized by the film mass transfer coefficient, $K_f$; (2) transfer of the solute protein through the pores by diffusion, described by the pore diffusion coefficient, $D_p$; (3) adsorption interaction between the solute protein and the adsorbent resin.

The purpose of the batch adsorption kinetic experiments is to quantify $K_f$ and $D_p$, and these parameters can be used in both understanding the mass transfer mechanism for the batch adsorption and simulating the vortex flow adsorption process.

The adsorbent resin is suspended in the liquid of the finite bath by agitation, and the bulk concentration of the adsorbate is taken to be uniform throughout the bath except in a thin
film of liquid surrounding each particle (McCoy and Liapis, 1991). A differential mass balance for the adsorbate in the fluid phase of the finite bath gives,

\[
\frac{dC_d}{dt} = \frac{3}{r_0} \frac{1}{\varepsilon} K_f [C_p(r_0, t) - C_d]
\]  

(4-14)

\[C_d = C_0 \quad t = 0\]  

(4-15)

A differential material balance for the adsorbate in the adsorbent particle is given by,

\[
\varepsilon_p \frac{\partial C_p}{\partial t} + \frac{\partial C_s}{\partial t} = \varepsilon_p D_p \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial C_p}{\partial r} \right)
\]  

(4-16)

\[C_p = 0 \quad t = 0 \quad 0 \leq r \leq r_0\]  

(4-17)

\[C_s = 0 \quad t = 0 \quad 0 \leq r \leq r_0\]  

(4-18)

For boundary conditions,

\[
\varepsilon_p D_p \frac{\partial C_p}{\partial r} \bigg|_{r=r_0} = K_f \left[ C_d - C_p(r_0, t) \right] \quad t > 0
\]  

(4-19)

\[
\frac{\partial C_p}{\partial r} \bigg|_{r=0} = 0 \quad t > 0
\]  

(4-20)

In these equations, \(C_d\) is the bath protein concentration, \(C_p\) is the protein concentration in the pore fluid, and \(C_s\) is the adsorbed protein concentration on the adsorbent resin, \(\varepsilon_p\) is the resin porosity, \(\varepsilon\) is the fluid voidage in the reactor, \(r_0\) is the radius of the adsorbent resin.

Since the adsorption interaction is a chemical reaction and assumed to be fast, the equilibrium between the protein in the pore fluid and the adsorbed protein is assumed; this allows one to use the following form of the chain rule,

\[
\frac{\partial C_s}{\partial t} = \left( \frac{dC_s}{dC_p} \right) \left( \frac{\partial C_p}{\partial t} \right)
\]  

(4-21)
The Langmuir adsorption equilibrium mechanism is used here at low concentrations,

\[ C_s = \frac{QKC_p}{1 + KC_p} \quad (4-22) \]

Therefore, the equation for the chain rule is

\[ \frac{\partial C_s}{\partial t} = \left( \frac{dC_s}{dC_p} \frac{\partial C_p}{\partial t} \right) = \frac{QK}{(1 + KC_p)^2} \left( \frac{\partial C_p}{\partial t} \right) \quad (4-23) \]

In order to solve the partial differential equations listed above, an orthogonal collocation method (Holland and Liapis, 1983) was used to discretize the space variable, \( r \), in Equations (4-16), (4-19), and (4-20) and convert these equations into ordinary differential equations. In applying the orthogonal method to solve the equations, the first step is to convert the partial differential equations to a dimensionless form. The dimensionless variables were defined as follows,

\[
\begin{align*}
C_d' &= \frac{C_d}{C_0}, & C_p' &= \frac{C_p}{C_0}, & C_s' &= \frac{C_s}{C_s(0)}, & C_0 &= \frac{QKC_0}{1 + KC_0}, \\
\rho &= \frac{r^2}{r_0^2}, & \tau &= \frac{tD_{p0}}{A_0}, & A_0 &= 4\pi r_0^2, & sh &= \frac{2r_0K_f}{\varepsilon_pD_p}.
\end{align*}
\]

Therefore, the dimensionless form of the differential equations is,

\[
\frac{dC_d'}{d\tau} = 6\pi \frac{1 - \varepsilon}{\varepsilon \varepsilon_p} \cdot sh \left( C_p' - C_d' \right)_{\rho=1} \quad (4-24)
\]

\[ C_d' = 1 \quad \tau = 0 \quad (4-25) \]

\[
\left( \varepsilon_p + \frac{QK}{(1 + KC_0C_p')}^2 \right) \frac{\partial C_p'}{\partial \tau} = 4\pi \varepsilon_p \left( 6 \frac{\partial C_p'}{\partial \rho} + 4\rho \frac{\partial^2 C_p'}{\partial \rho^2} \right) \quad (4-26)
\]

\[ C_p' = 0 \quad \tau = 0 \quad 0 \leq \rho \leq 1 \quad (4-27) \]
In the dimensionless equations shown above, the dimensionless concentrations, $C_d'$, $C_p'$, and $C_s'$, and the dimensionless radial position, $\rho$, are in a range of 0 to 1.

4.1.6 Orthogonal Collocation Method

Once the dimensionless differential equations are available, the next step is to discretize the equations. A general form of the orthogonal method is interpreted before the discretization of the batch adsorption equations is implemented.

If a dependent variable, $y(u)$, in a differential equation can be expressed as a polynomial of an independent variable, $u$, i.e.,

$$y(u_i) = \sum_{j=1}^{n} a_j u_i^{j-1} \quad (i = 1, 2, \ldots, n)$$

and then,

$$\frac{dy}{du}|_{u=u_i} = \sum_{j=1}^{n} a_j (j-1) u_i^{j-2} \quad (i = 1, 2, \ldots, n)$$

$$\frac{d^2y}{du^2}|_{u=u_i} = \sum_{j=1}^{n} a_j (j-1)(j-2) u_i^{j-3} \quad (i = 1, 2, \ldots, n)$$

The discrete points $u_i (i = 1, 2, \ldots, n)$ are the roots of the associated orthogonal Jacobi polynomial,

$$P_n(x) = \frac{(-1)^n}{\Gamma(n+1)} \frac{d^n}{dx^n} \left[ (1-x)^n x^n \right]$$

$$\frac{4}{sh} \frac{\partial C'_p}{\partial \rho} \bigg|_{\rho=1} = \left( C'_d - C'_p \right) \bigg|_{\rho=1} \quad (4-28)$$

$$\frac{\partial C'_p}{\partial \rho} \bigg|_{\rho=0} \text{ is finite} \quad (4-29)$$
where \( \Gamma(n) \) is the gamma function and for integers \( \Gamma(n+1) = n! \). In this work, an eight-point orthogonal Jacobi polynomial was chosen, i.e., \( n = 8 \), and the corresponding roots were 0.02, 0.10, 0.24, 0.41, 0.59, 0.76, 0.90, and 0.98. The set of equations represented by Equation (4-30) may be stated in a matrix form,

\[
y = Qa
\]

where,

\[
y = [y(u_1) \quad y(u_2) \quad ... \quad y(u_n)]^T
\]

\[
a = [a_1 \quad a_2 \quad ... \quad a_n]^T
\]

\[
Q = \begin{pmatrix}
Q_{11} & \cdots & Q_{1n} \\
\vdots & \ddots & \vdots \\
Q_{n1} & \cdots & Q_{nn}
\end{pmatrix}
\]

\[Q_{ij} = u_i^{j-1}\]  \hspace{1cm} (4-35)

Similarly, for the set of equations given by Equations (4-31) and (4-32)

\[
\frac{dy}{du} = Ca \quad C_{ij} = u_i^{j-2}(j-1)
\]

\[
\frac{d^2y}{du^2} = Da \quad D_{ij} = u_i^{j-3}(j-1)(j-2)
\]  \hspace{1cm} (4-37)

After Equation (4-34) has been solved for \( a \),

\[
a = Q^{-1}y
\]  \hspace{1cm} (4-38)

and this result may be used to eliminate \( a \) from Equations (4-36) and (4-37)

\[
\frac{dy}{du} = CQ^{-1}y = Ay, \quad A = CQ^{-1}
\]  \hspace{1cm} (4-39)

\[
\frac{d^2y}{du^2} = DQ^{-1}y = By, \quad B = DQ^{-1}
\]  \hspace{1cm} (4-40)
where the elements of $A$ and $B$ are numbered in the same manner shown for $Q$. By applying the multiplication rule to row $i$ of Equations (4-39) and (4-40), we obtain

$$
\frac{dy}{du} \bigg|_{u_i} = \sum_{j=1}^{n} A_{ij} y(u_j) \quad (4-41)
$$

$$
\frac{d^2 y}{du^2} \bigg|_{u_i} = \sum_{j=1}^{n} B_{ij} y(u_j) \quad (4-42)
$$

In the orthogonal collocation method, by using only the information about the discrete points, as is shown in Equations (4-41) and (4-42), the first and second order derivatives can be expressed as polynomials of dependent variables at these discrete points. Through this manipulation, an ordinary differential equation can be converted into an algebraic equation and a partial differential equation can be converted into an ordinary differential equation.

In applying the above approach to discretize the spatial variable, $\rho$, in the dimensionless modeling equations for the batch adsorption, in addition to internal points, two boundary conditions were considered. Therefore, $n+1$ discrete points (including one at $\rho = 1$ and $n$ orthogonal points) were chosen. The boundary condition at $\rho = 0$, i.e., Equation (4-29), can be satisfied by choosing a specific form for the polynomial shown in Equation (4-30). In this situation, the polynomial was

$$
C_p(\rho_i) = \sum_{j=1}^{n+1} a_j \rho_i^{j-1} \quad (i = 1, 2, \ldots, n+1) \quad (4-43)
$$

$$
\frac{dC_d}{d\tau} = 6\pi \frac{1-\varepsilon}{\varepsilon} \varepsilon_p \ast sh \left( C_{p,n+1} - C_d \right) \quad (4-44)
$$

$$
C_d \bigg|_{\tau = 0} = 1 \quad (4-45)
$$
\[
\left( \varepsilon_p + \frac{QK}{1 + KC_0 C_{p,i}^{\prime}} \right) \frac{dC_{p,i}^{\prime}}{d\tau} = 4\pi \rho_p \sum_{j=1}^{n+1} A_{i,j} C_{p,j}^{\prime} + 4\rho_p \sum_{l=1}^{n+1} B_{i,l} C_{p,l}^{\prime}
\]  
(4-46)

\(i = 1, 2, \ldots, n\)

\[C_{p,i}^{\prime} = 0 \quad \tau = 0 \quad (i = 1, 2, \ldots, n+1) \]  
(4-47)

\[\frac{4}{8^2} \sum_{l=1}^{n+1} A_{n+1,l} C_{p,l}^{\prime} = C_d^{\prime} - C_{p,n+1}^{\prime} \]  
(4-48)

The resulting ordinary differential equations were solved numerically by the backward differentiation formulas. The numerical integration was implemented by a MATLAB ordinary differential equation solving routine, ode15s.

4.1.7 Initial Estimate of Modeling Parameters

In solving the differential equations discussed previously, the values of the pore diffusion coefficient \(D_p\) and the film mass transfer coefficient \(K_f\) are needed. The initial guesses of these parameters were made with literature correlations. The modified Polson equation can be used to estimate the molecular diffusion coefficients of large molecules (Geankoplis, 1983a),

\[D_m = \frac{9.40 \times 10^{-15} T}{\mu M^{1/3}} \]  
(4-49)

where \(M\) is the molecular weight of the large molecule (> 1 kDa), \(\mu\) is the viscosity of the dilute aqueous solution (Pa•S), \(T\) is the temperature (K), and \(D_m\) is the molecular diffusion coefficient (m²/s). The following correlation between \(D_m\) and \(D_p\) was used to estimate \(D_p\) (Miyabe and Guiochon, 1999),
\[ D_p = \left( \frac{\varepsilon_p}{2-\varepsilon_p} \right)^2 D_m \]  

(4-50)

where \( \varepsilon_p \) is the porosity of the solid particle, and 0.5 was chosen in this work.

The film mass transfer coefficient can be estimated using Equation (4-51) (Geankoplis, 1983b). It is valid to estimate the mass transfer coefficient from the liquid phase to the surface of small solid particles,

\[
K_f = \frac{2D_m}{d_p} + 0.31Sc^{\frac{2}{3}} \left( \frac{\rho_p - \rho_f}{\rho_f^2} \right)^{\frac{1}{3}}
\]

(4-51)

where \( D_m \) is the molecular diffusion coefficient of the solute in the solution (m\(^2\)/s), \( d_p \) is the diameter of the solid particle (m), \( \mu \) is the viscosity of the solution (kg/(m\(\cdot\)s)), \( g \) is the gravity constant, 9.80665 m/s\(^2\), \( \rho_f \) is the density of the solution (kg/m\(^3\)), \( \rho_p \) is the density of the solid particle (kg/m\(^3\)), and \( Sc = \mu/(\rho_f \cdot D_m) \).

### 4.1.8 Simulation of Batch Adsorption Kinetics

The values of the pore diffusion coefficient \( D_p \) and the film mass transfer coefficient \( K_f \) were eventually obtained through the Nelder-Mead simplex nonlinear optimization algorithm (Lagarias et al., 1998; Press et al., 1995). The objective function was the sum of the squares of the errors between the modeled and experimental batch adsorption kinetics data. The Nelder-Mead simplex nonlinear optimization algorithm is also called downhill simplex method. It requires only function evaluations, not derivatives. Therefore, it is a very good method for this work. Since every evaluation of the objective function is based on the solution to the differential equations, it is impossible to get the derivative values for the objective function. An
example of the experimental and simulated batch adsorption kinetics data is shown in Figure 4.4. The modeling parameters and their values are listed in Table 4-2.

![Figure 4.4: Experimental and simulated batch adsorption kinetics data](image)

Initial BSA concentration is 1.52 g/L, the total volume of the suspension is 89 ml, and Streamline DEAE volume is 18 ml (0.2 in volume fraction)

Table 4-2: Modeling Parameters for Adsorption Kinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( K_f ) (m/s)</th>
<th>( D_p ) (m(^2)/s)</th>
<th>( \varepsilon_p )</th>
<th>( r_0 ) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>( 5.68 \times 10^{-6} )</td>
<td>( 5.45 \times 10^{-11} )</td>
<td>0.5</td>
<td>100</td>
</tr>
</tbody>
</table>
4.1.9 **Downhill Simplex Method**

The downhill simplex method was first put forward by Nelder and Mead (Nelder and Mead, 1965). This nonlinear optimization algorithm was used in this work for all the optimization calculations. A simplex is a geometrical figure consisting, in n dimensions, of n+1 points or vertices, all their interconnecting line segments, and polygonal faces. In two dimensions, a simplex is a triangle. In three dimensions, it is a tetrahedron. In general, a simplex is nondegenerate, i.e., it encloses a finite n-dimensional volume.

The downhill simplex method must be started not just with a single point, but with n+1 points, defining an initial simplex. It takes a series of steps, most steps just moving the point of the simplex where the function is largest through the opposite face of the simplex to a lower point. These steps are called reflections and they are constructed to conserve the volume of the simplex and hence maintain its nondegeneracy. When it can do so, the method expands the simplex in one or another direction to take larger steps. When it reaches a minimum value, the method contracts the simplex in the transverse direction. If there is a situation where the simplex is trying to pass through a lowest point, it contracts itself in all directions, pulling itself in around its lowest point. The basic movements are summarized in Figure 4.5 (Press et al., 1995). By following these moving steps, it terminates when the vector distance moved in a specific step is fractionally smaller in magnitude than some tolerance defined in the optimization process.
Figure 4.5: Possible outcomes for a step in the downhill simplex method

The simplex at the beginning of the step, a tetrahedron, is shown at the top. The simplex at the end of the step can be any of: (1) a reflection away from the highest point, (b) a reflection and expansion away from the highest point, (c) a contraction along one dimension from the highest point, and (d) a contraction along all dimensions towards the lowest point. An appropriate sequence of such steps always converges to a minimum of the function.
4.1.10 Vortex Flow Adsorption Model

The basic idea of vortex flow adsorption simulation is to include the film resistance and pore diffusion model and the Langmuir adsorption model into the modeling framework of the two-region vortex flow model to account for adsorptive interaction between protein and adsorbent resin. The species conservation equations for the vortex core and bypass regions are as follows,

\[
\frac{dC^b_i}{dt} = \frac{V_{ax}}{\varepsilon V_i^b} (C^b_{i-1} - C^b_i) + \frac{AD_i}{dV_i^b} \left[ (C^b_{i+1} - C^b_i) - (C^b_i - C^b_{i-1}) \right] \\
- \frac{K_{bc} S_i^{hc}}{V_i^b} (C^b_i - C^c_i) - \frac{1 - \varepsilon}{\varepsilon} \frac{3}{r_0} K_f \left( C^b_i - C^b_{i,p} (r_0, t) \right) \quad i = 1,\ldots,N
\]

\[
C^b_i = 0 \quad t = 0
\]

\[
\frac{dC^c_i}{dt} = \frac{K_{bc} S_i^{hc}}{V_i^c} (C^b_i - C^c_i) - \frac{1 - \varepsilon}{\varepsilon} \frac{3}{r_0} K_f \left( C^c_i - C^c_{i,p} (r_0, t) \right) \quad i = 1,\ldots,N
\]

\[
C^c_i = 0 \quad t = 0
\]

The species conservation equation for the adsorbent phase used in the batch adsorption simulation is employed,

\[
\varepsilon_p \frac{\partial C_p}{\partial t} + \frac{\partial C_s}{\partial t} = \varepsilon_p D_p \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial C_p}{\partial r} \right)
\]

\[
C_p = 0 \quad t = 0 \quad 0 \leq r \leq r_0
\]

\[
C_s = 0 \quad t = 0 \quad 0 \leq r \leq r_0
\]

For boundary conditions,

\[
\varepsilon_p D_p \left. \frac{\partial C_p}{\partial r} \right|_{r=r_0} = K_f \left[ C - C_p (t, r_0) \right] \quad t > 0
\]
The local protein concentrations in the pore fluid and in the adsorbent phase can be related by the following chain rule equation,

\[
\frac{\partial C_p}{\partial r} \bigg|_{r=0} = 0 \quad t > 0 \tag{4-60}
\]

The Langmuir adsorption mechanism is employed,

\[
C_s = \frac{Q KC_p}{1 + K C_p} \tag{4-62}
\]

Therefore, the equation for the chain rule is

\[
\frac{\partial C_s}{\partial t} = \left( \frac{dC_s}{dC_p} \right) \left( \frac{\partial C_p}{\partial t} \right) = \frac{Q K}{(1 + K C_p)^2} \left( \frac{\partial C_p}{\partial t} \right) \tag{4-63}
\]

It is necessary to note that Equations (4-56) to (4-63) are valid for both the vortex core and bypass regions at each stage along the axial direction in the vortex flow reactor and the subscript \( i \) and the superscripts \( b \) and \( c \) are not labeled for the concentration terms, \( C, C_s, C_p, \) and \( C_p(t, r_0) \).

The ordinary and partial differential equations listed above provide the basis for the simulation of the vortex flow adsorption process. As in the batch adsorption simulation, an orthogonal collocation method with eight internal points was applied to the space variable \( r \) in Equations (4-56), (4-59), and (4-60). The resultant nonlinear ordinary differential equations were solved numerically by the backward differentiation method. The numerical integration was implemented by a MATLAB ordinary differential equation solving routine, ode15s.
Since all the necessary parameters, e.g., Q, K, D_p, and K_f, were estimated from the batch adsorption simulation, the experimental breakthrough profile in vortex flow adsorption was fitted to the vortex flow adsorption model through the Nelder-Mead simplex nonlinear optimization algorithm. The objective function was the sum of the squares of the errors between the modeling and experimental data. Both the axial dispersion coefficient D_L and the vortex core-bypass mass transfer coefficient K_{bc} were obtained.
4.1.11 Notation

A  Annular cross-sectional area of vortex flow reactor, L$^2$
C$_d$  Bath protein concentration, M/L$^3$
C$_0$  Initial bath protein concentration, M/L$^3$
C$_i^b$  Concentration in the bypass region of stage i, M/L$^3$
C$_i^c$  Concentration in the vortex core region of stage i, M/L$^3$
C$_p$  Concentration in the pore fluid of adsorbent resin, M/L$^3$
C$_s$  Concentration in the solid phase of adsorbent resin, M/L$^3$
d  Annular gap width, L
D$_m$  Molecular diffusion coefficient, L$^2$/T
D$_L$  Bypass axial dispersion coefficient, L$^2$/T
D$_p$  Pore diffusion coefficient, L$^2$/T
D$_z$  Axial dispersion coefficient, L$^2$/T
K  Adsorption affinity constant, (M/L$^3$)$^{-1}$
K$_2$  Adsorption affinity constant, (M/L$^3$)$^{-1}$
K$_{bc}$  Vortex core- bypass mass transfer coefficient, L/T
K$_f$  Film mass transfer coefficient, L/T
L  Axial length of vortex flow reactor, L
N  Number of Stages along the axial direction of vortex flow reactor
Q  Adsorption saturated capacity, M/L$^3$
Q$_2$  Adsorption saturated capacity, M/L$^3$
r$_0$  Adsorbent particle radius, L
R$_i^b$  Reaction rate in the bypass region of stage i, M/(L$^3$-T)
R$_i^c$  Reaction rate in the vortex core region of stage i, M/(L$^3$-T)
S$_i^{bc}$  Interfacial area between vortex core and bypass at stage i, L$^2$
t$_m$  Mean residence time, T
U  Axial superficial velocity, L/T
U$_{ax}$  Axial superficial velocity, L/T
V$_{ax}$  Axial flowrate, L$^3$/T
V$_i^b$  Volume of the bypass region of stage i, L$^3$
\( V_i^c \) Volume of the vortex core region of stage \( i \), \( L^3 \)

\( Z_i \) Axial coordinate of the upper boundary of stage \( i \), \( L \)

\( \varepsilon \) Reactor voidage

\( \varepsilon_p \) Adsorbent pore voidage

\( \sigma^2 \) Variance of tracer RTD data, \( T^2 \)

\( \omega \) Angular velocity of inner cylinder, \( T^{-1} \)

\( Pe \) Peclet number (= \( U_{ax}L/\varepsilon D_z \) or \( U_{ax}L/D_z \))
4.2 Tracer Residence Time Distribution Study in Vortex Flow Reactor

4.2.1 Introduction

The performance of a vortex flow system varies with operating conditions, including rotation rate of the inner cylinder, axial loading flowrate, and adsorbent resin volume fraction. An objective of this research is to characterize the performance of the vortex flow system, particularly the axial dispersion, through tracer residence time distribution (RTD) experiments.

In the tracer study, two sets of experiments were conducted. The first group was to inject the tracer Alizarin Red S directly into the bottom vortex in the vortex flow reactor, and the second group was to fluidize Streamline sulphopropyl (SP) resin in the vortex flow reactor and then inject the tracer Alizarin Red S into the bottom vortex. Since there was no interaction between Alizarin Red S and Streamline SP, which was confirmed by the mass balance on Alizarin Red S, Streamline SP resin was regarded as inert.

Streamline SP was used as an inert resin considering that the physical properties of Streamline SP are the same as those of Streamline DEAE anion exchange resin except for the functional group. Therefore, by using the inert resin in the tracer RTD study, the axial dispersion in the actual vortex flow adsorption can be predicted. Due to the physical properties of Streamline SP, including particle size and particle density, the resin cannot be fluidized until the rotation rate of the inner cylinder is above approximately 200 rpm. So the tracer RTD experiments were conducted without resin under 150 rpm and with resin above 200 rpm.

4.2.2 The Effect of Rotation Rate of Inner Cylinder

Figure 4.6 shows the tracer RTD results at relatively low rotation rates, including 15 rpm, 30 rpm, 50 rpm, and 150 rpm. For the purpose of clarity, the result at the rotation rate of
80 rpm is not included. The peak time at each rotation rate is plotted in Figure 4.7. It indicates that the peak time decreased from 22.0 to 4.0 minutes when the rotation speed changed from 15 rpm to 150 rpm. However, the space time in the vortex flow reactor is 26.7 minutes based on the annular volume of 160 ml and the axial flowrate of 6 ml/min. It is apparent that the vortex flow reactor behaves like a plug flow reactor (PFR) at low rotation rates, such as 15 rpm and 30 rpm, under which tracer molecules flow out of the reactor with the average residence time close to the space time. Once the rotation rate goes up to 150 rpm, the reactor behaves like a continuous-stirred tank reactor (CSTR), with both early mixing and extended delay. The transition from plug flow performance to CSTR occurs around 50 rpm. In addition, since the same amount of tracer was injected in all the tracer experiments, the areas under all the RTD curves were equal.

The tracer RTD results at relatively high rotation rates are shown in Figure 4.8. Only the results at 200 rpm and 400 rpm are presented because the tracer RTD profiles overlap each other as rotation rate goes up to high values ranging from 200 rpm to 400 rpm. At this range of rotation rate, the vortex flow reactor performs exactly like a CSTR.

As is discussed previously, for a pulse tracer, the analytical solution to the one-dimensional model is an infinite series, so the tracer RTD profiles under different rotation rates were simulated only through the two-region vortex flow model. In Figure 4.9, the simulated and experimental tracer RTD profiles at a low rotation rate of 50 rpm and a high rotation rate of 200 rpm are presented. By adjusting two modeling parameters, $K_{bc}$ and $D_L$, the model can simulate the experimental data very well. Therefore, the two-region model can describe the main mass transport and axial dispersion features of the vortex flow reactor very well.
Figure 4.6: Tracer RTD results at low rotation rates of inner cylinder

Axial flowrate: 6 ml/min, tracer: 0.2 ml of 2 g/L Alizarin Red S.

Figure 4.7: Peak time of tracer RTD curves at low rotation rates of inner cylinder

The error bars represent the standard deviations of at least duplicate runs.
Figure 4.8: Tracer RTD results at high rotation rates of inner cylinder

Axial flowrate: 6 ml/min, Streamline SP resin volume: 30 ml, tracer: 0.2 ml of 2 g/L Alizarin Red S.

Figure 4.9: Two-region vortex flow modeling results

At rotation rate of 50 rpm, \( D_L = 4.50 \times 10^{-6} \text{ m}^2/\text{s} \), \( K_{bc} = 5.00 \times 10^{-3} \text{ m/s} \); at 200 rpm, \( D_L = 1.30 \times 10^{-4} \text{ m}^2/\text{s} \), \( K_{bc} = 2.55 \times 10^{-5} \text{ m/s} \). Only the main parts of the experimental and modeling results are shown here for the purpose of clarity.
Another approach to analyzing the axial dispersion in the vortex flow reactor is through the tanks-in-series model. In this model, the number of CSTRs in series, which give approximately the same RTD as the vortex flow reactor, can be determined. The number of tanks, \( n \), is related to the mean residence time, \( t_m \), and the variance in residence time, \( \sigma^2 \), from the tracer RTD data (Fogler, 1999),

\[
n = \frac{t_m^2}{\sigma^2}
\]  \hspace{1cm} (4-64)

Figure 4.10 shows the number of equivalent CSTRs in the vortex flow reactor under different rotation rates. It is clearly shown that, when the rotation rate was very low, e.g., 15 rpm, the number of CSTRs was 9, however, if the rotation rate went to 80 even 150 rpm, the number of CSTRs was close to 1. It means that the vortex flow reactor behaves like a plug flow reactor at low rotation rates and the whole reactor performs like a CSTR at high rotation rates.

As discussed in the two-region vortex flow model, for the configuration of the vortex flow reactor used in this work, the whole reactor can be divided into 17 stages along the axial direction with each stage as high as the annular gap width. Each vortex has two boundaries, one is the inflow boundary and the other is the outflow boundary. The radial velocity is higher at the outflow boundary than the inflow boundary. As a consequence, the mass transfer is much better at the outflow boundary. In this sense, every two neighboring vortices with a common outflow boundary can be regarded as a unit or a contacting stage. Therefore, there are 9 units in the vortex flow reactor. This number is exactly the same as the number of equivalent CSTRs in the vortex flow reactor when the rotation rate is low, e.g., 15 rpm. The low number of CSTRs at high rotation rates, e.g., 80 rpm and 150 rpm, means that the axial dispersion between units is very strong so that the whole reactor behaves like a single CSTR.
Figure 4.10: The number of CSTRs at different rotation rates of inner cylinder

When the one-dimensional dispersion convective model and the two-region vortex flow model were applied to fit the tracer RTD data, the axial dispersion coefficients were obtained at different rotation rates. Figures 4.11 and 4.12 show the fitted axial dispersion coefficients from the two models, respectively. In both cases, when the rotation rate was below 150 rpm, the fitted axial dispersion coefficient was very sensitive to the rotation rate. However, if the rotation rate was above 200 rpm, the axial dispersion coefficient was almost constant, independent of the rotation rate.
Figure 4.11: Fitted axial dispersion coefficients from one-dimensional dispersion convective model at different rotation rates.

The error bars represent the standard deviations of duplicate runs.

Figure 4.12: Fitted axial dispersion coefficients from two-region vortex flow model at different rotation rates.

Each point represents the mean value obtained from duplicate runs.
If the absolute values of the fitted axial dispersion coefficients from the two models are compared to each other, the values were almost one order of magnitude higher with the two-region vortex flow model. In the one-dimensional dispersion convective model, rapid mixing within each stage (including vortex core and bypass) is assumed and there is neither radial dispersion nor azimuthal dispersion. Therefore, only axial dispersion is considered. In fact, the mixing within each stage is slow, so narrow bands of tracer dye form at the interface between vortices (Campero and Vigil, 1997; Desmet et al., 1996a) when the tracer is injected in the outer layer of the vortices, i.e., the bypass region in the two-region vortex flow model. In the two-region vortex flow model, it is assumed that there is finite mass transfer between vortex core and bypass and no mass transfer between two neighboring vortex core regions. The axial dispersion occurs between two neighboring bypass regions. Due to the finite mass transfer between vortex core and bypass regions, when the tracer is injected into the bypass region, the majority of the tracer stays in the bypass region and the tracer sees a well mixed region with a volume of $V^b$ instead of $V^b + V^c$ assumed in the one-dimensional model. It is noted that $V^b$ is the bypass region volume and $V^c$ is the vortex core region volume. Therefore, the axial dispersion coefficient is higher from the two-region model than that from the one-dimensional model, which agrees with the literature result (Campero and Vigil, 1997). In this sense, the two-region model is more precise than the one-dimensional model in describing the distinct features of the vortex flow system.
If Figure 4.11 is redrawn using the relative Taylor number, \( \frac{Ta}{Ta_c} \), instead of the rotation rate, Figure 4.13 is obtained. The Taylor number, \( Ta \), is defined as,

\[
Ta = \frac{\omega \cdot R_i \cdot d}{\nu}
\]  

(4-65)

where \( \omega \) is the angular velocity of the inner cylinder and \( \nu \) is the kinematic viscosity. Like the Reynolds number, the Taylor number represents the ratio of the inertial force to the viscous force. For the configuration of the vortex flow reactor used in this work, \( Ta_c \) is calculated as 78.6 by Equation (4-66) (Esser and Grossmann, 1996), very close to 77.7 calculated from Equation (4-67) (Lee and Lueptow, 2001).

\[
Ta_c = \frac{1}{0.1556^2} \left( \frac{1+\eta}{2\eta} \right)^{0.5}
\]  

(4-66)

\[
Ta_c = 41.02 \left( \frac{1-\eta}{\eta} \right)^{0.5} + 25.75 \left( \frac{1-\eta}{\eta} \right)^{0.5} + 1.85 \left( \frac{1-\eta}{\eta} \right)^{1.5}
\]  

(4-67)

where \( \eta = R_i/R_o \). Only when the Taylor number, \( Ta \), exceeds the critical Taylor number, \( Ta_c \), does the instability appear as counter-rotating toroidal vortices stacked in the annulus. With the increase of the Taylor number, there are different vortex flow regimes (Sczechowski et al., 1995): Taylor vortex flow, wavy and spiraling vortex flow, turbulent vortex flow, and turbulent flow without vortices, as listed in Table 4-3. Vortex structures, mass transfer features, and axial dispersion characteristics are different in different vortex flow regimes. Figure 4.13 suggests that: in the Taylor vortex flow regime, the axial dispersion coefficient is low and very sensitive to the change in the rotation rate; in the wavy and spiraling vortex flow regime, the varying
trend of the axial dispersion coefficient becomes flat; in the turbulent vortex flow regime, the axial dispersion coefficient is almost constant even though the rotation rate varies.

![Figure 4.13: Fitted axial dispersion coefficients from one-dimensional dispersion convective model put in the relative Taylor number coordinate](image)

**Table 4-3: Flow Regimes in Vortex Flow Reactor**

<table>
<thead>
<tr>
<th>Ta</th>
<th>Flow Regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_a &lt; 15T_a) c</td>
<td>Taylor Vortex Flow</td>
</tr>
<tr>
<td>(15T_a &lt; 30T_a) c</td>
<td>Wavy Vortex and Spiraling Vortex Flow</td>
</tr>
<tr>
<td>(30T_a &lt; 160T_a) c</td>
<td>Turbulent Vortex Flow</td>
</tr>
<tr>
<td>(\text{&gt; } 250 T_a) c</td>
<td>Turbulent Flow</td>
</tr>
</tbody>
</table>

Based on the one-dimensional dispersion convective model, an empirical correlation was developed between the Peclet number, Pe, and the operating parameters, e.g., the axial Reynolds number, Re\(_{z_a}\) and the Taylor number, Ta, as well as the geometrical dimensions of the vortex flow reactor (Moore and Cooney, 1995),
\[ P e^{-1} = 7.2 \times 10^{-3} \left( \frac{d}{R_i} \right)^{0.28} (2Ta)^{1.05} (2Re_{ax})^{-0.83} \left( \frac{2d}{L} \right) \] (4-68)

where \( Re_{ax} = \frac{U_{ax}d}{\nu} \). In this study, the axial dispersion coefficients were calculated with this correlation under different rotation rates. As is shown in Figure 4.14, the changing trend of the axial dispersion coefficient with the rotation rate of the inner cylinder is similar among different modeling approaches.

![Simulated axial dispersion coefficients from different models](image)

**Figure 4.14: Simulated axial dispersion coefficients from different models**

Therefore, by varying the rotation rate of the inner cylinder, the vortex flow reactor can span from a plug-flow reactor with weak axial dispersion in the Taylor vortex flow regime to a CSTR with strong axial dispersion in the turbulent vortex flow regime. In this sense, the vortex flow reactor provides much flexibility in operation.
4.2.3 The Effect of Axial Loading Flowrate

In a vortex flow system, the rotation rate of the inner cylinder is a very important operating variable in determining the performance of the vortex flow reactor. Therefore, the previous discussion is focused on the effect of the rotation rate on the axial dispersion. However, the axial flowrate determines the processing time and throughput of the vortex flow adsorption process. It is important to know whether the axial flowrate affects axial dispersion as well.

Tracer RTD experiments were performed under different axial flowrates ranging from 6 to 24 ml/min. As is shown in Figure 4.15, the majority of the tracer flowed through the vortex flow reactor more quickly at a high flowrate than at a low flowrate. However, unlike the RTD curves at low rotation rates, the peak time for all the RTD curves at different axial flowrates was ~2.5 minutes. If the processing time is normalized by the space time, as shown in Figure 4.16, the tracer RTD curves at different axial flowrates almost overlapped each other. Therefore, a fast flow-through of the tracer at a high flowrate was not due to a high axial dispersion but a fast convective motion. If the one-dimensional dispersion convective model was used to derive the axial dispersion coefficient from the tracer RTD data, as shown in Figure 4.17, with the increase of the axial flowrate, the axial dispersion coefficient slightly increased.

Therefore, although the axial flowrate is very important in determining the processing time and throughput of vortex flow adsorption, it is not so important to affect the axial dispersive behavior in the vortex flow reactor as much as the rotation rate of the inner cylinder does.
Figure 4.15: Tracer RTD results at different axial flowrates

Rotation rate of inner cylinder: 250 rpm, Streamline SP resin volume: 30 ml, tracer: 0.2 ml of 2 g/L Alizarin Red S.

Figure 4.16: Tracer RTD results at different axial flowrates with the dimensionless time as the horizontal axis
Figure 4.17: Fitted axial dispersion coefficients at different flowrates from one-dimensional dispersion convective model

4.2.4 The Effect of Adsorbent Resin Volume Fraction

In addition to the rotation rate and the axial flowrate, another operating variable that could affect the performance of vortex flow adsorption is the adsorbent resin volume fraction. In packed column chromatography, the axial dispersion is low compared to expanded bed adsorption. Therefore, it is very natural to postulate that, if the adsorbent resin volume fraction is increased in the vortex flow system, the axial dispersion could be decreased.

As is shown in Figure 4.18, tracer RTD experiments were conducted at a range of adsorbent resin volume fraction from 0.06 up to 0.56, and the later was very close to the resin volume fraction of 0.6 in packed bed chromatography (McCoy and Liapis, 1991; Thömmes et al., 1996). With the increase of the resin volume fraction, although the magnitude of the tracer RTD peak increased slightly, the peak time was almost fixed at ~4 minutes. It seems that the axial dispersion cannot be attenuated by increasing the adsorbent resin volume fraction. If the
one-dimensional dispersion convective model was applied to derive the axial dispersion coefficient from the tracer RTD data, as shown in Figure 4.19, with the increase of the adsorbent resin volume fraction, the axial dispersion was almost invariant.

In the vortex flow adsorption system, the rotation rate of the inner cylinder has to be higher than a minimum fluidization rotation rate to maintain the adsorbent resin fluidized. Once the resin is fluidized, the motion of the particles follows the streamline of vortices. Therefore, by manipulating the adsorbent resin volume fraction, the axial dispersive behavior in the vortex flow reactor cannot be attenuated. However, in packed bed chromatography, the resin is packed, so the loading fluid flows through the void in the column and the axial dispersion is quite low.

![Figure 4.18: Tracer RTD results at different resin volume fractions](image)

Rotation rate of inner cylinder: 200 rpm, axial loading flowrate: 6 ml/min, resin: Streamline SP, and tracer: 0.2 ml of 2 g/L Alizarin Red S.
4.2.5 Summary

Through tracer RTD experiments, the axial dispersion occurring in the vortex flow system was explored under different operating conditions. It was demonstrated that, among the operating variables (including the rotation rate of the inner cylinder, the axial flowrate, and the adsorbent resin volume fraction), the rotation rate of the inner cylinder is the most important in determining the axial dispersion in the vortex flow system. A Taylor vortex flow regime is most desired if low axial dispersion is necessary. Once the operation goes to the wavy and turbulent vortex flow regimes, the change of the axial dispersion due to the rotation rate is negligible.

Figure 4.19: Fitted axial dispersion coefficients at different resin volume fractions from one-dimensional dispersion convective model
4.3 Adsorption Studies in Vortex Flow System

4.3.1 Introduction

In the previous section, the vortex flow system was characterized as a chemical reactor. The study is very useful in identifying how far or close the vortex flow reactor is to an ideal PFR or CSTR at specific operating conditions. In applying the vortex flow adsorption system to recovering biochemical products directly from crude cell extract, the axial dispersion needs to be understood in a complex context with the adsorption mechanism and the mass transfer of the adsorbate. In this section, the characterization of the vortex flow system as an adsorption column is discussed. The breakthrough or dynamic capacity of the model system was measured under different operating conditions. The advantage of a model system study is its simplicity and usefulness in predicting the adsorptive behavior in the practical application of the vortex flow adsorption system.

4.3.2 Breakthrough Capacity

The dynamic capacity, $Q_B$, also called the breakthrough capacity, is defined as the total amount of protein continuously loaded to the column per unit settled adsorbent at $V = V_B$ (Chang and Chase, 1996a),

$$Q_B = \frac{C_0 \cdot V_b}{V_s}$$  \hspace{1cm} (4-69)

where $Q_B$ is the dynamic or breakthrough capacity (g/L), $C_0$ is the inlet concentration of protein (g/L), $V_b$ is the volume (L) of the loaded protein solution at the breakthrough point ($C/C_0$), and $V_s$ is the volume of the settled adsorbent in the column (L). As an alternative to the equilibrium capacity, the dynamic capacity is the capacity of an adsorption column or reactor
under defined operating conditions of flowrate, pH, salt concentration, sample concentration, and so on (Coligan et al., 1995). For vortex flow adsorption, rotation rate should be included into these operating variables. In addition, since vortex flow adsorption is a fluidized adsorption, the column voidage or the adsorbent volume fraction should also be included. Therefore, the dynamic capacity can be used to characterize vortex flow adsorption performance under different operating conditions including rotation rate, axial loading flowrate, and adsorbent volume fraction.

In this work, the 10% breakthrough capacity of BSA with Streamline DEAE anion exchange resin was measured under different operating conditions. The 10% breakthrough capacity was used since it is a practical capacity of the column to ensure high recovery of the target protein (Coligan et al., 1995). Figure 4.20 gives an example of the breakthrough profiles of vortex flow adsorption under different axial flowrates. When the breakthrough point, \( C/C_0 \), where \( C \) is the outlet concentration and \( C_0 \) is the inlet loading concentration, goes up to 0.1, the corresponding loaded amount of BSA per unit settled Streamline DEAE adsorbent resin in the vortex flow reactor is the so-called 10% breakthrough capacity.
4.3.3 The Effect of Axial Loading Flowrate

In Figure 4.21, the 10% breakthrough capacity at different axial flowrates is obtained from Figure 4.20 and plotted with the axial flowrate. It is shown that with the increase of the axial flowrate, the breakthrough capacity decreased. With the increase of the axial flowrate, the corresponding residence time becomes shorter and the BSA molecules do not have sufficient time to diffuse into the resin pores and become adsorbed. The simulation described later shows that the pore diffusion resistance is dominant in the overall vortex flow adsorption process.
Figure 4.21: Breakthrough capacities of BSA by Streamline DEAE at different axial loading flowrates

Rotation rate: 200 rpm, Streamline DEAE resin volume fraction: 0.19. The error bars represent the standard deviations of triplicate runs.

4.3.4 The Effect of Adsorbent Resin Volume Fraction

As is shown in Figure 4.22, the breakthrough capacity increased with the increase of the volume fraction of the Streamline DEAE resin. However, the changing trend became flat at high adsorbent resin volume fractions. When the volume fraction of the adsorbent resin is high, the resin is distributed densely in the annulus. In this case, when the BSA molecules pass through the vortex flow reactor, the possibility is higher for the protein molecules to be adsorbed on the resin. On the contrary, if the resin distribution is dilute in the annulus, the possibility of being adsorbed becomes lower.
Figure 4.22: Breakthrough capacities of BSA by Streamline DEAE at different adsorbent resin volume fractions

Rotation rate: Rotation rate: 200 rpm, axial flowrate: 12 ml/min. The error bars represent the standard deviations of duplicate runs.

4.3.5 The Effect of Rotation Rate of Inner Cylinder

In addition, as is shown in Figure 4.23, in the range of the rotation rate of 200-500 rpm, the breakthrough capacity was almost constant at a fixed axial flowrate. This range of the rotation rate was chosen since the Streamline DEAE adsorbent resin is fluidized completely only above 200 rpm. From the tracer RTD experiments at 200-500 rpm, it was concluded that the vortex flow reactor is operated in the turbulent vortex flow regime and that the axial dispersion is almost constant and independent of the rotation rate. Therefore, the constant axial dispersion renders the breakthrough capacity invariant.

Worthy of mention, the critical Taylor number, $T_{a_c}$, is changed when the adsorbent resin is added to the fluid (Ali et al., 2002). It is very likely that this change of $T_{a_c}$ affects the lower and upper boundaries of the vortex flow regimes listed in Table 4-3. Therefore, a
modified Taylor number expressed with the physical properties of the suspension instead of the fluid is more appropriate to the suspension system.

![Graph of Breakthrough capacities of BSA by Streamline DEAE at different rotation rates of inner cylinder]

**Figure 4.23: Breakthrough capacities of BSA by Streamline DEAE at different rotation rates of inner cylinder**

Streamline DEAE resin volume fraction: 0.19. The error bars represent the standard deviations of triplicate runs.

### 4.3.6 Analysis and Simulation of Vortex Flow Adsorption

The above discussion is focused on the effect of the operating variables on the breakthrough capacity. However, from a process point of view, it is most important to know how far or close the vortex flow adsorption process is to a possible optimal case.

In Figure 4.21, if the linear regression curve fitting the experimental data is extended to the zero flowrate, the breakthrough capacity is 64.8 g/L. Under this ideal situation, since there is no axial flow, the adsorbed BSA is at equilibrium with the fluid BSA. So, the 10% breakthrough capacity can be calculated using the Langmuir equation with \( Q = 81.78 \text{ g/L} \), \( K = 33.27 \text{ L/g} \), \( C_0 = 1.5 \text{ g/L} \), and the resin volume fraction of 0.19 (30 ml in volume),
This calculated maximum breakthrough capacity without axial flow is comparable to the extended experimental value.

In Figure 4.22, the maximum adsorbent volume fraction which can be reached in the vortex flow reactor is about the same as that in a packed column, which is 0.6 (McCoy and Liapis, 1991; Thömmes et al., 1996). At this resin volume fraction, the fitting curve shown in Figure 4.22 gives a breakthrough capacity of 68.8 g/L. Similarly, the maximum 10% breakthrough capacity can be calculated as 68.2 g/L if 0.6 is used instead of 0.19 in the above calculation shown in Equation (4-70). It is very close to 68.8 g/L obtained from the fitting curve.

In Figure 4.23, if the rotation rate of the inner cylinder can be reduced so that there is no axial dispersion, there would exist an equilibrium state between the adsorbed BSA and the fluid BSA, which is also the ideal situation in Figure 4.21. The maximum 10% breakthrough capacity can be calculated as 68.8 g/L as well.

Therefore, for a specific adsorption system, the adsorption mechanism, i.e., adsorption capacity and adsorption affinity, is defined. By either extending the experimental data or considering some optimal cases, it indicates that the performance of the vortex flow adsorption process can be optimized to some extent by manipulating operating variables including rotation rate of the inner cylinder, axial loading flowrate, and adsorbent volume fraction.

Additionally, the two-region vortex flow adsorption model described earlier was used to simulate the breakthrough profiles of vortex flow adsorption under different operating
conditions. An example of the comparison between the simulated and experimental results is given in Figure 4.24. The results suggest that the two-region vortex flow adsorption model successfully describes the main features of vortex flow adsorption and simulates its breakthrough profile. The main simulation parameters and their values are listed in Table 4-4.

In Figure 4.24, a simulated breakthrough profile is also shown with a zero axial dispersion coefficient. This is like the situation discussed above with either a zero axial flowrate or a very low rotation rate, however, not reaching adsorption equilibrium. By comparing the breakthrough profiles with and without axial dispersion in vortex flow adsorption, it is very clear that the axial dispersion is a very important factor in affecting or determining the breakthrough capacity in vortex flow adsorption.

![Figure 4.24: Vortex flow adsorption modeling result](image)

Rotation rate: 200 rpm, axial flowrate: 12 ml/min, Streamline DEAE resin volume fraction: 0.19.
### Table 4-4: Vortex Flow Adsorption Modeling Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_p$</td>
<td>m²/s</td>
<td>$5.45 \times 10^{-11}$</td>
</tr>
<tr>
<td>$K_f$</td>
<td>m/s</td>
<td>$5.68 \times 10^{-6}$</td>
</tr>
<tr>
<td>$D_L$</td>
<td>m²/s</td>
<td>$3.50 \times 10^{-6}$</td>
</tr>
<tr>
<td>$K_{bc}$</td>
<td>m/s</td>
<td>$2.46 \times 10^{-5}$</td>
</tr>
<tr>
<td>$Q$</td>
<td>g/L</td>
<td>81.78</td>
</tr>
<tr>
<td>$K$</td>
<td>(g/L)^{-1}</td>
<td>33.27</td>
</tr>
</tbody>
</table>

In the vortex flow adsorption process, there are three consecutive mass transfer steps when the solute protein moves from the bulk fluid to the adsorbent resin: (1) transfer of the solute protein from the bulk fluid to the opening of the pores, characterized by the film mass transfer coefficient, $K_f$. (2) transfer of the solute protein through the pores by diffusion, described by the pore diffusion coefficient, $D_p$. (3) adsorption interaction between the solute protein and the adsorbent resin. The adsorption interaction is a fast step since it is a chemical reaction in nature. The Biot number, Bi, was calculated for two sequential steps: the film mass transfer and the pore diffusion steps. The Biot number is defined as (Deen, 1998),

$$Bi = \frac{K_f \cdot r_0}{D_p}$$  \hspace{1cm} (4-71)$$

where $r_0$ is the Streamline DEAE resin radius with an average value of 100 µm. For the above simulation example, the Biot number was calculated as 10.4, showing that the pore diffusion is much slower than the film mass transfer. Therefore, the overall vortex flow adsorption process is dominated by the pore diffusion step.
4.3.7 Performance Analysis of Vortex Flow Adsorption

In the preceding discussion, vortex flow adsorption is studied as a unit operation. The following discussion focuses on the effect of the axial loading flowrate on the performance of the vortex flow adsorption system from a process point of view.

There are three important parameters in evaluating adsorption processes: dynamic capacity, volumetric throughput, and recovery yield. The dynamic capacity and the recovery yield are concerned with the process efficiency while the volumetric throughput is concerned with the processing time. The breakthrough capacity is already defined and investigated. In Equation (4-72), the volumetric throughput (g of BSA/L of vortex flow reactor-Hr) is defined as the amount of the loaded protein per unit reactor volume and processing time,

\[ P = \frac{C_0 \cdot V_b}{t_c \cdot V_r} = \frac{C_0 \cdot V_{ax}}{V_r} \]  

(4-72)

where \( P \) is the volumetric throughput, \( t_c \) is the processing time, \( V_r \) is the effective volume of the vortex flow reactor, \( V_{ax} \) is the axial loading flowrate. In Equation (4-73), the recovery yield is defined as the percentage of the loaded protein that is recovered in the adsorption process,

\[ R = \left(1 - \frac{C_{out} \cdot V_b}{C_0 \cdot V_b}\right) \times 100\% \]  

(4-73)

where \( C_{out} \) is the average outlet protein concentration during the loading process before the breakthrough point.

In this work, the effect of the volumetric throughput on both the breakthrough capacity and the recovery yield was studied. As is shown in Figure 4.25, where the axial loading flowrate ranged from 6 to 24 ml/min, the breakthrough capacity changed distinctly when the volumetric throughput varied. Therefore, there exists a trade off between the breakthrough
capacity and the volumetric throughput from a practical operation point of view. That is to say, if the adsorbent resin needs to be fully used and a high breakthrough capacity is desired, the volumetric throughput needs to be lowered. On the contrary, if adsorption needs to be processed quickly, the breakthrough capacity should be sacrificed to a low level. However, as is shown in Figure 4.26, in the same range of the volumetric throughput, the recovery yield only changed slightly. The recovery yield is quite high due to a low breakthrough point of 10%.

Figure 4.25: Vortex flow adsorption performance analysis: breakthrough capacity vs. volumetric throughput.

Rotation rate: 200 rpm, Streamline DEAE resin volume: 30 ml (0.19 in volume fraction). The error bars represent the standard deviations of triplicate runs.
4.3.8 Summary

The breakthrough capacity of the model system of BSA with Streamline DEAE was measured at different operating conditions, including the rotation rate of the inner cylinder, the axial loading flowrate, and the adsorbent resin volume fraction. The results indicate that the breakthrough capacity is almost independent of the rotation rate in the turbulent vortex flow regime due to the constant axial dispersion. The breakthrough capacity decreases with the increase of the axial loading flowrate since the overall adsorption process is controlled by the pore diffusion of BSA protein molecules. In addition, to increase the adsorbent resin volume fraction is a good approach to increasing the breakthrough capacity.
4.4 Conclusions

The performance of vortex flow reactor was studied through tracer RTD experiments and two modeling approaches: a one-dimensional dispersion convective model and a two-region vortex flow model. It was concluded that the axial dispersive behavior in the vortex flow system is distinct in different vortex flow regimes. The axial dispersion deteriorates with the increase of the rotation rate in the Taylor and wavy vortex flow regimes. Therefore, the Taylor vortex flow regime is preferred in order to minimize the axial dispersion.

Vortex flow adsorption was characterized using the BSA and Streamline DEAE system. The effect of the operating variables, including inner cylinder rotation rate, axial loading flow rate, and adsorbent volume fraction, on the dynamic capacity was explored.

The benefit of vortex flow adsorption is its advantage of dealing with fermentation broth or homogenized cell culture without first separating particulates, e.g., cells, cell debris, and solid components in the feedstock. In the application of vortex flow adsorption to recovering biochemical products directly from crude cell extract, it is necessary to maintain sufficient differences in size and density between adsorbent resin and cell materials to keep the adsorbent resin inside the vortex flow reactor and allow the cellular materials to flow out. The adsorbent resin must be heavier than the cells that have a wet density of around 1.05 g/ml. However, heavy adsorbent resins need a high rotation rate to be fluidized so that the vortex flow system is operated in the wavy or turbulent vortex flow regimes. In these operation regimes, the axial dispersion is very high and almost independent of the rotation rate.
5 Application of Vortex Flow Adsorption to Protein Recovery

5.1 Production of Intein $\alpha_1$-Antitrypsin Fusion Protein

5.1.1 Introduction

In this research, recombinant human $\alpha_1$-antitrypsin was expressed in *E. coli* as a C-terminal fusion to a modified Sce VMA intein (Chong *et al.*, 1998a) containing a chitin-binding domain (CBD). Therefore, the fusion protein can be recovered by a chitin resin and induced to undergo *in vitro* peptide bond cleavage that specifically releases $\alpha_1$-antitrypsin from the fusion protein bound on the chitin resin. The recovery yield of a target protein during the intein-mediated purification is determined by three major factors: (1) minimal *in vivo* cleavage; (2) efficient binding of the fusion protein to affinity resin; and (3) high *in vitro* intein-mediated cleavage efficiency (Cantor and Chong, 2001; Zhang *et al.*, 2001).

In order for the intein to be useful for protein purification, the cleavage activity of the intein has to be attenuated *in vivo* but remain inducible *in vitro* (Chong *et al.*, 1998a). The thiol-induced *in vitro* cleavage at the intein N-terminus is mediated by the intein-catalyzed N-S acyl rearrangement reaction. This requires correct folding of both the target protein and the intein to create a favorable conformation at the splice junction (Zhang *et al.*, 2001). Misfolded target protein/intein would inhibit the N-S acyl rearrangement reaction thereby decreasing the thiol-induced *in vitro* cleavage efficiency and/or expose the splice junction to hydrolysis resulting in *in vivo* cleavage (Zhang *et al.*, 2001). Misfolded proteins produced in *E. coli* cells often form insoluble aggregates, i.e., inclusion bodies. Only soluble proteins can be effectively purified by the intein system.
Low induction temperatures may reduce the formation of inclusion bodies, improve the folding and solubility of the fusion protein, and increase *in vitro* cleavage efficiency of the intein (Cantor and Chong, 2001; Xu *et al.*, 2000). The expression level of the fusion protein could be affected by induction temperature as well. Low induction temperatures (15-20°C) sometimes increase the expression of the soluble fusion precursor and the final yield of the target protein. In other cases, the induction temperature makes no difference (Chong *et al.*, 1998a). Therefore, different induction temperatures should be tested for each target protein in order to achieve optimal protein expression. However, low induction temperatures (15-20°C) could make the protein synthesis slow compared to the commonly used induction temperatures, i.e., 30°C or 37°C. In addition, a long induction time could be used to increase the expression level of the fusion protein, however, could lead to the variation in the expression of the recombinant protein in *E. coli* cells as well.

### 5.1.2 Experimental Design

The objective of this work is to investigate: (1) cell growth rate; (2) the fusion protein expression level; and (3) *in vivo* cleavage of the fusion protein with different induction times under different induction temperatures. Through this work, an optimal combination of induction temperature and induction time is expected to obtain. The induction temperatures and times were chosen according to Figure 5.1. Induction temperatures were above 25°C since both cell growth and protein synthesis are slow at low temperatures.
5.1.3 Cell Growth

In Figure 5.2, the growth profiles of *E. coli* cells harboring the plasmid pT12AT1 are compared among three different induction temperatures, 25°C, 30°C, and 35°C. During the first 4 hours after induction, the cell density, quantified by OD at 600 nm, increased with the time. The cell density also increased with the increase of the induction temperature. After 4 hours, *E. coli* cells still grew at 25°C. However, OD\textsubscript{600} started to decline after 5 hours. At 30°C, the cells stopped dramatic growth after 4 hours and finally started to degrade after 5 hours. At the induction temperature of 35°C, after 4 hours of induction, the cells started to degrade. If only cell growth is considered, 30°C is a good induction temperature because the cell growth at this temperature is faster than at 25°C and also there is no significant improvement if the induction temperature changes from 30°C to 35°C. At 30°C, the induction duration of 4 hours is appropriate since *E. coli* cells stop dramatic growth after this time.
5.1.4 Fusion Protein Expression

The expression level of the intein α1-antitrypsin fusion protein was investigated at different induction temperatures. In Figure 5.3, a SDS-PAGE assay of soluble *E. coli* cell extract is shown for the induction temperature of 30°C before induction and at different induction times. The results suggest that, with the increase of the induction time, the fusion protein expression level increased. The same assay was performed at 25°C and 35°C.

Based on the SDS-PAGE assay, at each induction temperature, a relative fusion expression level at different induction times was calculated as the ratio of the expression level to that at the induction of 3 hours. Therefore, the relative fusion expression level has no units. Since all the soluble cell extract samples at different induction temperatures cannot be assayed on a single polyacrylamide gel, the samples at the same induction temperature were assayed on
a single gel. The relative fusion expression level was used in order to correct the background differences between gels.

Figure 5.3: SDS-PAGE analysis of soluble cell extract from *E. coli* harboring pT12AT1 before and after induction at 30°C

As is shown in Figure 5.4, at 25°C, after 4 hours, the fusion expression still increased with the induction time. At 30°C, the fusion expression almost leveled off after 4 hours and started to drop after 5 hours. At 35°C, after 3 hrs, the expression almost leveled off with slight decrease. The combination of the induction temperature of 30°C and the induction duration of 4 hours is still a good choice if both the expression level and the expression time need to be optimized simultaneously.
Figure 5.4: Intein α1-antitrypsin fusion protein expression at different induction times under different induction temperatures

In addition to the expression level of the fusion protein, the fusion protein productivity was also explored at different induction temperatures. The fusion protein productivity is defined as the expression level of the fusion protein within unit induction time. In Figure 5.5, the fusion protein productivity at different induction times is compared between different induction temperatures. At each induction temperature, the productivity was normalized with the value at the induction time of 3 hours. At 25°C, the fusion productivity still increased after 4 hours. At 30°C, the fusion productivity reached a maximum at 4 hours and started to decline after this time. For the induction temperature of 35°C, after 3 hours, the fusion productivity started to drop continuously with the induction time. These results indicate that the combination of the induction temperature of 30°C and the induction time of 4 hours is also good as far as the fusion protein productivity is concerned.
5.1.5 *In vivo* Cleavage of Fusion Protein

Another consideration in optimizing the induction condition is *in vivo* cleavage of the fusion protein. *In vivo* cleavage leads to the loss of the intein segment from the fusion protein and makes it impossible to recover the target protein. As is shown in Figure 5.3, at the induction temperature of 30°C, the protein bands corresponding to the molecular weight of α1-antitrypsin (44 kD) became dark with the increase of the induction time. It seems that the amount of *in vivo* cleaved α1-antitrypsin increased with the induction time. In order to confirm this observation, a Western blotting assay was made for the same samples analyzed by SDS-PAGE in Figure 5.3. As Figure 5.6 indicates, with the increase of the induction time, the fusion protein expression level increased. However, there was no protein band corresponding to *in vivo* cleaved α1-antitrypsin for all the *E. coli* soluble cell extracts. Therefore, the protein bands
at the molecular weight of 44 kD detected in SDS-PAGE were probably due to other *E. coli* proteins but not *in vivo* cleaved α1-antitrypsin. Therefore, *in vivo* cleavage is not a problem when the induction conditions are optimized for the expression of the fusion protein. In this research, only soluble cell extract was used to recover and purify α1-antitrypsin, and it is likely that *in vivo* cleavage exists for the inclusion body.

![Figure 5.6: Western blotting analysis for soluble cell extract from *E. coli* harboring pT12AT1 before and after induction at 30°C](image)

**Figure 5.6: Western blotting analysis for soluble cell extract from *E. coli* harboring pT12AT1 before and after induction at 30°C**

### 5.1.6 Summary

The induction conditions, including induction temperature and induction time, were optimized as far as the cell growth rate, the fusion protein expression level, and the fusion protein productivity are concerned. A combination of induction temperature of 30°C and induction time of 4 hours is regarded as an optimal condition. *In vivo* cleavage of the fusion protein is not a problem for the soluble cell extract under the experimental conditions investigated in this work.
5.2 Preparation of Chitin Resin

5.2.1 Introduction

In the application of the vortex flow adsorption system, the intein \( \alpha 1 \)-antitrypsin fusion protein expressed in *E. coli* was chosen as a model protein. Due to the chitin-binding domain (CBD) in the fusion protein, the chitin resin was used to recover the fusion protein.

Currently, chitin resin is only commercially available from New England Biolabs (Beverly, MA). It has a particle density of around 1.0 g/ml and a particle diameter of 50-100 \( \mu \)m. It was developed mainly for packed column chromatography. The tentative experiments of vortex flow adsorption with this chitin resin showed that: (1) the beads are soft and very easily broken, and thereby aggregated or reshaped, when exposed to the rotation of the inner cylinder; (2) the density of the chitin beads is very close to that of water; therefore, in the application of the vortex flow adsorption system for the crude cell extract, it is very hard to separate them from cellular materials with a wet density of 1.05 g/ml.

In this work, two approaches were explored to prepare chitin beads that can be used in the vortex flow system. The chitin beads should be robust, and the density should be higher than that of the cellular material but not very high because they should be easily fluidized at moderate rotation rates.

5.2.2 Chitin and Chitosan

Chitin is one of the most abundant organic materials, being second only to cellulose in nature (Roberts, 1992). Chitin is poly \([\beta-(1 \rightarrow 4)-2\text{-acetamido}-2\text{-deoxy-D-glucopyranose}]\). The principal derivative of chitin is chitosan, produced by alkaline deacetylation of chitin (Roberts, 1992). Chitosan is poly \([\beta-(1 \rightarrow 4)-2\text{-amino}-2\text{-deoxy-D-glucopyranose}]\). The structures of chitin
and chitosan are shown in Figure 5.7. Chitosan also occurs naturally in some fungi but its occurrence is much less widespread than that of chitin. Chitosan can be converted to chitin by acetic anhydride to change the C(2)-amine group to a C(2)-acetamido group.

![Figure 5.7: Structure of polysaccharides chitin and chitosan](image)

The unfavorable property for chitin to be exploited is that chitin cannot be dissolved in aqueous solutions, even acidic solutions. Compared to chitin, chitosan can be dissolved in some acidic solutions; for instance, chitosan can be dissolved in acetic acid solutions.

### 5.2.3 Procedure for Preparing Chitin Resin

Considering different solubility properties of chitin and chitosan, chitosan is used as the initial substance to prepare chitin beads. In the final step, acetic anhydride is added to change C(2)-amine group to C(2)-acetamido group.

The basic procedure for preparing the chitin beads that can be used in the vortex flow system is: (a) choose robust core beads (such as glass, silica, metal, etc.) with chemical groups
(such as amine, hydroxyl, etc.) to be used to react with activating reagents; (b) select bi-
functional activating reagents which not only could activate the chemical groups on the core 
beads but also could react with the functional groups of the adsorbent molecules; and (c) coat 
the adsorbent molecules on the core beads through the activating reagents.

In this work, the glutaraldehyde and divinyl-sulphone activation methods were used to 
coat chitosan and thus convert it to chitin. The composite beads with chitin coated on the 
surface were used for the adsorption of the intein fusion protein.

5.2.4 Glutaraldehyde Method

Glutaraldehyde can be used to immobilize amine-containing compounds through 
several different reaction schemes (Ternynck and Avrameas, 1972; Weston and Avrameas, 
1971). As is shown in Figure 5.8 (Hermanson, 1996), one approach makes use of a 
glutaraldehyde polymer containing vinyl bonds to modify a matrix containing amine or amide 
bonds and leave the formyl groups free for subsequent coupling to a ligand (Hermanson et al., 

Amine functional glass beads (Polysciences) are high surface-area soda lime glass 
beads that are functionalized by reaction with the appropriate organosilane. Prior to the 
organosilane treatment, the surfaces of the glass particles are etched to increase the surface 
area. They are capable of coupling with peptides, dyes, and other compounds. The amine glass 
beads have a particle diameter range of 30-50 µm and a particle density range of 2.42-2.50 
g/ml.
In preparing chitin beads with glutaraldehyde method, firstly 10 g amine functional glass beads were activated by 100 ml 25% (v/v) glutaraldehyde solution at 37°C for 18 hours. Once activated, the beads were washed with phosphate buffer saline solution (PBS: 0.02 M phosphate buffer, 0.15 M NaCl, pH 7.4). 100 ml 0.5% (w/v) chitosan (low molecular weight,
Fluke) solution in 0.5% (v/v) acetic acid was mixed with the beads at 37°C for ~12 hours, chitosan being coated on the activated beads through glutaraldehyde. The residual chitosan solution was washed off. 100 ml acetic anhydride was added to convert the amine group in the chitosan molecule to the acetamido group in the chitin molecule. Finally, chitin coated beads were washed with PBS solution until the suspension went to a neutral pH condition. The chemical mechanism for this procedure is shown in Figure 5.9. The activation condition of 37°C and 18 hours is necessary to produce a glutaraldehyde polymer with vinyl bonds to activate the amine functional glass beads.

It is apparent that the amine groups in the chitosan molecule were used to coat chitosan onto the amine functional glass beads and the remaining amine groups were converted into the acetamido groups in the chitin molecule. Therefore, if most of the amine groups on chitosan were used for coating chitosan, the concentration of the amine groups that were left and could be converted to the acetamido groups decreased. This means that the concentration of the acetamido groups on the surface of the glass beads would be low. This could affect the adsorption capacity of the chitin-coated glass beads for the fusion protein.
Figure 5.9: Chemical mechanism of glutaraldehyde method for preparing chitin beads
5.2.5 Divinyl Sulphone Method

Divinyl sulphone (DVS) is a bi-functional reagent used to activate agarose and other hydroxyl-containing matrices (Porath, 1974). DVS introduces reactive vinyl groups into the matrix that couple to amines, alcohols, thiols, and phenols (Hermanson et al., 1992). DVS-activated matrices are especially well suited for immobilization of proteins (e.g., antibodies, antigens, and enzymes) (Lihme et al., 1986) and carbohydrates (e.g., monosaccharides and polysaccharides) (Allen and Johnson, 1977; Fornstedt and Porath, 1975; Porath et al., 1975).

In this work, the starting material was DVS-activated agarose glass beads (Upfront Chromatography, Denmark). The beads are composed of a glass core coated with a layer of crosslinked agarose activated by DVS. Due to the cross-linking of the agarose matrix during the activation procedure, this matrix is particularly mechanically stable. The concentration of the functional vinyl group on the beads is 17 mmol/kg. The beads have a particle density of ~1.5 g/ml and a particle diameter range of 40-100 μm.

For the DVS-activated agarose glass beads, chitin beads were prepared according to the following protocol, illustrated in Figure 5.10. 100 ml 0.5% (w/v) chitosan (low molecular weight, Fluke) solution in 0.5% (v/v) acetic acid was mixed with 30 ml DVS-activated agarose glass beads at 30°C for ~12 hours to allow chitosan to be coated on the beads. The residual chitosan solution was washed off. The washed beads were incubated with 100 ml acetic anhydride at 30°C for ~12 hours to convert chitosan to chitin. In this step, the C (2)-amine group on the chitosan molecules was converted to a C (2)-acetamido group to generate chitin molecules. Finally, chitin coated beads were washed with deionized water until the suspension went to a neutral pH condition.
Figure 5.10: Chemical mechanism of divinyl sulphone method for preparing chitin beads
In the DVS activation method, the functional groups used for coating chitosan are hydroxyl groups not amine groups. Additionally, if possible, all the amine groups on chitosan molecules can be converted to acetamido groups. Therefore, the concentration of coated chitin on the beads is higher compared to the amine functional glass beads; hence the adsorption capacity for the intein fusion protein could be higher.

### 5.2.6 Adsorption of Intein Fusion Protein by Chitin Resin

In order to check whether a layer of chitin was coated on the amine functional glass beads or the DVS-activated agarose glass beads, the adsorption of the intein fusion protein by these beads was studied. Maltose binding protein (MBP) intein fusion protein was employed since it is the best scenario for the intein-mediated protein purification system. Therefore, any effect related to the target protein can be avoided and the adsorption properties of chitin beads can be determined independently. The schematic structure of the MBP intein fusion protein and the molecular weight of each component are shown in Figure 5.11.

![Figure 5.11: Schematic structure of MBP intein fusion protein](image)

Batch adsorption experiments were carried out for the chitin resins prepared by both the glutaraldehyde and DVS methods. After washing, the cleavage and stripping steps were
processed parallel for the chitin resins bound with the fusion protein. MBP (42 kDa) was cleaved from the chitin resins through the DTT-induced cleavage at the N-terminus of the intein. Since the stripping step was carried out immediately after the adsorption step, all the bound proteins could be eluted from the chitin resins, including the fusion protein (97 kDa) and MBP.

The SDS-PAGE assay results in Figures 5.12 and 5.13 show that MBP was specifically cleaved from the fusion protein bound on the chitin resins. In the stripping samples, the adsorbed fusion protein was eluted from the chitin resins as well as MBP and other non-specifically adsorbed proteins. In addition, the batch adsorption was also conducted for the chitin resin from New England Biolabs (NEB) as a comparison control.

![Figure 5.12: SDS-PAGE assay for the adsorption of MBP intein fusion protein by chitin resin prepared with glutaraldehyde method](image)

Clarified soluble cell extract containing MBP fusion protein was incubated with chitin resin prepared by glutaraldehyde method for 3 hours. The supernatant after this step was unbound cell extract. Chitin resin was washed with column buffer (20 mM Hepes, 500 mM NaCl, pH 8.0). Cleavage solution (30 mM DTT in column buffer) and stripping solution (2% SDS aqueous solution) were incubated with parallel samples of washed chitin resin for 3 hours.
Figure 5.13: SDS-PAGE assay for the adsorption of MBP intein fusion protein by chitin resin prepared with DVS method

Clarified soluble cell extract containing MBP fusion protein was incubated with chitin resin prepared by DVS method for 5 hours. The supernatant after this step was unbound cell extract. Chitin resin was washed with column buffer (20 mM Hepes, 500 mM NaCl, pH 8.0). Cleavage solution (30 mM DTT in column buffer) and stripping solution (2% SDS aqueous solution) were incubated with parallel samples of washed chitin resin for 3.5 hours. Chitin resin from NEB was also used as a comparison control.

In Figures 5.12 and 5.13, for each chitin resin, the MBP band in the cleavage sample and the fusion protein and MBP bands in the stripping sample were quantified. The adsorption capacity was calculated as the sum of the fusion protein and MBP eluted in the stripping sample since the same volume of the chitin resin was used in the experiments. In addition, the cleavage efficiency was calculated as the percentage of the adsorbed fusion protein that was cleaved as MBP in the cleavage sample. In calculating the adsorption capacity and cleavage efficiency, the fusion protein band volume was adjusted based on the molecular weights of the
fusion protein and MBP since the band volume is proportional to the molecular weight of the protein. The protein band quantification results are listed in Table 5-1.

Table 5-1: Adsorption of MBP Intein Fusion Protein by Chitin Resins

<table>
<thead>
<tr>
<th></th>
<th>Protein Band Volume</th>
<th>Comparison Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cleavage</td>
<td>Stripping</td>
</tr>
<tr>
<td></td>
<td>MBP</td>
<td>MBP</td>
</tr>
<tr>
<td>Glutaraldehyde Chitin Resin</td>
<td>22.5</td>
<td>37.6</td>
</tr>
<tr>
<td>DVS Chitin Resin</td>
<td>46.3</td>
<td>36.1</td>
</tr>
<tr>
<td>NEB Chitin Resin</td>
<td>58.0</td>
<td>71.3</td>
</tr>
</tbody>
</table>

For the glutaraldehyde chitin resin, both the adsorption capacity and cleavage efficiency were low compared to the DVS chitin resin. As is discussed previously, in the glutaraldehyde activation method, low adsorption capacity was expected as a consequence of a low concentration of acetamido groups on the surface of the resin.

For the DVS chitin resin, the adsorption capacity was almost the same as the NEB chitin resin. The NEB chitin resin is porous, with a high specific surface area; the composite chitin resin prepared by the DVS method has only a thin layer of chitin coated on the surface. Therefore, it is not appropriate to use the resin volume as a comparison basis. However, it can at least be confirmed that the chitin resin prepared by the DVS activation method is efficient to be used for the adsorption of the fusion protein. More important, the DVS chitin resin is robust, and therefore appropriate for the vortex flow adsorption system.
5.2.7 Summary

The glutaraldehyde and divinyl sulfide methods were developed to prepare chitin resins. The prepared chitin resins are appropriate for the vortex flow adsorption system since matrices with the inclusion of a glass core were used to provide the required high density and robustness. Through the adsorption capacity study for the two chitin resins, it was concluded that the DVS method is more efficient in obtaining a chitin resin with a high adsorption capacity. Therefore, the DVS chitin resin was used in this research.
5.3 Adsorbent Resin Fluidization

5.3.1 Introduction

An important practical problem related to the application of vortex flow adsorption is the fluidization of the adsorbent resin. Unlike expanded bed adsorption, there is no liquid distributor at the bottom of the vortex flow reactor; hence axial flow cannot be distributed evenly across the whole cross-sectional area. Therefore, in vortex flow adsorption, the rotation of the inner cylinder, instead of the axial flow, is used to facilitate the fluidization of the adsorbent resin. Therefore, the rotation speed needs to be high enough to overcome the sedimentation or settling velocity of the resin particles, which is described by the Stokes’ law in the creeping flow regime (Seville et al., 1997),

\[ v_t = \frac{d_p^2 (\rho_p - \rho_f)g}{18 \mu} \]  

(5-1)

where \( v_t \) is settling velocity, \( d_p \) is resin particle diameter, \( \rho_p \) is resin particle density, \( \rho_f \) is fluid density, and \( \mu \) is fluid viscosity. Worthy of mention, the Stokes’ law may not be valid at operating conditions used in vortex flow adsorption, it is used here only for a qualitative understanding of how the sedimentation velocity is affected by the physical properties of the particle and the fluid.

The objective of this work is to explore and correlate the minimum fluidization rotation rate with the physical properties of the adsorbent resin and the fluid. Similar to the Stokes’ law, the minimum fluidization rotation rate should be a function of the particle properties (e.g., particle size and particle density) and the fluid properties (e.g., fluid viscosity and fluid density), i.e.,
\[ R_{\text{min}} = f(\rho_p, \rho_f, d_p, \mu) \]  \hspace{1cm} (5-2)

If only the physical properties of the particles are taken into account, a tentative correlation could be,

\[ R_{\text{min}} = s \left( \rho_p - \rho_f \right)^a d_p^b \]  \hspace{1cm} (5-3)

where \( a, b, \) and \( s \) are correlation parameters. Two groups, \( \rho_p-\rho_f \) and \( d_p \), are employed as in the Stokes’ law. In order to incorporate the physical properties of the fluid into this correlation, a viscosity group is added,

\[ R_{\text{min}} = s \left( \rho_p - \rho_f \right)^a d_p^b \mu^c \]  \hspace{1cm} (5-4)

where the parameter \( c \) is used to take the fluid viscosity into account. The correlating work is to determine the values of the parameters \( a, b, c, \) and \( s \) based on the measurements of the minimum fluidization rotation rates under different operating conditions.

### 5.3.2 Measurements of Minimum Fluidization Rotation Rates

In order to implement the correlation, two groups of experiments were conducted. One group was to measure the minimum fluidization rotation rate by keeping the physical properties of the fluid fixed and varying the physical properties of the adsorbent resin, including particle density and particle diameter. From the particles available commercially, e.g., adsorbent resin or media, it is almost impossible to obtain a population of resins with the same particle size and different particle density or with the same particle density and different particle size. The actual situation is that the particle size and the particle density change simultaneously from one resin to the other. In addition, there exist a particle size distribution and a particle density distribution for any resin. In the correlation, the average particle diameter and density were
used to represent the resin. Deionized water was used at room temperature to provide constant physical properties of the fluid.

The other group was to measure the minimum fluidization rotation rate for a fixed resin in the fluid with the physical properties varied. The similar situation exists for the fluid in that it is impossible to separate the change of the fluid viscosity from the fluid density. The real situation is that both the fluid density and the fluid viscosity change simultaneously. In these experiments, Streamline SP was used as the resin. Sucrose solutions at different concentrations were used as the fluids. The density of the sucrose solution at any temperature between 0°C and 30°C is correlated to the temperature and the sucrose concentration by Equation (5-5) (Dawson et al., 1986),

\[
\rho_T = (B_1 + B_2 T + B_3 T^2) + (B_4 + B_5 T + B_6 T^2) Y + (B_7 + B_8 T + B_9 T^2) Y^2
\]  

(5-5)

where \(\rho_T\) is the density of a sucrose solution, \(T\) is the temperature in °C, and \(Y\) is the weight fraction of sucrose in the solution. \(B_i\)'s (\(i = 1\) to \(9\)) are the correlation parameters and their values are listed in Table 5-2. In Figure 5.14, the viscosity of the sucrose solution is plotted with the concentration in wt % at 20°C (Dawson et al., 1986).

| Table 5-2: Parameters in Correlation of Sucrose Density |
|----------------|----------------|----------------|----------------|
| \(B_1\)        | 1.00037        | \(B_4\)        | 0.389824       | \(B_7\)        | 0.170976       |
| \(B_2\)        | \(3.96805 \times 10^{-5}\) | \(B_5\)        | \(-1.05789 \times 10^{-3}\) | \(B_8\)        | \(4.75301 \times 10^{-4}\) |
| \(B_3\)        | \(-5.85133 \times 10^{-6}\) | \(B_6\)        | \(1.23928 \times 10^{-5}\)  | \(B_9\)        | \(-8.92397 \times 10^{-6}\) |
The minimum fluidization rotation rates were measured for several resins in the vortex flow reactor. The operating conditions were: axial flowrate 18 ml/min, the effective vortex flow reactor volume 160 ml, and resin volume 25 ml. Only the physical properties, including the particle diameter and the particle density, not the functional group of the adsorbent resin, were considered. In all the experiments, deionized water was used as a loading fluid.

As listed in Table 5-3, with the increase of either the particle diameter or the particle density, the minimum rotation rate required to fluidize the adsorbent resin increased. For Streamline DEAE and SP adsorbent resins, which have a particle density of 1.2 g/ml and a particle diameter range of 100-300 µm, the minimum rotation rate was 226 rpm. For the DVS-activated agarose glass beads, which have a particle density of 1.5 g/ml and a particle diameter range of 40-100 µm, the minimum rotation rate was measured to be 313 rpm.
Table 5-3: Minimum Fluidization Rotation Rates for Adsorbent Resins

<table>
<thead>
<tr>
<th>Resin</th>
<th>Company</th>
<th>Particle Density (g/ml)</th>
<th>Particle Size (µm)</th>
<th>Fluidization Rotation Rate (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin Resin</td>
<td>New England Biolabs</td>
<td>1.0</td>
<td>50 - 100</td>
<td>97</td>
</tr>
<tr>
<td>DFF-100</td>
<td>Sigma</td>
<td>1.0</td>
<td>45 - 165</td>
<td>95</td>
</tr>
<tr>
<td>MEP</td>
<td>Biosepra</td>
<td>1.1</td>
<td>80 - 100</td>
<td>156</td>
</tr>
<tr>
<td>Streamline DEAE or SP</td>
<td>Amersham Bioscience</td>
<td>1.2</td>
<td>100 - 300</td>
<td>226</td>
</tr>
<tr>
<td>Amino Glass Bead</td>
<td>Polyscience</td>
<td>2.42 - 2.50</td>
<td>30 - 50</td>
<td>321</td>
</tr>
<tr>
<td>DVS Agarose Glass Bead</td>
<td>Upfront Chromatography</td>
<td>1.5</td>
<td>40 - 100</td>
<td>313</td>
</tr>
<tr>
<td>Stainless Steel DEAE</td>
<td>Upfront Chromatography</td>
<td>2.5 - 3.0</td>
<td>40 - 100</td>
<td>481</td>
</tr>
</tbody>
</table>

In addition, the minimum fluidization rotation rates were measured for Streamline SP resin in sucrose solutions with different concentrations. The operating conditions were: axial flowrate 0 ml/min, the effective vortex flow reactor volume 160 ml, and resin volume 30 ml. The results are listed in Table 5-4 together with the physical properties of the sucrose solutions.

Table 5-4: Minimum Fluidization Rotation Rates in Sucrose Solutions with Different Concentrations

<table>
<thead>
<tr>
<th>Conc. (wt %)</th>
<th>Density (g/ml)</th>
<th>Viscosity (cp)</th>
<th>Rotation Rate (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.9988</td>
<td>1.00</td>
<td>226</td>
</tr>
<tr>
<td>5.06</td>
<td>1.0182</td>
<td>1.15</td>
<td>192</td>
</tr>
<tr>
<td>10.06</td>
<td>1.0382</td>
<td>1.34</td>
<td>203</td>
</tr>
<tr>
<td>20.00</td>
<td>1.0806</td>
<td>1.96</td>
<td>165</td>
</tr>
<tr>
<td>30.01</td>
<td>1.1269</td>
<td>3.21</td>
<td>161</td>
</tr>
<tr>
<td>40.20</td>
<td>1.1776</td>
<td>6.32</td>
<td>169</td>
</tr>
<tr>
<td>44.98</td>
<td>1.2027</td>
<td>9.49</td>
<td>157</td>
</tr>
</tbody>
</table>
5.3.3 Correlation of Minimum Fluidization Rotation Rates

The measured minimum fluidization rotation rates were fitted to the correlation in Equation (5-4), and the correlation parameters a, b, c, and s were obtained. The fitting approach was to minimize the objective function of the sum of the squares of the errors between the measured and correlated rotation rates. The minimization of the objective function was implemented by a Matlab routine fminsearch. The values of the correlation parameters are listed in Table 5-5. The correlation is now,

\[ R_{\text{min}} = 182.70 \left( \rho_p - \rho_f \right)^{0.42} d_p^{0.15} \mu^{0.26} \]  

(5-6)

A higher exponential value for \( \rho_p - \rho_f \) than that for \( d_p \) shows that the effect of the density difference between the particle and the fluid is more important than the particle size in determining the minimum fluidization rotation rate. This is different from the Stokes’ law, where an exponential value of 2 for \( d_p \) is higher than the value of 1 for \( \rho_p - \rho_f \). Therefore, the flow regime in the vortex flow reactor is different from the creeping flow under which the Stokes’ law is valid. As discussed in the tracer study, at the range of the rotation rates for fluidizing the adsorbent resins, the vortex flow reactor is operated in the wavy or turbulent vortex flow regime.

Table 5-5: Parameters in Correlation of Minimum Fluidization Rotation Rate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>0.42</td>
<td>0.15</td>
<td>0.26</td>
<td>182.70</td>
</tr>
</tbody>
</table>
5.3.4 Comparison of Measured and Correlated Minimum Fluidization Rotation Rates

In Figure 5.15, the measured and correlated minimum fluidization rotation rates are compared for several adsorbent resins with physical properties listed in Table 5-3. It is clearly shown that the correlation in Equation (5-6) captures the main features of the fluidization process facilitated by the rotation of the inner cylinder in the vortex flow reactor. The errors between the measured and correlated values are within 15%.

![Graph showing measured and correlated minimum fluidization rotation rates](image)

**Figure 5.15: Measured and correlated minimum fluidization rotation rates for adsorbent resins**

The measured and correlated minimum fluidization rotation rates are compared for Streamline SP resin in sucrose solutions with different concentrations in Figure 5.16. Both the density and viscosity of sucrose solutions at different concentrations are listed in Table 5-4. As is shown in Figure 5.16, the measured data can be fitted to the correlation equation quite well. The errors between the measured and correlated values are within 25%, which does not include the situation for the 45% (wt) sucrose solution with the viscosity of 9.5 cp. It seems that the
correlation in Equation (5-6) is not valid at high viscosity since the flow pattern is possibly different with a very viscous solution.

Figure 5.16: Measured and correlated minimum fluidization rotation rates in sucrose solutions with different concentrations

5.3.5 Complexity of Particle Fluidization Process in Vortex Flow Reactor

In the vortex flow adsorption system, the adsorbent resin can be fluidized due to the rotation of the inner cylinder. Therefore, the rotation rate of the inner cylinder must be high enough to overcome the sedimentation velocity of the adsorbent resin. Vortex is formed as a secondary flow pattern due to the rotation of the inner cylinder as well. Above a critical rotation rate, circular Couette flow bifurcates into vortices. Since the primary circular Couette flow is in the tangential direction, it must be the axial velocity component of vortices that overcomes the sedimentation velocity of the adsorbent resin to allow it to be fluidized. If there exists a simple relation between the rotation rate of the inner cylinder and the axial velocity component of vortices, it would be easy to compare the axial velocity component with the
sedimentation velocity of the particles and determine whether or not the particles can be fluidized. In fact, to solve the Navier-Stokes equation for the velocity components in the vortex flow system is a very complex task. In addition, it is complex to determine the sedimentation velocity of the particles at different operating regimes. In this work, a simple Stokes’ law was used to describe the sedimentation velocity and it is valid only for the creeping flow. The sedimentation velocity at other operating regimes is much more complex (Seville et al., 1997).

Another difficulty in quantifying the minimum fluidization rotation rates is from the characterization of particles. Currently, almost all the physical properties of particles are described with a distribution range and mean value. There is no further information about the distribution. In addition, the adsorbent particles are normally porous. When the particles are fluidized in the fluid, the pores of particles are filled with the fluid. The actual particle density in determining the minimum fluidization rotation rate is a combination of the particle and fluid density. All these make it difficult to obtain an accurate correlation for the minimum fluidization rotation rate.

5.3.6 Summary

In this work, instead of exploring the intrinsic mechanism of the fluidization process occurring in the vortex flow system, a simplified empirical approach based on the Stokes’ law was employed. In the correlation, the mean physical properties of particles were used. An empirical correlation is very useful not only in predicting the minimum fluidization rotation rate from the physical properties of the adsorbent particles and the fluid but also in designing the adsorbent particles to optimize the operating conditions, especially the rotation rate of the inner cylinder, for the vortex flow adsorption process.
5.4 Recovery of Intein $\alpha_1$-Antitrypsin ($\alpha_1$-AT) Fusion Protein

5.4.1 Introduction

The benefit of vortex flow adsorption is its ability to deal with fermentation broth or homogenized cell culture without first separating cellular materials, i.e., cells or cell debris. In this work, recombinant human $\alpha_1$-antitrypsin ($\alpha_1$-AT) was expressed in *E. coli* as a C-terminal fusion to a modified intein containing a chitin-binding domain. Therefore, the fusion protein can be recovered by chitin resin affinity adsorption. Vortex flow adsorption was explored as an integrative technology to directly recover the intein $\alpha_1$-AT fusion protein from crude *E. coli* cell culture with the fusion protein released.

5.4.2 Intein $\alpha_1$-Antitrypsin Fusion Protein System

In this research, human $\alpha_1$-antitrypsin was fused as a target protein to the C-terminus of a modified *Sce* VMA intein containing a chitin-binding domain (CBD) (Chong *et al.*, 1996). In the fusion protein, the CBD allows the binding of the fusion protein to a chitin resin. The intein-mediated cleavage is induced by the incubation of the chitin resin-bound fusion protein with DTT. DTT cleaves the thiol ester bond formed by an N-S acyl rearrangement at the intein N-terminal cysteine, which subsequently triggers peptide cleavage at the intein C-terminal asparagine (Chong *et al.*, 1998a). As a result, $\alpha_1$-AT is released from the chitin resin without using any protease and separated from its fusion partner on the chitin resin that purifies the fusion protein. A small N-terminal peptide (N-extein) providing a favorable translational start can be purified after the cleavage reaction and removed by dialysis (Chong *et al.*, 1998a). The
schematic structure of the intein α1-AT fusion protein and the molecular weight of each component are shown in Figure 5.17.

![Schematic structure of intein α1-AT fusion protein](image)

**Figure 5.17: Schematic structure of intein α1-AT fusion protein**

5.4.3 Interaction between *E. coli* Cell Debris and Chitin Resin

In the application of vortex flow adsorption to recovering biochemical products, a practical concern is whether there exists adsorptive interaction between adsorbent resin and cellular material, which could reduce the availability of the adsorbent binding sites for the desired protein.

Retention of cells or cell debris can result only from mechanical retardation in the fluid void in the vortex flow adsorption system. If a pulse of cells or cell debris is applied to the vortex flow reactor, mechanical retardation results in a distorted response peak with a significantly increased mean residence time compared to a non-retarded tracer molecule (Feuser *et al.*, 1999). The total response peak area, however, would not differ from the input pulse if no adsorption occurs, and any cell or cell debris applied to the reactor would pass through sooner or later. If there were adsorptive interaction between cell debris and adsorbent resin, it would be detected by a reduction in the overall response peak area. The cell debris recovery is defined to describe the percentage of the cell debris applied to the vortex flow
reactor that can flow out eventually. Theoretically, if the interaction between cell debris and adsorbent resin is strong, the cell debris recovery is low.

Figure 5.18 shows the results of cell debris pulse-response experiments with the Streamline DEAE resin and the chitin resin. When the same amount of *E. coli* cell debris was applied to the vortex flow reactor, the response area for the chitin resin was bigger than that for Streamline DEAE. Figure 5.19 shows the recovery of *E. coli* cell debris for the two resins. For Streamline DEAE, only 48.2% of the loaded cell debris eventually flowed out of the vortex flow reactor. Therefore, the interaction between Streamline DEAE and *E. coli* cell debris is very strong. Since Streamline DEAE is an anion exchange resin and *E. coli* cell debris was suspended in Tris buffer at pH 8, *E. coli* cell debris must be negatively charged at pH 8 and prone to adsorption to positively charged anion exchange resin. This agrees with the general expectation that cell surface is negatively charged at neutral pH (Feuser *et al.*, 1999). For the chitin resin prepared by the DVS method, around 95.6% of the cell debris applied to the vortex flow reactor was recovered. This means that the interaction between *E. coli* cell debris and the chitin resin is almost negligible. In this study, the chitin resin was used to adsorb the intein α1-AT fusion protein through a chitin-binding domain on the intein segment. Therefore, when vortex flow adsorption is applied to directly recovering the fusion protein from crude *E. coli* cell extract without first separating cell debris, the adsorption capacity of the chitin resin for the fusion protein cannot be affected by the presence of *E. coli* cell debris.
20 ml of *E. coli* cell debris suspension at OD$_{600}$ = 0.5 in Tris buffer (0.1 M Tris, 5 mM EDTA, pH 8.0) with released intracellular components removed was used as a pulse tracer.

The error bars represent standard deviations of triplicate runs.

**Figure 5.18:** Cell debris pulse-response experiment results in vortex flow reactor

**Figure 5.19:** *E. coli* cell debris recovery in the pulse-response experiments for both Streamline DEAE anion exchange resin and chitin resin

The error bars represent standard deviations of triplicate runs.
5.4.4 Fluidization of Chitin Resin

In addition to the adsorptive interaction between the chitin resin and *E. coli* cell debris, another practical problem related to the application of vortex flow adsorption to recovering the intein α1-antitrypsin fusion protein is the fluidization of the chitin resin.

For the chitin resin used in this work, it has a particle density of ~1.5 g/ml and a particle diameter range of 40-100 µm. The minimum rotation rate to fluidize the chitin resin was experimentally measured to be 320 rpm. Therefore, in all the vortex flow adsorption experiments, the rotation speed of the inner cylinder must be higher than 320 rpm in order to fluidize the chitin resin completely.

5.4.5 Vortex Flow Adsorption Process

There are a total of four steps in the whole vortex flow adsorption process: loading/adsorption, washing, cleavage, and stripping. In the loading of the crude disrupted cell extract, the fusion protein is adsorbed by the chitin resin due to the affinity interaction between the CBD on the fusion protein and the chitin resin. The other components in the crude cell extract, including cell debris, flow out of the reactor. The subsequent washing step is used to wash off non-adsorbed proteins and cell debris both on the surface of the chitin resin and in the fluid void in the vortex flow reactor. The cleavage step is used to cleave α1-AT from the chitin resin. The stripping step is used to elute the intein segment, non-cleaved fusion protein, and other non-specifically adsorbed proteins from the chitin resin. Both the cleavage and stripping steps are used to quantify how much fusion protein is adsorbed in the adsorption step, i.e., the efficiency of the adsorption step. Since our main purpose is to explore vortex flow adsorption
as a method for recovering proteins from non-clarified cell extract, we are more interested in the loading/adsorption step instead of the cleavage and stripping steps.

5.4.6 Evaluation of Vortex Flow Adsorption Performance

Two process parameters, capture efficiency and cleavage efficiency, were used to evaluate the performance of vortex flow adsorption. The capture efficiency is defined as the percentage of the loaded fusion protein in the crude *E. coli* cell extract that is adsorbed in the loading/adsorption step. The adsorbed fusion protein is either cleaved in the cleavage step as α1-AT or eluted in the stripping step as non-cleaved fusion protein. The cleavage efficiency is defined as the percentage of the adsorbed fusion protein that is recovered as α1-AT in the cleavage step. The cleavage efficiency depends on the cleavage mechanism. The N-terminal cleavage of the N-extein is induced by DTT. The subsequent C-terminal cleavage of α1-AT is induced by the N-terminal cleavage. The C-terminal cleavage needs a basic condition (Chong *et al.*, 1998b), and pH 8 was used in this work. Both the capture efficiency and the cleavage efficiency were determined through the SDS-PAGE and Western blotting assays.

Figure 5.20 shows the measurements for the outlet stream of the vortex flow reactor during the loading and washing steps. After 10 minutes’ loading, unbound proteins (A280) and cell debris (OD600) were almost washed off within 2 hours. Only when the readings of the UV detector and the Chem2000-UV-Vis spectrometer went to zero did the cleavage step start.
Figure 5.20: Outlet stream measurements during loading/adsorption and washing steps in vortex flow adsorption process for recovery of intein α₁-antitrypsin fusion protein

Figure 5.21 shows the results of the SDS-PAGE and Western blotting assays for clarified loading sample, cleavage sample, and stripping sample in the vortex flow adsorption process. The flow-through samples during the loading/adsorption and washing steps were analyzed by SDS-PAGE qualitatively not quantitatively and are not shown in Figure 5.21. In SDS-PAGE, proteins are separated and assayed based on protein size, i.e., molecular weight. Specifically, the molecular weights for the fusion protein, the intein, and α₁-antitrypsin are 100.6 kDa, 55 kDa, and 44 kDa, respectively. All these proteins can be detected in the Coomassie blue stained polyacrylamide gels. The N-extein cannot be detected due to its small size with a molecular weight of 1.6 kDa. Since it is very likely that two different proteins with the same or close molecular weights are in a single protein band in the gels, a question about SDS-PAGE is whether or not some co-migrating proteins were also counted when the fusion protein and α₁-antitrypsin were quantified. Therefore, Western blotting was used as an
alternative assay method since it is based on the specific affinity interaction between antigen and antibody. In the Western blotting assay, only the intein α1-AT fusion protein and α1-antitrypsin can be detected.

**Figure 5.21: SDS-PAGE and Western blotting assays for vortex flow adsorption**

Clarified cell extract, cleavage sample, and stripping sample in one run of vortex flow adsorption were analyzed at least twice in the assays. N-extein (MW: 1.6 kD) is too small to be detected in the gels.

Figure 5.22 summarizes the capture efficiency and cleavage efficiency of vortex flow adsorption from SDS-PAGE and Western blotting assays. The capture efficiency and cleavage efficiency were 5.9% and 20.8%, respectively, from the SDS-PAGE assay. However, they were 26.2% and 21.4%, respectively, from the Western blotting assay. The capture efficiency calculated from the Western blotting assay was around 4.5 times that from the SDS-PAGE assay. It is apparent that, in the SDS-PAGE assay, some co-migrating proteins were counted as the fusion protein in the crude cell extract sample, which leads to a very low capture efficiency. Therefore, the Western blotting assay results are more believable. The cleavage efficiency was
almost the same between the two assays. Therefore, almost no other proteins co-migrated with α1-AT or the fusion protein in the cleavage and stripping samples. Since the capture efficiency represents the performance of vortex flow adsorption, the capture efficiency of 26.2% obtained from the Western blotting assay is very encouraging since only one unit operation, vortex flow adsorption, was used in the whole downstream recovery process.

![Capture efficiency and cleavage efficiency from SDS-PAGE and Western blotting assays](image)

**Figure 5.22: Capture efficiency and cleavage efficiency from SDS-PAGE and Western blotting assays**

The error bars are standard deviations of two independent VFA runs.

### 5.4.7 Non-Clarified vs. Clarified *E. coli* Cell Extract

In the vortex flow adsorption system, there are three major components, i.e., *E. coli* cell debris, the chitin resin, and the fusion protein. As is shown in Figure 5.23, there is affinity interaction between the chitin resin and the fusion protein. In addition, the outcome of the *E. coli* cell debris pulse-response experiments confirmed that the interaction between cell debris
and chitin resin is negligible. In the interaction network, the remaining question is whether there exists interaction between the fusion protein and cell debris that would affect the free concentration of the fusion protein available for the adsorption of the chitin resin.

![Interaction network between components in vortex flow adsorption system](image)

**Figure 5.23: Interaction network between components in vortex flow adsorption system**

In order to answer this question, the vortex flow adsorption process was conducted by loading clarified *E. coli* cell extract instead of non-clarified *E. coli* cell extract. By using clarified *E. coli* cell extract, the interaction between cell debris and the fusion protein can be avoided if it exists. From the SDS-PAGE assay results in Figure 5.24, the capture efficiency was 6.9% for the clarified *E. coli* cell extract, which was consistent with the value of 5.9% for the non-clarified cell extract. Therefore, the presence of cell debris significantly affects neither the free concentration of the fusion protein nor the adsorption of the fusion protein by the chitin resin.
5.4.8 N-terminal Amino Acid Sequence Analysis

As is discussed above, in the SDS-PAGE assay, there are proteins that co-migrate with the fusion protein in the cell extract. In order to explore what proteins existed in the protein bands corresponding to the fusion protein on polyacrylamide gels, N-terminal amino acid sequencing was conducted for these protein bands. In addition, N-terminal amino acid sequencing can also be used to check whether the fusion of human α1-antitrypsin to the C-terminus of the intein may lead to an N-terminus sequence change in the cleaved α1-antitrypsin.

For the fusion protein bands, there were two dominant sequences: one was MKIEEGKLVI, which is exactly the N-terminal sequence of MBP, from which the N-extein, i.e., the initial 12 amino acids at the N-terminus of the fusion protein, was derived; the other was AVTNVAELNALVER, which was determined to be the N-terminal sequence of alcohol dehydrogenase (MW: 96 kDa) of *E. coli* through protein database search. The encouraging
result was that alcohol dehydrogenase was dominant in the crude cell extract sample and the fusion protein was dominant in the cleavage and stripping samples. Therefore, in the SDS-PAGE assay, alcohol dehydrogenase was mistakenly taken into account in quantifying the fusion protein in the crude cell extract, which leaded to a low capture efficiency.

In addition, the sole N-terminal sequence of the cleaved α1-antitrypsin was determined to be AGHMDPQGDAAQKTDT. The first three residues (AGH) were added when the gene encoding human α1-antitrypsin was inserted into the frame of the plasmid pTYB12 through the NdeI site, and the remaining residues (MDPQGDAAQKTDT) were exactly the N-terminal sequence of human α1-antitrypsin whose code was from the plasmid pEAT8-137.

5.4.9 Activity Assay for Vortex Flow Adsorption Process

In this study, porcine pancreatic elastase was used to measure the activity, i.e., elastase inhibitory capacity (EIC), of α1-antitrypsin and the intein α1-AT fusion protein. The units for EIC are milli-absorbance unit per second (mAU/Sec). The EIC was measured for both the crude cell extract and the cleavage sample, and the results are shown in Figure 5.25. For the clarified crude cell extract, the specific activity was 0.3 EIC/(mg total protein), and it means that the concentration of the fusion protein relative to the total protein was very low in the cell extract. After adsorption and cleavage, the specific activity was dramatically improved to be 205.2 in the cleavage sample.

As was reported previously (Laska, 2001b), the N-terminal region in α1-antitrypsin is flexible and disordered and could not be assigned coordinates in the crystal structure. In the fusion protein, α1-antitrypsin is fused to the C-terminus of the intein. Therefore, it is
reasonable to assume that the existence of the intein segment could not affect the folding of $\alpha_1$-antitrypsin and its activity.

**Figure 5.25: Activity assay for vortex flow adsorption process**

The specific activity of $\alpha_1$-antitrypsin or fusion protein was measured in EIC per unit amount of total protein for clarified crude cell extract and cleavage sample. The error bars represent standard deviations of triplicate VFA runs.

**5.4.10 Summary**

In this research, the intein $\alpha_1$-antitrypsin fusion protein was used as a model system to demonstrate that vortex flow adsorption not only can capture the fusion protein from crude cell extract but also can efficiently purify $\alpha_1$-antitrypsin. The purification in this application was due to both the specific affinity between the chitin resin and the CBD on the fusion protein as well as the specific DTT-induced peptide cleavage at the N- and C- terminus of the intein. Therefore, vortex flow adsorption has proven that the primary steps, i.e., clarification,
concentration, and purification in the conventional downstream process, can be integrated to efficiently recover and purify biochemical products.
5.5 Theoretical Analysis of Vortex Flow Adsorption Performance

5.5.1 Introduction

In the above discussion, vortex flow adsorption is explored to recover recombinant fusion protein directly from crude *E. coli* cell extract without first clarifying cells or cell debris. The capture efficiency in the vortex flow adsorption process depends on many factors.

One category of factors is related to operating variables of vortex flow adsorption, including rotation rate of the inner cylinder, axial loading flowrate, and adsorbent resin volume fraction. In the vortex flow adsorption system, the rotation rate of the inner cylinder is very important in that it not only facilitates the fluidization of the adsorption resin but also determines both the axial dispersion in the vortex flow reactor and the vortex flow regime in which the system is operated. The detailed study about the effect of the operating conditions on the performance of vortex flow adsorption was discussed previously with a BSA and Streamline DEAE system.

The other category of factors is related to the adsorption mechanism between the fusion protein and the chitin resin, e.g., adsorbent capacity and adsorption affinity. If the adsorption mechanism can be described by the Langmuir mechanism,

\[ A + L \xrightleftharpoons[\frac{k_2}{k_1}]{k_1} AL \]  \hspace{1cm} (5-7)

where A is a protein, L is an adsorbent resin, and AL represents the adsorbed protein. By assuming elementary reactions, the rate of the adsorption reaction may be described by Equation (5-8),

\[ \frac{dC_s}{dt} = k_1 C(\Omega - C_s) - k_2 C_s \]  \hspace{1cm} (5-8)
At equilibrium,
\[ C_s = \frac{QKC}{1 + KC} \]  
(5-9)

where \( C_s \) is the adsorbed protein concentration, \( C \) is the fluid protein concentration, \( Q \) is the saturated adsorbent capacity for the adsorbate protein, and \( K \) is the affinity constant which represents the affinity interaction between the adsorbent and the adsorbate. \( K \) is the ratio of the forward and backward reaction rate coefficients, \( k_1 \) and \( k_2 \).

\[ K = \frac{k_1}{k_2} \]  
(5-10)

It is apparent that the parameters \( Q, K, k_1, \) and \( k_2 \) could describe the adsorption mechanism. These parameters are very important in determining the capture efficiency of the vortex flow adsorption process.

As discussed previously, the intein \( \alpha \)-1-antitrypsin fusion protein can be adsorbed to the chitin resin through the CBD on the intein segment. However, the fusion protein cannot be purified through the chitin resin affinity adsorption alone because there is nonspecific adsorption of other cellular proteins. Additional purification steps are needed to purify the fusion protein first in order to measure the adsorption kinetics and equilibrium parameters for the fusion protein and chitin resin system.

In this work, instead of characterizing the fusion protein and chitin resin system in detail, a well-studied adsorption system was cited: \( \beta \)-galactosidase and anti-\( \beta \)-galactosidase immobilized on non-porous glass coated beads. This is an affinity adsorption system with non-porous adsorbent resin, which is also the situation for the fusion protein and chitin resin system. Therefore, by studying this alternative system with a modeling approach, it could be
understood how the adsorption mechanism \(Q, K, k_1,\) and \(k_2\) and the operating conditions affect the capture efficiency in the vortex flow adsorption system.

5.5.2 Modeling Equations

In the BSA and Streamline DEAE system, a film resistance and pore diffusion model was used to describe the mass transfer of the solute protein from the bulk fluid to the binding sites of the porous adsorbent resin. In a non-porous adsorption system, the adsorption model is different and described for a batch adsorption system (McCoy and Liapis, 1991) in the following. For the bulk fluid,

\[
\frac{dC_a}{dt} = \frac{1 - \varepsilon}{\varepsilon} \frac{3}{r_0} K_f \left( C_{dp} - C_a \right)
\]  
(5-11)

For the adsorbent phase,

\[
\frac{dC_s}{dt} = \frac{3}{r_0} K_f \left( C_a - C_{dp} \right)
\]  
(5-12)

From the Langmuir adsorption mechanism,

\[
\frac{dC_s}{dt} = k_1 C_{dp} \left( Q - C_s \right) - k_2 C_s
\]  
(5-13)

By combining Equations (5-12) and (5-13),

\[
C_{dp} = \frac{3K_f}{r_0} \frac{C_a + k_2 C_s}{\frac{3K_f}{r_0} + k_1 \left( Q - C_s \right)}
\]  
(5-14)

The fluid protein concentration on the resin surface, \(C_{dp}\), in Equations (5-11) and (5-12) can be described by Equation (5-14). Equations (5-11) and (5-12) can be solved together with appropriate initial conditions to get the time profiles for \(C_a\) and \(C_s\).
If the two-region vortex flow model and the non-porous adsorption model are combined, the following equations can be used to simulate vortex flow adsorption with non-porous adsorbent. The species conversation equations for the bypass and vortex core regions are,

\[
\frac{dC_b^i}{dt} = \frac{V_{as}}{\varepsilon V_b^i} (C_{i-1}^b - C_i^b) + \frac{AD_L}{dV_b^i} \left[ (C_{i+1}^b - C_i^b) - (C_i^b - C_{i-1}^b) \right] - \frac{K_{bc} S_{bc}^b}{V_b^i} (C_i^b - C_i^c) - \frac{1 - \varepsilon}{\varepsilon} \frac{3}{r_0} K_f \left( C_i^b - C_{i,dp}^b \right)
\]  
(Bypass Region)  \hspace{1cm} (5-15)

\[
C_i^b = 0 \hspace{1cm} t = 0  
\]  
(5-16)

\[
\frac{dC_i^c}{dt} = \frac{K_{bc} S_{bc}^c}{V_i^c} (C_i^b - C_i^c) - \frac{1 - \varepsilon}{\varepsilon} \frac{3}{r_0} K_f \left( C_i^c - C_{i,dp}^c \right)
\]  
(Vortex core)  \hspace{1cm} (5-17)

\[
C_i^c = 0 \hspace{1cm} t = 0  
\]  
(5-18)

For the adsorbent phase,

\[
\frac{dC_{i,s}^b}{dt} = \frac{3K_f}{r_0} \left( C_i^b - C_{i,dp}^b \right)  
\]  
(Bypass region)  \hspace{1cm} (5-19)

\[
C_{i,s}^b = 0 \hspace{1cm} t = 0  
\]  
(5-20)

\[
\frac{dC_{i,s}^c}{dt} = \frac{3K_f}{r_0} \left( C_i^c - C_{i,dp}^c \right)  
\]  
(Vortex core)  \hspace{1cm} (5-21)

\[
C_{i,s}^c = 0 \hspace{1cm} t = 0  
\]  
(5-22)

The correlations between the adsorbed concentration and the fluid bulk concentration are from Equation (5-14),
\[
C_{i,dp}^b = \frac{3K_f C_i^b + k_2 C_{i,s}^b}{\frac{r_0}{3K_f} + k_1(Q - C_{i,s}^b)} \quad \text{(Bypass region)} \quad (5-23)
\]
\[
C_{i,dp}^c = \frac{3K_f C_i^c + k_2 C_{i,s}^c}{\frac{r_0}{3K_f} + k_1(Q - C_{i,s}^c)} \quad \text{(Vortex core)} \quad (5-24)
\]

where \( i = 1, \ldots, N \). \( N \) is the number of stages along the axial direction of vortex flow reactor and is 17 for the reactor configuration used in this work. By solving this set of ordinary differential equations through a MATLAB ordinary differential equation solving routine, ode15s, the protein concentrations in the bulk fluid and on the adsorbent resin in both vortex core and bypass regions can be obtained.

### 5.5.3 Model System and Parameters

For the β-galactosidase and anti-β-galactosidase resin system, the adsorption mechanism parameters (McCoy and Liapis, 1991) are listed in Table 5-6. In the theoretical analysis, these parameters and the baseline case described in Table 5-7 were used together to investigate the change of the capture efficiency when these parameters vary. As listed in Table 5-7, the operating conditions, i.e., rotation rate, axial loading flowrate, and adsorbent resin volume fraction, are the same as those used in the recovery of the fusion protein from the crude cell extract by vortex flow adsorption. The axial dispersion coefficient \( D_L \) and the vortex core-bypass mass transfer coefficient \( K_{bc} \) are from the simulation of the breakthrough capacity of the BSA and Streamline DEAE system at the same operating conditions. The rest of the parameters in Table 5-7 are from the literature (McCoy and Liapis, 1991).
Table 5-6: Parameters for Adsorption Mechanism

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Q, g/L</th>
<th>K, L/g</th>
<th>(k_1), L/g-s</th>
<th>(k_2), s(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-porous glass coated beads</td>
<td>0.34</td>
<td>19.12</td>
<td>(6.19 \times 10^{-2})</td>
<td>(3.20 \times 10^{-3})</td>
</tr>
<tr>
<td>Porous silica beads</td>
<td>2.20</td>
<td>4540</td>
<td>(2.35 \times 10^{-2})</td>
<td>(5.17 \times 10^{-6})</td>
</tr>
</tbody>
</table>

Table 5-7: Modeling Parameters for the Baseline Case

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotation Rate</td>
<td>rpm</td>
<td>300</td>
</tr>
<tr>
<td>(V_{ax})</td>
<td>m(^3)/s</td>
<td>(10^{-7})</td>
</tr>
<tr>
<td>(\varepsilon)</td>
<td>------</td>
<td>0.81</td>
</tr>
<tr>
<td>(C_0)</td>
<td>g/L</td>
<td>(6.20 \times 10^{-3})</td>
</tr>
<tr>
<td>(K_f)</td>
<td>m/s</td>
<td>(4.38 \times 10^{-6})</td>
</tr>
<tr>
<td>(D_L)</td>
<td>m(^2)/s</td>
<td>(5.00 \times 10^{-6})</td>
</tr>
<tr>
<td>(K_{bc})</td>
<td>m/s</td>
<td>(2.22 \times 10^{-5})</td>
</tr>
<tr>
<td>Q</td>
<td>g/L</td>
<td>0.34</td>
</tr>
<tr>
<td>(K)</td>
<td>(g/L(^{-1}))</td>
<td>19.12</td>
</tr>
<tr>
<td>(k_1)</td>
<td>(g/L(^{-1})/s)</td>
<td>(6.19 \times 10^{-2})</td>
</tr>
<tr>
<td>(k_2)</td>
<td>s(^{-1})</td>
<td>(3.20 \times 10^{-3})</td>
</tr>
<tr>
<td>(r_0)</td>
<td>m</td>
<td>(8.60 \times 10^{-5})</td>
</tr>
</tbody>
</table>

The capture efficiency also depends on the loading time or amount of the sample. If the loading time is short, the capture efficiency could be high due to unoccupied binding sites on the adsorbent resin. On the contrary, if the sample is overloaded, the protein molecules pass through the reactor without being adsorbed due to lack of adsorption sites. In this work, the loading time was fixed at 40 minutes to explore the effect of other parameters.
5.5.4 The Effect of Q

The adsorbent capacity, Q, is very important in determining the capture efficiency in the vortex flow adsorption process. The difference in the value of Q may reflect the difference in the total amount of anti-β-galactosidase that can be coupled to different supports (e.g., porous silica beads and non-porous glass coated beads) that have different available specific surface area (i.e., surface area per unit volume of beads). As Figure 5.26 indicates, if Q can be improved from 0.34 g/L for non-porous resin to 2.2 g/L for porous resin with a high specific surface area, the capture efficiency could be doubled from 41% to 85%. In fact, it is impossible for non-porous resin to have a very high adsorbent capacity due to a very low specific surface area. However, the data shown in Figure 5.26 suggest that a strategy to improve the capture efficiency is to improve the coating concentration of the ligand on the matrix surface, i.e., the density of the binding sites.

![Figure 5.26: Capture efficiency at different adsorbent capacities](image_url)
5.5.5 The Effect of $K$ and $k_1$

The difference in $K$ values may be evidence for various conformational changes occurring in the anti-$\beta$-galactosidase structure when it is immobilized on different supports (Liapis, 1989). These variations may affect the ability of anti-$\beta$-galactosidase to bind adsorbate to varying extents (McCoy and Liapis, 1991). With a high value of $K$, the affinity of the adsorbent to the adsorbate is high. $K$ is the ratio of the forward and backward adsorption reaction rate coefficients, $k_1$ and $k_2$. In the vortex flow adsorption process, fast adsorption kinetics is advantageous for the protein to be adsorbed by the adsorbent resin before the protein molecules pass through the reactor either by axial convection or dispersion.

Listed in Table 5-8, three cases with different adsorption kinetics were considered in this work. Cases A and B are real situations, and case C is an imaginary situation. Between case A and case C, $k_1$ is fixed and $K$ is increased almost 240 times. As is shown in Figure 5.27, the capture efficiency is increased from 41% to 81%. Between case B and case C, $K$ is fixed and $k_1$ is increased about 2.6 times. The capture efficiency is, however, increased from 61% to 81%. Therefore, favorable adsorption affinity and/or fast adsorption kinetics are very important for improving the capture efficiency in the vortex flow adsorption process. The capture efficiency is more sensitive to the adsorption kinetics.

Table 5-8: Three Cases with Different Adsorption Kinetics

<table>
<thead>
<tr>
<th>Case</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$</td>
<td>(g/L)$^{-1}$</td>
<td>19.12</td>
<td>4.54 $\times 10^3$</td>
</tr>
<tr>
<td>$k_1$</td>
<td>(g/L)$^{-1}$/s</td>
<td>6.19 $\times 10^{-2}$</td>
<td>2.35 $\times 10^{-2}$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>s$^{-1}$</td>
<td>3.20 $\times 10^{-3}$</td>
<td>5.17 $\times 10^{-6}$</td>
</tr>
</tbody>
</table>
5.5.6 The Effect of Rotation Rate

In the above discussion, the focus is on the effect of the adsorption mechanism on the capture efficiency. In this section, the effect of operating conditions, especially the rotation rate of the inner cylinder, on the capture efficiency is explored. As is known from the tracer RTD study, the axial dispersion coefficient is very sensitive to the change of the rotation rate in the Taylor vortex flow regime. Therefore, the axial dispersion coefficients obtained from the modeling of the tracer RTD data are used to explore how the capture efficiency varies with the axial dispersion coefficient or the rotation rate of the inner cylinder in the Taylor vortex flow regime.

In Table 5-9, the axial dispersion coefficient $D_L$ and the vortex core-bypass mass transfer coefficient $K_{bc}$ at different rotation rates are listed. The axial dispersion coefficient increases dramatically with the increase of the rotation rate. When the rotation rate increases from 15 rpm to 150 rpm, the axial dispersion coefficient increases almost 2000 times.
However, as Figure 5.28 suggests, the capture efficiency only decreases from 49% to 40%. It is apparent that, although the axial dispersion changes dramatically in the Taylor vortex flow regime, the resultant change of the capture efficiency is relatively small. Worthy of mention, the fluidization of the adsorbent resin is neglected for the time being. It is impossible for the adsorbent resin to be fluidized at very low rotation rates, e.g., 15 rpm.

**Table 5-9: Axial Dispersion Coefficient $D_L$ and Vortex Core-Bypass Mass Transfer Coefficient $K_{bc}$ at Different Rotation Rates**

<table>
<thead>
<tr>
<th>Rotation Rate</th>
<th>rpm</th>
<th>15</th>
<th>50</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_L$</td>
<td>m$^2$/s</td>
<td>$1.00 \times 10^{-8}$</td>
<td>$4.50 \times 10^{-6}$</td>
<td>$2.00 \times 10^{-5}$</td>
</tr>
<tr>
<td>$K_{bc}$</td>
<td>m/s</td>
<td>$1.60 \times 10^{-4}$</td>
<td>$5.00 \times 10^{-3}$</td>
<td>$1.00 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

**Figure 5.28: Capture efficiency at different rotation rates of inner cylinder**

As is known, the rotation rate of 15 rpm is in the Taylor vortex flow regime, and the rotation rate of 150 rpm is very close to the turbulent vortex flow regime. The tracer RTD study shows that the axial dispersion coefficient is almost constant and independent of the...
rotation rate in the turbulent vortex flow regime; hence the capture efficiency is expected to be
invariant. Therefore, the capture efficiency does not change too much when vortex flow
adsorption is operated at different flow regimes, which is different from the situation for the
axial dispersion.

5.5.7 Summary

The performance of the vortex flow adsorption process was evaluated theoretically with
an alternative affinity adsorption system, β-galactosidase and anti-β-galactosidase resin. The
adsorption mechanism, e.g., Q, K, k₁, and k₂, plays a very important role in determining the
capture efficiency. In addition, by exploring the capture efficiency at different rotation rates, it
was found that the capture efficiency is not so sensitive as the axial dispersion coefficient to the
change of the rotation rate.

In the direct recovery of the fusion protein by the vortex flow adsorption system, as is
suggested from the theoretical analysis, the capture efficiency can be improved if adsorbent
capacity Q, adsorption affinity constant K, or adsorption kinetics k₁ and k₂ could be improved.

For the chitin resin used in this study, it can be fully fluidized only when the rotation
rate is above 320 rpm, at which vortex flow adsorption is operated in the turbulent vortex flow
regime. Although the physical properties of the adsorbent resin can be manipulated so that the
resin may be completely fluidized in the Taylor vortex flow regime, the improvement of the
capture efficiency is not dramatic based on the above discussion.
6 Design and Optimization of Vortex Flow Adsorption System

6.1 Introduction

Different aspects of vortex flow adsorption are investigated in this research, e.g., axial dispersion, dynamic capacity, adsorbent resin fluidization, cell debris and adsorbent resin interaction, intein-mediated protein purification, direct recovery of fusion protein by vortex flow adsorption, as well as theoretical analysis of the performance of the vortex flow adsorption process, etc. Based on all this information, process design and optimization of vortex flow adsorption is discussed in this section.

6.2 Determination of Adsorption Mechanism

For an adsorption system, the adsorption mechanism includes adsorption equilibrium and adsorption kinetics. Both the Langmuir and bi-Langmuir mechanisms can be used to describe the adsorption equilibrium. For the adsorption kinetics, the film resistance and pore diffusion mechanism can be employed for porous adsorbent resin; only the film resistance should be considered for non-porous adsorbent resin. Therefore, both adsorption equilibrium and adsorption kinetics experiments should be conducted to find out what adsorption mechanism is valid for a specific adsorption system.

6.3 Design of Adsorbent Resin

In addition to the adsorption mechanism, the physical properties of adsorbent matrix are very important in the vortex flow adsorption system. The physical properties of particular interest include particle size and particle density as well as the porosity of the matrix.
Based on the porosity of the adsorbent matrix, there are non-porous adsorbent resins and porous adsorbent resins. For the non-porous adsorbent resin, the bottleneck of mass transfer, the pore diffusion step, can be avoided. However, the specific surface area for this resin is quite low, which affects the adsorbent capacity, i.e., the concentration of the binding sites on the adsorbent surface. For the porous adsorbent resin, the adsorbent capacity is high due to a high specific surface area. However, the overall adsorption process is controlled by the slow pore diffusion step. Therefore, there exists a trade off between the adsorbent capacity and the adsorbate mass transfer in choosing the adsorbent matrix.

In the vortex flow adsorption system, in order to process fermentation broths or homogenized cell cultures without first separating particulate materials, sufficient differences in size and density between adsorbent resin and cell materials are necessary to implement solid-solid separation between adsorbent resin and cellular materials. Therefore, adsorbent resin must be heavier than cells that have a wet density of \( ~1.05 \) g/ml. However, as is indicated from Figure 6.1, which is based on the correlation obtained previously,

\[
R_{\text{min}} = 182.70 \left( \rho_p - \rho_f \right)^{0.42} d_p^{0.15} \mu^{0.26}
\]  

(6-1)

an adsorbent resin with a high density or a large size needs a high rotation rate to be fluidized. At a high rotation rate, the vortex flow reactor is operated in the flow regimes with high axial dispersion, which is not beneficial to the capture efficiency of the vortex flow adsorption system. Therefore, the optimization of the vortex flow adsorption system eventually results in the optimization of the physical properties of the adsorbent resin, i.e., particle size and particle density.
Figure 6.1: Contour plot of minimum fluidization rotation rate with particle density and particle diameter

The number labeling each curve is the value of the minimum fluidization rotation rate.

The purpose of optimizing the physical properties of adsorbent resin is to maintain sufficient differences in size and density between adsorbent resin and cellular materials to assure the solid-solid separation between adsorbent resin and cellular materials. Simultaneously, the differences cannot be very big to make adsorbent resin too heavy or too big to be fluidized at a low rotation rate. High rotation rates would lead to high axial dispersion that deteriorates the performance of the vortex flow adsorption process.

6.4 Design of Vortex Flow Reactor

In this research, a vortex flow reactor with fixed geometric dimensions was used to study the performance of vortex flow adsorption. In fact, the geometric dimensions could determine both the critical Taylor number to induce vortex flow and the operating regime.
As is discussed previously, the critical Taylor number to produce vortices, $Ta_c$, can be calculated with Equation (6-2) (Esser and Grossmann, 1996),

$$Ta_c = \frac{1}{0.1556^2} \frac{(1 + \eta)^2}{2\eta \sqrt{(1 - \eta)(3 + \eta)}}$$  \hspace{1cm} (6-2)

In Figure 6.2, the critical Taylor number, $Ta_c$, is plotted for different $\eta$ values. When the radius ratio is less than 0.46, the critical Taylor number decreases with the increase of the radius ratio; however, when the radius ratio is above 0.46, the critical Taylor number increases with the increase of the radius ratio. When the radius ratio is around 0.46, the critical Taylor number reaches a minimal value of 70. For the configuration of the vortex flow reactor used in this work, $\eta = 0.68$ and the corresponding critical Taylor number is 79.

![Figure 6.2: Critical Taylor number $Ta_c$ for vortex flow reactor with different geometric dimensions](image)

The solid cycle represents the case for the configuration of the vortex flow reactor used in this work.
If the geometric dimensions of vortex flow reactor can be adjusted in the design process, especially the radius ratio of the inner and outer cylinders, the critical Taylor number could be changed. As a consequence, the lower and upper boundaries of different vortex flow regimes listed in Table 4-3 would be changed. It is likely that the same rotation rate of the inner cylinder could produce turbulent vortex flow in one configuration of vortex flow reactor and Taylor vortex flow in another. Consequently, at the same operating conditions, the mass transfer and axial dispersion could be different in the vortex flow systems with different geometric dimensions.

### 6.5 Operation of Vortex Flow Adsorption

In this research, the effect of the operating conditions (e.g., rotation rate of the inner cylinder, axial flowrate, and adsorbent resin volume fraction) on the performance of vortex flow adsorption is focused upon. Among these operating variables, the rotation rate of the inner cylinder is pivotal in that the rotation of the inner cylinder induces the secondary vortex flow pattern and facilitates the fluidization of the adsorbent resin. Simultaneously, the rotation of the inner cylinder significantly affects axial dispersion. As is known, the minimum rotation rate for fluidizing the adsorbent resin can be optimized by manipulating the physical properties of the adsorbent resin. In addition, the critical Taylor number to induce the secondary vortex flow pattern could be optimized by adjusting the geometric dimensions of the vortex flow reactor. Supposedly, an optimal rotation rate to lower the axial dispersion in the vortex flow reactor could be obtained by manipulating the physical properties of the adsorbent resin and the geometric dimensions of the vortex flow reactor.
6.6 Summary

To sum up, designing and optimizing a vortex flow adsorption system should be implemented with a systems engineering approach. The major purpose of the vortex flow adsorption process is to integrate the early steps in conventional downstream processing, i.e., clarification, concentration, and purification, into a new single unit operation to simplify the whole process. As shown in Figure 6.3, the optimization of the vortex flow adsorption process eventually requires manipulating the geometric dimensions of vortex flow reactor, the physical properties of adsorbent resin (i.e., particle density, particle size, and the porosity of the support), and adsorption mechanism. All these are considered through different modeling parameters in the two-region vortex flow adsorption model.
7 Contributions and Conclusions

One strategy to reduce costs in manufacturing a biochemical product is simplification of downstream processing. Biochemical product recovery often starts from fermentation broth or cell culture. In conventional downstream processing, the initial steps are clarification, concentration, and purification. Simplification of downstream processing may be achieved by reducing the number of unit operations. An integrative technology seeks to combine steps into a new single unit operation to simplify the whole process.

Vortex flow occurs in the annular gap between an inner rotating solid cylinder and an outer stationary cylindrical shell. Above a critical rotation rate, circular Couette flow bifurcates to a series of counter-rotating toroidal vortices. By suspending adsorbent resin in the vortices, a novel unit operation, vortex flow adsorption (VFA), is created. In VFA, the rotation of the inner cylinder facilitates the fluidization of the adsorbent resin. Similar to expanded bed processes, VFA has a high fluid void fraction. Therefore, it can be used to recover biochemical products directly from fermentation broths or cell homogenates without first separating cells or cell debris.

Since vortex flow adsorption is a novel application of vortex flow, it is necessary to characterize its behavior especially the axial dispersion or back mixing. In the vortex flow system, the rotation rate of the inner cylinder is very important in determining its performance. Therefore, tracer residence time distribution (RTD) studies were carried out at different rotation rates.

As an alternative to the equilibrium capacity, the dynamic capacity is the capacity of an adsorption column or reactor under defined operating conditions. The adsorptive behavior in
the vortex flow system can be characterized by measuring the dynamic capacity under different operating conditions, including rotation rate, axial loading flowrate, and adsorbent volume fraction. In this work, a model system of bovine serum albumin (BSA) and Streamline DEAE anion exchange resin was used.

In addition to the experimental approaches, two modeling approaches were employed to characterize and simulate vortex flow adsorption. One model was a one-dimensional dispersion convective model. It was used to calculate the axial dispersion coefficient from the tracer RTD data. The other model was a two-region vortex flow model. In this model, the whole reactor is divided into stages each with an axial height equal to the annular gap width and at each stage there are two regions: vortex core region and bypass region. Therefore, two coefficients, the vortex core-bypass mass transfer coefficient, $K_{bc}$, and the axial dispersion coefficient, $D_L$, were used in the modeling. Through the two-region model, not only the axial dispersion coefficient can be obtained but also the species concentrations in both the vortex core and bypass regions at each stage can be simulated. In the simulation of the vortex flow adsorption system, a film resistance and pore diffusion model was used to describe the mass transfer of the solute from the bulk fluid to the pores of the adsorbent resin.

In the application of the vortex flow adsorption system to a real biochemical system, recombinant human $\alpha_1$-antitrypsin ($\alpha_1$-AT) was expressed in *E. coli* as a C-terminal fusion to a modified intein containing a chitin-binding domain. Therefore, the fusion protein can be recovered by chitin resin. Furthermore, the intein can be induced to undergo *in vitro* peptide bond cleavage to specifically release $\alpha_1$-AT from the chitin resin. The experimental results
indicated that vortex flow adsorption not only can efficiently recover the fusion protein from the crude cell extract containing cell debris but also can purify α1-AT.

To sum up, this research characterizes the performance of vortex flow adsorption and explores its application to directly recovering recombinant intein α1-AT fusion protein from crude *E. coli* cell extract. Vortex flow adsorption is an efficient method for integrating the primary clarification, concentration, and purification steps to simplify conventional downstream processing.

The specific contributions of this work are:

- The vortex flow reactor was improved by modifying the drive shaft and the outlet area. The vortex flow system with the new configuration can be used to study vortex flow adsorption with neither resin accumulation at the outlet nor wobbling problem.
- Tracer residence time distribution (RTD) studies were carried out at different rotation rates of the inner cylinder to explore the axial dispersive behavior in the vortex flow reactor. It was concluded that the axial dispersion is distinct in different vortex flow regimes. In the Taylor vortex flow regime, the axial dispersion is low and sensitive to the change of the rotation rate; in the wavy vortex flow regime, the axial dispersion is almost flat with the increase of the rotation rate; in the turbulent vortex flow, the axial dispersion coefficient is almost constant and independent of the rotation rate. Therefore, by manipulating the rotation rate of the inner cylinder, the performance of vortex flow adsorption, especially the axial dispersion, can be adjusted and controlled.
- The breakthrough capacity of the BSA and Streamline DEAE system was measured at different operating conditions. The results indicated that the breakthrough capacity is
almost independent of the rotation rate in the turbulent vortex flow regime due to the invariant axial dispersion. The breakthrough capacity decreases with the increase of the axial loading flowrate since the overall adsorption process is controlled by the pore diffusion of the BSA molecules in the adsorbent Streamline DEAE resin. In addition, increasing the adsorbent resin volume fraction is a good approach to increasing the breakthrough capacity.

- Two modeling approaches were used for simulating the vortex flow adsorption process: a one-dimensional dispersion convective model and a two-region vortex flow model. The two-region model was used not only for obtaining the axial dispersion coefficient but also for simulating the species concentrations along the axial direction in the vortex flow system.

- A recombinant form of human α1-antitrypsin (α1-AT) was expressed in E. coli as a C-terminal fusion to a modified intein containing a chitin-binding domain. The effect of the induction temperature was explored regarding to the E. coli cell growth, the fusion protein expression, and the fusion protein productivity.

- The glutaraldehyde and divinyl sulfide methods were developed to prepare a chitin resin that is robust and heavy enough to be used in the vortex flow adsorption system. Since the divinyl sulfide method is more efficient in obtaining a chitin resin with a high adsorbent capacity, it was used in the entire work.

- The minimum fluidization rotation rates were measured for adsorbent resins with different physical properties (e.g., particle density and particle size) in fluids with varying physical properties (e.g., fluid viscosity and density). An empirical approach, instead of exploring
the intrinsic mechanism of the fluidization process occurring in the vortex flow reactor, was followed. A correlation based on the Stokes’ law was developed.

- Vortex flow adsorption (VFA) was applied to directly recovering the fusion protein from crude *E. coli* cell extract. The results suggested that VFA not only captured the fusion protein but also purified α1-AT. It was concluded that vortex flow adsorption is an integrative technology to combine the primary clarification, concentration, and purification steps to simplify conventional downstream processing.

- A theoretical analysis was conducted to explore the effects of the adsorption mechanism and the operating conditions on the capture efficiency of the vortex flow adsorption system. It was concluded that the capture efficiency does not change dramatically when vortex flow adsorption is operated in different vortex flow regimes, which is different from the situation for the axial dispersion. Among the adsorption mechanism parameters (including Q, K, k₁ and k₂), the capture efficiency is very sensitive to the adsorption capacity Q and the adsorption kinetics k₁ and k₂.

- Some suggestions were put forward regarding the design and optimization of the vortex flow adsorption system. The rotation rate plays a very important role in determining the performance of vortex flow adsorption since the rotation of the inner cylinder not only induces the appearance of the secondary vortex flow pattern but also facilitates the fluidization of the adsorbent resin. Eventually, the optimization of the performance of the vortex flow adsorption process relies on the optimization of the geometric dimensions of the vortex flow reactor and the physical properties of the adsorbent resin.
8 Recommended Future Work

8.1 Characterization of Adsorption Mechanism between Fusion Protein and Chitin Resin

In applying vortex flow adsorption to directly recovering the fusion protein from crude *E. coli* cell extract, the adsorption mechanism between the fusion protein and the chitin resin is unknown. In the adsorption process between the cell extract and the chitin resin, in addition to the affinity adsorption of the fusion protein by the chitin resin, there is also non-specific adsorption of other components. Therefore, additional purification steps are necessary in order to purify the fusion protein. With the pure fusion protein, the adsorption mechanism, including the adsorption equilibrium and kinetics, between the fusion protein and the chitin resin, can be explored. Based on the adsorption mechanism, it would be possible to explore what exactly led to a capture efficiency of 26% in the vortex flow adsorption process. Furthermore, it would also be possible to separate the effect of the operating conditions from the effect of the adsorption mechanism on the performance of the vortex flow adsorption system. Just as the theoretical analysis of the vortex flow adsorption process for the β-galactosidase and anti-β-galactosidase resin system, it is feasible to study the effect of the adsorption mechanism and the operating conditions on the performance of the vortex flow adsorption of the fusion protein from the crude cell extract.

8.2 Simulation of Fluid Velocity in Vortex Flow System

The advantage of vortex flow adsorption is its ability to process fermentation broths or cell homogenates without first separating cellular materials. The adsorbent resin must be fluidized to allow for the passage of cells or cell debris. Therefore, it is necessary to determine
the minimum rotation rate for fluidizing the adsorbent resin. In this work, an empirical
correlation was developed to relate the minimum fluidization rotation rate to the physical
properties of the adsorbent resin and the fluid. The limitation of this correlation is that it is
empirical and based on the measurements in a vortex flow reactor with fixed geometric
dimensions. Therefore, a general correlation is desirable which is valid for any adsorbent resin
and vortex flow system.

As is known, the particles can be fluidized only when the velocity of the surrounding
fluid is above the sedimentation velocity of the particles. In the vortex flow system, above a
critical rotation rate, circular Couette flow bifurcates to a series of counter-rotating toroidal
vortices. In addition to the global tangential velocity, there are axial and radial velocities in the
vortices. It is desirable to calculate the fluid velocity either analytically or numerically at
different rotation rates in the vortex flow system with different geometric dimensions. In this
way, the axial velocity component can be compared to the sedimentation velocity of the
adsorbent resin to determine whether the resin can be fluidized. The expected outcome is that
the minimum fluidization rotation rate could be generally expressed as a function of the
geometric dimensions of the vortex flow reactor and the physical properties of the adsorbent
resin and the fluid. This general expression is very useful in designing a vortex flow system and
optimizing its performance.

8.3 Improvement of Vortex Flow Model

In this work, a two-region vortex flow model was employed to investigate the
performance of the vortex flow adsorption system. In the two-region model, there are a vortex
core region and a bypass region at each stage, and the volume ratio between two regions is
assumed to be constant. In the real situation, this ratio varies with the operating conditions, especially the rotation rate of the inner cylinder. Therefore, a three-parameter model with the axial dispersion coefficient, the mass transfer coefficient between the vortex core and the bypass region, and the volume ratio between two regions, should be developed to study the performance of the vortex flow adsorption system.

8.4 Scaling-up of Vortex Flow Adsorption System

In this research, all the experiments were conducted in a bench scale vortex flow reactor with an effective volume of 160 ml. If the vortex flow adsorption process were to be carried out in a pilot or manufacturing scale, scale-up of the vortex flow reactor would be necessary.

In the scaling-up, the reactor height would be increased. In order to smoothly transfer the rotating motion from the magnetic drive to the far end of the inner cylinder, the bearings holding the drive shaft need to have a large diameter and be separated by a long distance to avoid any wobbling when the vortex flow reactor is operated at a high rotation rate.

In the vortex flow system, heat is produced due to the rotation of the inner cylinder. It would be disadvantageous for a biochemical system if heat were accumulated in the system. A cooling system is desired for the vortex flow system to remove the heat. An option is to use a cylindrical shell instead of a solid cylinder for the inner cylinder; hence heat can be removed through heat exchange with a cooling media, e.g., the water.
9 Bibliography


