A Systematic Framework to Optimize and Control Monoclonal Antibody Manufacturing Process

Ying Fei Li

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2018
Abstract

A Systematic Framework to Optimize and Control Monoclonal Antibody Manufacturing Process

Ying Fei Li

Since the approval of the first therapeutic monoclonal antibody in 1986, monoclonal antibody has become an important class of drugs within the biopharmaceutical industry, with indications and superior efficacy across multiple therapeutic areas, such as oncology and immunology. Although there has been great advance in this field, there are still challenges that hinder or delay the development and approval of new antibodies.

For example, we have seen issues in manufacturing, such as quality, process inconsistency and large manufacturing cost, which can be attributed to production failure, delay in approval and drug shortage. Recently, the development of new technologies, such as Process Analytical Tools (PCT), and the use of statistical tools, such as quality by design (QbD), Design of Experiment (DoE) and Statistical Process Control (SPC), has enabled us to identify critical process parameters and attributes, and monitor manufacturing performance.

However, these methods might not be reliable or comprehensive enough to accurately describe the relationship between critical process parameters and attributes, or still lack the ability to forecast manufacturing performance. In this work, by utilizing multiple modeling approaches, we have
developed a systematic framework to optimize and control monoclonal antibody manufacturing process.

In our first study, we leverage DoE-PCA approach to unambiguously identify critical process parameters to improve process yield and cost of goods, followed by the use of Monte Carlo simulation to validate the impact of parameters on these attributes. In our second study, we use a Bayesian approach to predict product quality for future manufacturing batches, and hence mitigation strategies can be put in place if the data suggest a potential deviation. Finally, we use neural network model to accurately characterize the impurity reduction of each purification step, and ultimately use this model to develop acceptance criteria for the feed based on the predetermined process specifications. Overall, the work in this thesis demonstrates that the framework is powerful and more reliable for process optimization, monitoring and control.
Contents

List of Tables .......................................................................................................................... v
List of Figures ........................................................................................................................... vi
Abbreviations .......................................................................................................................... x

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

1.1 Overview of Drug Development Process ................................................................. 3

1.2 Monoclonal Antibodies .............................................................................................. 6

1.2.1 Monoclonal Antibody Structure ........................................................................... 7

1.2.2 Manufacturing of Monoclonal Antibodies ............................................................ 8

1.3 Manufacturing Issues ................................................................................................. 12

1.3.1 Biopharmaceutical Manufacturing Principles ..................................................... 12

1.3.2 Overview of Product Failure ............................................................................... 12

1.3.3 Product Specification, Process Characterization and Validation, Process Monitoring .......................................................................................................................... 14

1.3.3.1 Product Specification ...................................................................................... 14

1.3.3.2 Process Characterization ............................................................................... 19

1.3.3.3 Process Validation .......................................................................................... 21

1.3.3.4 Process Monitoring ....................................................................................... 23

1.3.4 Process Optimization to Reduce Cost of Goods ................................................... 24

1.4 Aims & Organization of Thesis ................................................................................... 28
## 2 Integrating Design of Experiment and Principal Component Analysis to Reduce Downstream Cost of Goods in Monoclonal Antibody Production

2.1 Introduction

2.2 Method

2.2.1 Monoclonal Antibody Production Process

2.2.2 Process Simulation Software – SuperPro

2.2.3 Design of Experiment (DoE)

2.2.4 Principal Component Analysis

2.2.5 Monte Carlo Simulation

2.3 Case Study

2.3.1 Overview of the study

2.3.2 Key Assumptions

2.3.3 Results

2.3.3.1 Monte Carlo Simulation Results at Current Downstream Operating Conditions

2.3.3.2 Design of Experiments to explore the impact of selected DSP parameters on downstream cost of goods

2.3.3.3 Principal Component Analysis to confirm or refine DoE results

2.3.3.4 Monte Carlo simulation results at optimized process conditions

2.4 Conclusion

## 3 Leveraging Bayesian Approach to Predict Drug Manufacturing Performance
3.1 Introduction ........................................................................................................................................54
3.2 Methodology .....................................................................................................................................56
  3.2.1 Data Collection ..........................................................................................................................57
  3.2.2 Conventional and Bayesian Approach for Mean Prediction ....................................................58
3.3 Results ..............................................................................................................................................63
  3.3.1 Conventional and Bayesian Approaches result in Different Predicted Means .........................63
  3.3.2 Prior and Likelihood Mean Ratio vs Bayesian Predicted Mean ...............................................67
  3.3.3 Impact of Uncertainty in Likelihood Data on Bayesian Predicted Mean .................................69
3.4 Summary and Conclusion ..............................................................................................................72

4 Neural Network to Understand Process Capability and Process Intermediate Acceptance
   Criteria in Monoclonal Antibody Production Process .....................................................................75
  4.1 Introduction .....................................................................................................................................75
  4.2 Methodology .....................................................................................................................................78
  4.2.1 Overview of Monoclonal Antibody Production Process ..........................................................78
  4.2.2 Data Collection ..........................................................................................................................81
  4.2.3 Approach to Estimate Process Capability ..................................................................................81
  4.2.3.1 Conventional Approach .........................................................................................................81
  4.2.3.2 Alternative Approach – Neural Network ..............................................................................82
     4.2.3.2.1 Neural Network Model Selection and Evaluation .........................................................83
     4.2.3.2.1.1 Training Algorithm .......................................................................................................85
     4.2.3.2.1.2 Hidden Layer and Nodes ..............................................................................................86
  4.2.4 Comparison between Conventional and Neural Network Approaches ..................................87
List of Tables

Table 1.1 Global Sales for the Top 50 Pharmaceutical Companies by Molecule Type (2009 – 2014) .................................................................................................................................................. 8
Table 1.2 Selected List of Approved Antibodies Produced in CHO cells......................... 10
Table 1.3 Typical release tests used for monoclonal antibody products.......................... 16
Table 1.4 Example of Risk Assessment for Quality Attributes in Each Unit Operation..... 18
Table 1.5 Steps in Testing the Robustness of a Process................................................... 23
Table 1.6 Cost Breakdown for Monoclonal Antibody Production................................. 26
Table 2.1 Parameters and Associated Ranges for DoE Study........................................... 40
Table 2.2 P-values from DoE Analysis.............................................................................. 46
Table 2.3 Parameters and Associated Ranges for Monte Carlo Simulation...................... 51
Table 3.1 Impact of the Mean Ratio on the Predicted Means............................................ 65
Table 4.1 Neural network Algorithm and Number of Nodes in Each Process................. 95
Table 4.2 Conventional Model Equations......................................................................... 96
Table 4.3 SSE of Model using Conventional and Neural Network Approaches............. 97
Table 4.4 Feed Acceptance Limit Calculated using the Internal Model, Conventional and Neural Network Model........................................................... 101
Table 4.5 Comparison of Error% between Conventional and Neural Network Model.... 101
List of Figures

Figure 1.1 Overview of Drug Development Process ......................................................... 4
Figure 1.2 Phase Transition Success Rates for All Diseases, All Modalities .................... 6
Figure 1.3 Basic Structure of a Monoclonal Antibody ..................................................... 9
Figure 1.4 Overview of a Typical Monoclonal Antibody Upstream Process (USP) ........ 11
Figure 1.5 Overview of a Typical Monoclonal Antibody Downstream Process (DSP) .... 12
Figure 1.6 Illustration of an Approach for Setting Specifications for Product Quality Attributes .................................................................................................................... 19
Figure 1.7 Overview of Process Characterization ............................................................. 21
Figure 1.8 Lifecycle Approach to Process Validation ...................................................... 23
Figure 1.9 Example of SuperPro Design Model of Monoclonal Antibody Production Process ........................................................................................................................................ 27
Figure 2.1 Systematic Approach to Facilitate Decision Making during Monoclonal Antibody Development to Reduce Cost of Goods .................................................................................................................. 33
Figure 2.2 Typical Monoclonal Antibody Manufacturing Process .................................. 35
Figure 2.3 Monoclonal Antibody Production Process Flowsheet .................................. 37
Figure 2.4 Impact of Titer on the Overall Cost per Gram of Drug Substance ................. 43
Figure 2.5 Loading Plot from PCA Analysis ..................................................................... 47
Figure 2.6 Frequency Distribution for Downstream Cost of Goods of Base Case and Optimized Conditions .................................................................................................................. 50
Figure 2.7 Cumulative Distribution Function of Downstream COGs at Base Case and Optimized Condition .................................................................................................................. 53
Figure 3.1 Data Analysis with Conventional and Bayesian Approach……………………..59
Figure 3.2 Overview of Probability Distribution Generation Process……………………..61
Figure 3.3 Statistical Process Control Diagram of Protein A Chromatography Yield……..65
Figure 3.4 Probability Distribution of Prior, Likelihood and Predicted Means of Protein A Chromatography Yield with Bayesian Approach..................................................66
Figure 3.5 Statistical Process Control Diagram of IEX Chromatography Yield…………..67
Figure 3.6 Probability Distribution of Prior, Likelihood and Predicted Means of IEX Chromatography Yield with Bayesian Approach..................................................68
Figure 3.7 Statistical Process Control Diagram of HCP Impurity Level in Protein A Eluate..............................................................................................................................71
Figure 3.8 Probability Distribution of Prior, Likelihood and Predicted Means of Protein A Eluate HCP Level..............................................................................................................72
Figure 4.1 Typical Monoclonal Antibody Manufacturing Process.................................80
Figure 4.2 Overview of Protein Aggregate Formation.....................................................81
Figure 4.3 Overview of Neural Network.........................................................................85
Figure 4.4 Neural Network Architecture.......................................................................86
Figure 4.5 Chromatography Feed vs. Product Aggregate Levels in Purification Processes from four different products.................................................................93
Figure 4.6 Chromatography Feed vs Product Aggregate Reduction Levels in all Four Processes.........................................................................................................................94
Figure 4.7 Observed vs. Model Predicted Aggregate Level Calculated using Conventional and Neural Network Approaches.................................................................99
Figure 4.8 Model Predicted Aggregate level vs Residuals from Conventional and Neural Network Approaches........................................................................................................100

Figure 4.9 Feed and Product Aggregate Level, with Product Specification, and Associated Feed Acceptance Limits Derived from Internal Model, Conventional Model and Neural Network Model.........................................................................................................................102
Acknowledgements

First and foremost, I would like to acknowledge and express my sincere gratitude to my advisor, Professor Venkat Venkatasubramanian. Professor Venkat has been one of the greatest mentors I have in both my research and professional development. I highly appreciate his guidance and strong support in overcoming the many challenges along the way. His insightful advices, openness to new ideas, and constant encouragement have truly made this journey a very joyful ride.

I would also like to extend my deep gratitude to my thesis committee members, Professor Alissa Park, Professor Daniel Esposito, Professor Garud Iyengar and Professor Jay Sethuraman. Their guidance and advice have been truly insightful and helped shape this thesis, and I really appreciate the time and effort that they have put in.

Finally, I would like to thank my amazing families, my grandparents, my parents and my husband, for their unconditional love and unceasing support throughout this journey, and gives me the strength to follow my dreams!
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEX</td>
<td>Anion – Exchange Chromatography</td>
</tr>
<tr>
<td>BLA</td>
<td>Biological Licensure Application</td>
</tr>
<tr>
<td>CEX</td>
<td>Cation-Exchange Chromatography</td>
</tr>
<tr>
<td>COG</td>
<td>Cost of Goods</td>
</tr>
<tr>
<td>CPP</td>
<td>Critical Process Parameters</td>
</tr>
<tr>
<td>CQA</td>
<td>Critical Quality Attributes</td>
</tr>
<tr>
<td>DoE</td>
<td>Design of Experiment</td>
</tr>
<tr>
<td>DSP</td>
<td>Downstream Processing</td>
</tr>
<tr>
<td>FDA</td>
<td>United States Food and Drug Administration</td>
</tr>
<tr>
<td>HCP</td>
<td>Host Cell Proteins</td>
</tr>
<tr>
<td>HIC</td>
<td>Hydrophobic Interaction Chromatography</td>
</tr>
<tr>
<td>HMW</td>
<td>High Molecular Weight Specifies</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization Technical Requirement for Registration of Pharmaceuticals for Human Use</td>
</tr>
<tr>
<td>IND</td>
<td>Investigational New Drug Application</td>
</tr>
<tr>
<td>LMW</td>
<td>Low Molecular Weight Species</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal Antibody</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal Component Analysis</td>
</tr>
<tr>
<td>QbD</td>
<td>Quality by Design</td>
</tr>
<tr>
<td>UFDF</td>
<td>Ultrafiltration/Diafiltration</td>
</tr>
<tr>
<td>USP</td>
<td>Upstream Processing</td>
</tr>
<tr>
<td>VF</td>
<td>Virus Filtration</td>
</tr>
</tbody>
</table>
1. Introduction

Monoclonal antibodies (mAb) have become an increasingly important class of therapeutic agents that contribute to several major advances in pharmacotherapy. Over the past decade, FDA has approved more than 15 new therapeutic antibody products spanning four major therapeutic areas [1], with 52% in immunology, 36% in oncology, 4% in infectious diseases, and 8% in other areas [2]. For antibody products that are currently in development, more than half (59%) are in the area of oncology [2]. In the past few years, FDA granted breakthrough designation and accelerated approval of several oncology mAbs such as Keytruda® (Merck) and Opdivo® (Bristol – Myers Squibb). These mAb drugs have shown breakthrough results in the treatment of melanoma and small cell lung cancer. In fact, the continued interest in antibody product development is partially driven by the rapid advancement of our understanding of diseases at a molecular level. Compared to small molecule products, mAbs often show higher efficacy and better safety, because they provide the most rapid route to a clinical proof-of-concept for activating, inhibiting or blocking disease targets, with minimal off target effects [3]. The market for monoclonal antibodies is rapidly expanding – the global sales revenue for all monoclonal antibody products was nearly $75 billion in 2013, and it represents the industry segment with the highest growth rate over the last decade [4, 5].

To respond to the increasing market demand and competition for mAbs, biopharmaceutical companies, in collaboration with academia, are looking to streamline the drug development process, implement stringent control of product quality and optimize manufacturing processes to reduce production cost. However, the development and manufacture of biopharmaceutical
drugs is a highly complex endeavor that is heavily regulated [6, 7]. This has stimulated discussions within the industry on the best route for monoclonal antibody development.

The recent advancement in expression technology has significantly increased the cell culture titer, and hence improved the productivity of the mAb upstream production. However, this has shifted the bottleneck to the downstream production, where the increase in downstream production cost can potentially negate the gain in titer and overall process throughput. Hence, biopharmaceutical companies explore different strategies to reduce the cost for mAb downstream processing. This gives rise to a significant opportunity for a decisional framework capable of comparing multiple optimization strategies and guiding decision makers towards the selection of an effective strategy.

In addition to reduce manufacturing cost through process optimization, biopharmaceutical companies focus on ensuring process and quality consistency, and ultimately minimizing process deviation and batch failures. To achieve this, it is imperative for these companies to develop methodologies to monitor and predict manufacturing performance. Hence, should a potential deviation be suspected, mitigation strategies can be developed in advance to minimize the risk. Furthermore, to ensure product consistency, and drug product meeting the final specification for safety and efficacy, it requires a development of appropriate specification for process intermediates in a complex multistep process. Although advanced qualitative methods, mathematical and statistical models have been used in various aspects of pharmaceutical drug development and manufacturing, such as clinical [8], and product quality risk quantification [9], application of such tools in predicting manufacturing performance, or setting process
intermediates specification, are yet to be developed. Consequently, the aim of this thesis is the development of a decisional framework for manufacturing process optimization, and quantitative tools to ensure manufacturing consistency and product quality.

The introductory chapter provides an overview of the biopharmaceutical industry, the increased demand for monoclonal antibody drugs, and the intense pressure within the industry, as more products, including novel and generic products, are coming to the market. Section 1.1 provides an overview of the drug development process in the biopharmaceutical industry, from basic discovery, preclinical, clinical and large scale commercialization. Section 1.2 focuses on monoclonal antibody as therapeutic drugs and reviews the current approved mAb drugs, their structure and the production process to manufacture mAb drugs. Section 1.3 discusses the various issues and complexities in mAb manufacturing, and how the regulatory agencies mandate the use of process characterization, process validation and monitoring to ensure process robustness and product quality. There is an overview of the different tools and approaches that biopharmaceutical companies utilize for process characterization, validation and monitoring, to reduce product failures. In addition to ensuring product consistency and quality, this section discusses how biopharmaceutical companies try to reduce manufacturing cost through process optimization, and thus make their products more cost effective. Finally, in Section 1.4 the aims and organization of the thesis are presented.

1.1 Overview of Drug Development Process
Developing a new drug from original idea to the launch of a finished product is a complex process which can take 12-15 years and cost in excess of $1 billion [10]. This process consists of four major steps: discovery and development, preclinical research, clinical research, and final approval (Fig 1.1). Discovery and development can be further broken down into four steps: target identification, target validation, lead identification and lead optimization. Target identification and validation is the process of identifying a potential target for a therapeutic drug for a particular disease or condition that does not have a treatment or for which existing therapeutic agents are lacking in some way [11]. After the target has been identified and validated, multiple drug candidates are then developed and further optimized. Drug candidate leads must be shown to reach the target and modulate its activity in vivo while acting within acceptable safety margins [11].

Fig 1.1 Overview of drug development process

Before testing a drug in human, research must be conducted in animals to determine whether it has the potential to cause serious harm, or toxicity. During this preclinical stage, studies in
animal models are combined with cellular and biochemical assays to understand the pharmacological and toxicity profile in at least two animal species. Early dosage studies are also performed to guide dose selection in the upcoming first in human studies [11].

Clinical trials consist of three major phases: Phase I, II and II. In phase I studies, the leading drug candidate is usually tested in healthy volunteer, at different dosages, to assess the safety of the drug. Phase I clinical trials often last several months to a year, and about 60% of experimental drugs pass this phase of testing (Fig 1.2). Upon the successful completion of phase I studies, the drug candidate is then tested in the target patient population to evaluate the safety and efficacy. Most phase II studies are randomized trials where one group of patients receive the experimental drug, while a second “control” group receives a standard treatment or placebo. This allows the investigators to assess the relative safety and effectiveness of the new drug against placebo or standard therapy.

Phase II studies can last from several months to two years, and about one-third of experimental drugs success complete this phase of testing. Phase III studies often involve randomized and blind testing in several hundred to several thousand patients. This is the largest scale of testing of all phases and it can last for several years. Results from Phase III studies should provide the pharmaceutical company and the FDA with a more thorough understanding of the effectiveness of the drug, the benefits and the range of possible adverse effects. Of the experimental drugs that enter the phase III studies, about 60% of them successfully pass this stage (Fig 1.2). Once phase III is complete, a pharmaceutical company can request FDA approval for marketing the drug.
The required production quantities of the experimental drug increase as the developmental stage progresses from discovery through development to large-scale manufacturing. Design and optimization of production scale-up, also known as process development, requires careful testing and monitoring using a wide range of different techniques including protein purification, analysis, quantification and identification tools at every step.

After final FDA approval of a new drug, large-scale manufacturing can begin. Pharmaceutical manufacturing requires continual monitoring of all aspects of the process: raw ingredients, manufacture, packaging, and storage, to ensure process consistency and product quality.

1.2 Monoclonal Antibodies
Since the approval and commercialization of the first therapeutic monoclonal antibody in 1986, Orthoclone OKT3, which was used for prevention of kidney transplant rejection, therapeutic monoclonal antibodies and antibody-related products such as Fc-fusion protein, antibody fragments and antibody-drug conjugates have become the dominant class within the biopharmaceutical industry [14]. Monoclonal antibody products today are approved for a variety of indications, such as cancer, inflammatory/autoimmune diseases, transplant rejection and infectious diseases.

Some of the well-known monoclonal antibody drugs include Avastin, Humira, Herceptin, Enbrel, Remicade, Rituxan, Yervoy, Keytruda and Opdivo. Annual approvals of monoclonal antibody products have increases significantly since 1982. Based on an approval rate of approximately four monoclonal antibody products per year, it is anticipated that there will be 70 or more monoclonal antibody products on the market by 2020 [14].

In addition to an increased approval rate, global sales of monoclonal antibody products have grown from ~$38 billion in 2008 to almost $63 billion in 2013 [14]. Compared to other class of therapeutic drugs, monoclonal antibody has become the dominant segment with the strongest growth (Table 1.1) [16]. Sales per gram of mAb range from $1000 per gram to $50,000 per gram depending on dosage [17, 18]. Biopharmaceuticals are so expensive partly due to the high cost of manufacturing and the relatively lower bioavailability, and consequently frequent administration of high doses.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Molecule</td>
<td>411</td>
<td>414</td>
<td>415</td>
<td>405</td>
<td>394</td>
<td>394</td>
<td>-4%</td>
</tr>
<tr>
<td>Therapeutic Protein</td>
<td>65</td>
<td>68</td>
<td>70</td>
<td>72</td>
<td>74</td>
<td>76</td>
<td>17%</td>
</tr>
<tr>
<td>Monoclonal Antibody</td>
<td>38</td>
<td>43</td>
<td>48</td>
<td>53</td>
<td>58</td>
<td>62</td>
<td>63%</td>
</tr>
<tr>
<td>Vaccine</td>
<td>21</td>
<td>22</td>
<td>24</td>
<td>25</td>
<td>27</td>
<td>28</td>
<td>33%</td>
</tr>
</tbody>
</table>

Table 1.1 Global sales for the top 50 pharmaceutical companies by molecule type (2009 – 2014) [16]

1.2.1 Monoclonal Antibody structure

Each monoclonal antibody is composed of two identical heavy polypeptide chains (Fig 1.3). These chains are each held together by disulfide bonds. Both the heavy and light chains are made up of folded regions, called domains. Light chains (LC) contain one variable region (denoted V for variable) and one constant region (denoted C for constant). Similarly, heavy chains (HC) contain one variable and three or four constant regions depending on the antibody isotype [15].
The amino acid sequences in the variable regions are varied among antibodies from different B-cell lineages. However, the constant regions are relatively conserved across different classes of antibodies. In addition to the basic structure, antibodies are naturally glycosylated along the heavy chain [15]. The pattern of glycosylation often have a strong impact on the actions of the antibody, including proper secretion, kinetic in circulation, and the chemistry related to proper radiolabel or linker attachment.

1.2.2. Manufacture of Monoclonal Antibodies

Mammalian cells are typically used for expression of all commercial therapeutic mAbs, and grown in suspension culture in large bioreactors [17]. Majority of commercial mAbs are derived from Chinese Hamster Ovary, or CHO cells. Several characteristics have made CHO
cell the most dominant cell line in antibody production, such as rapid growth, high expression, and the ability to be adapted for growth in chemically-defined media [17]. Table 1.2 shows a selected list of commercial mAbs produced in CHO cells [18]. A typical bioreactor production process lasts for 7 – 14 days with periodic feeds added to the bioreactor. These fed-batch processes will accumulate mAb titers of 1-5 g/L, with some companies reporting 10-15 g/L for extended culture durations. Production bioreactor volumes can range from 5 kL to 25 kL.

<table>
<thead>
<tr>
<th>Product</th>
<th>Indication</th>
<th>Marketing company</th>
<th>First US approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab(Rituxan™)</td>
<td>Chronic lymphocytic leukemia</td>
<td>Genentech, Biogen Idec</td>
<td>1997</td>
</tr>
<tr>
<td>Trastuzumab(Herceptin™)</td>
<td>Breast cancer</td>
<td>Genentech</td>
<td>1998</td>
</tr>
<tr>
<td>Alemtuzumab(Campath™)</td>
<td>Chronic lymphocytic leukemia</td>
<td>Genzyme</td>
<td>2001</td>
</tr>
<tr>
<td>Omalizumab(Xolair™)</td>
<td>Allergic asthma</td>
<td>Genentech</td>
<td>2003</td>
</tr>
<tr>
<td>Bevacizumab(Avastin™)</td>
<td>Colorectal cancer</td>
<td>Genentech</td>
<td>2004</td>
</tr>
<tr>
<td>Panitumumab(Vectibix™)</td>
<td>Colorectal cancer</td>
<td>Amgen</td>
<td>2006</td>
</tr>
<tr>
<td>Tocilizumab(Actemra™)</td>
<td>Rheumatoid arthritis</td>
<td>Chugai/Roche</td>
<td>2010</td>
</tr>
</tbody>
</table>

Table 1.2 Selected list of approved antibodies produced in CHO cells [18]

A typical cell culture process, or upstream process (USP), consists of a number of unit operations, including cell bank vial thaw, seed train, inoculum train, production culture and primary recovery, or harvest (Fig 1.4). After the cell bank vial is thawed, the cells will grow in the seed train. When the cells reach a specific density, they are then inoculated. During the inoculum train, the cell will continue to grow and expand. The cells are then transferred to the production bioreactor, which ranges from 5 kL to 25 kL, for commercial production. Appropriate media and process control need to be set in place to ensure growth, viability and productivity.
Upon the completion of the production bioreactor, the harvested cell culture bulk is processed by centrifugation, followed by depth filtration to remove the mammalian cells. This step is known as primary recovery, and the resulting harvested cell culture fluid (HCCF) is then clarified by a series of downstream purification steps.

Figure 1.5 shows an example of a monoclonal antibody downstream purification process (DSP). The HCCF is initially clarified by the Protein A chromatography step. This step typically operates in bind-and-elute mode, in which the monoclonal antibody initially binds to the Protein A resin, while the impurities, such as host cell protein (HCP), DNA and aggregates pass through. The low-pH product elution step at the end of the Protein A also acts as a virus inactivation step by denaturing enveloped viruses.

![Fig 1.4 Overview of a typical monoclonal antibody upstream process (USP)](image)
Subsequently, the Protein A eluate is further polished by two additional ion exchange steps. The ion exchange steps can operate either in bind-and-elute or flow-through mode, where the product of interest either bind to the resin initially, and the impurity will flow through, or vice versa. The most common forms of ion exchange chromatography are anion exchange (AEX) and cation exchange (CEX). While both of these polishing steps are utilized to remove impurities, such as HCP, DNA, leached Protein A ligand and aggregate, the AEX step can further reduce adventitious viruses.

After the two ion exchange steps, the product stream is then processed through a virus filtration step (VF), another step dedicated for virus reduction. This step utilizes nanofiltration to remove viruses by size exclusion. Finally, an ultrafiltration/diafiltration step is carried out to concentrate the product stream to a specified concentration and formulate it, in order to achieve efficacy and bioavailability.

Fig 1.5 Overview of a typical monoclonal antibody downstream process (DSP)
1.3 Manufacturing Issues

1.3.1 Biopharmaceutical Manufacturing Principles

The real goal of biopharmaceutical manufacturing is to provide product that reflects the material used in clinical trials regarding safety and efficacy, in a consistent way [20]. There might be changes throughout clinical manufacturing and commercial production, due to various reasons, such as changes in scale, manufacturing site and raw material source. However, these changes should only have minimal impact on product quality and overall output.

To achieve a robust manufacturing process, it is imperative to ensure that the process is well characterized, in other words, the relationships between critical process parameters (CPP) and critical quality attributes (CQA) are well understood. Furthermore, to ensure consistency from batch to batch, process control tools are often utilized to track the performance of each manufacturing batch, and to identify any potential trend.

1.3.2. Overview of product failure

Pharmaceutical product failure can often be attributed to three critical areas: medical utility (efficacy), safety and industrialization (manufacturing and quality) [21]. One frequent reason for product failure is that the product doesn’t show efficacy in clinical trials [20]. Ideally, we would want to pick up this issue early on in the development phase, such as Phase II studies.
However, in many cases, it has taken a large and costly pivotal study to reveal lack of efficacy [20]. Hence it is important to choose the right indication, appropriate clinical end points and select a good study design to support the benefits of a drug candidate [22]. In addition to efficacy, any safety concerns during the clinical trials, or even post registration, can delay the product to the market, stop the further development and even recall the product from the market.

Although manufacturing issues are usually not the major cause of product failure [23], manufacturing problems can significantly delay product approval for complex biotechnology drugs [20]. The lack of manufacturing consistency or the initiation of manufacturing changes late in product development [24] has delayed approval of monoclonal antibody products [20]. Some of the typical manufacturing issues include failure to comply with the final drug substance or drug product specification, lack of process and product consistency, contamination, and other types of deviations.

In the past, manufacturing cost has rarely been the cause of product failure. However, as the competition in the biopharmaceutical industry becomes more and more intense recently, and the drug market becomes more crowded, having a high manufacturing cost might make the products, especially if it’s not first to market, less competitive, or in the worst case, not be able to generate sufficient profit to justify the development. Hence, while companies are putting in a lot of efforts to ensure efficacy, safety and manufacturing robustness, more and more companies are starting to also focus their development to optimize their manufacturing processes to improve cost of goods.
1.3.3. Product Specification, Process Characterization and Validation, Process Monitoring

To ensure a robust and consistent manufacturing process, the triad of process control (e.g. raw material acceptance criteria, in-process testing, defined setpoints and operating range for process parameters and defined process and hold times), process validation and product testing are the basis for current manufacturing of monoclonal antibodies and the majority of pharmaceuticals [20].

1.3.3.1. Product Specification

The safety and efficacy of biopharmaceutical products are paramount to their successful commercialization. Product specifications have long been regarded a safeguard with respect to product efficacy and safety [25]. A specification is defined as a list of tests, references to analytical procedures and appropriate acceptance criteria to which drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use [26]. Specifications lead to a tradeoff between the likelihood a consumer will acquire a suboptimal dose and the likelihood a lot will fail release [20]. Hence, it is important to focus on relevant attributes and set acceptance criteria that are relevant to risks. Consumers can then receive high quality product without manufacturers incurring unnecessary failed lots [20].
Table 1.3 shows a list of typical release tests that have been reported in various literatures for monoclonal antibody manufacturing [25, 27]. These tests include various indicators, such as quantity (measured by protein concentration), purity (measured by various modes of chromatography), identity (measured by electrophoresis and peptide mapping), potency (measured by antigen binding assay), impurities (measured by DNA, HCP and endotoxin assays), and general properties (pH, volume and color). For each of these critical attributes, a specification is set for the final product, as well as some of the intermediate products.

Each specification has three key elements [25, 28, 29]:

- **A Critical quality attribute or process parameter** that the specification is targeting
- **An analytical method** that is used to perform the test

<table>
<thead>
<tr>
<th>Test</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentration by A280 absorbance (mg/mL)</td>
<td>Quantity</td>
</tr>
<tr>
<td>Percent purity by high-performance size-exclusion chromatography (HP SEC)</td>
<td>Purity (size)</td>
</tr>
<tr>
<td>Ion-exchange (IEC) purity</td>
<td>Purity (charge)</td>
</tr>
<tr>
<td>Percent deamidation by percent IEC</td>
<td>Purity (charge)</td>
</tr>
<tr>
<td>Capillary zone electrophoresis (CZE)</td>
<td>Identity</td>
</tr>
<tr>
<td>Peptide mapping</td>
<td>Identity</td>
</tr>
<tr>
<td>Antigen binding assay or other appropriate</td>
<td>Potency</td>
</tr>
<tr>
<td>Host cell proteins (mg/mg)</td>
<td>Impurities</td>
</tr>
<tr>
<td>Residual DNA (pg/mg)</td>
<td>Impurities</td>
</tr>
<tr>
<td>Endotoxin (EU/mg)</td>
<td>Impurities</td>
</tr>
<tr>
<td>pH</td>
<td>General</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>General</td>
</tr>
<tr>
<td>Appearance</td>
<td>General</td>
</tr>
</tbody>
</table>

Table 1.3 Typical release tests used for monoclonal antibody products [25].
• **Qualitative or quantitative acceptance criteria** that determine acceptance or rejection of a manufacturing lot

The first step to identify critical process parameters or quality attributes is to perform a risk assessment, and understand the relationship of each parameter and attribute with respect to clinical efficacy and safety. For each unit operation in both upstream and downstream processes, each of the process parameters, as well as, quality attributes, is assessed in terms of its impact on safety and efficacy, in case the process falls out of the pre-specified range. The risk score can be both quantitative and qualitative.

Table 1.4 shows an example of risk assessment of quality attributes in various process steps. The scores are given based on previous process knowledge, literature and process characterization studies, which will be discussed in subsequent sections. The quality attributes or process parameters that have a high risk, or sometime medium risk, are categorized as critical quality attributes (CQA), or critical process parameters (CPP).
<table>
<thead>
<tr>
<th>Critical quality attribute (risk level):</th>
<th>Unit operations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preculture &amp; expansion</td>
</tr>
<tr>
<td>Appearance (M)</td>
<td>Low</td>
</tr>
<tr>
<td>Impurities (II)</td>
<td>Medium</td>
</tr>
<tr>
<td>Protein content (H)</td>
<td>Low</td>
</tr>
<tr>
<td>Immunoreactivity (II)</td>
<td>Low</td>
</tr>
<tr>
<td>Purity (H)</td>
<td>Low</td>
</tr>
<tr>
<td>pH and ionic strength (M)</td>
<td>Low</td>
</tr>
<tr>
<td>Amino acid content/ratio (H)</td>
<td>Medium</td>
</tr>
<tr>
<td>Bioburden (H)</td>
<td>Low</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In-process controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill weight check (M)</td>
</tr>
<tr>
<td>Visual inspection (M, L)</td>
</tr>
</tbody>
</table>

Table 1.4 Example of risk assessment for quality attributes in each unit operation [30]

After identifying CQAs, specifications need to be set for these CQAs to ensure the final drug substance or drug product confers safety and efficacy. Figure 1.6 illustrates an approach for setting specifications for product quality attributes. Setting product specifications requires sifting through a variety of data from various sources, such as clinical studies, animal models, pharmacokinetic studies, characterization of process capability, analytical method capability, etc. [25].
While it is very common to use process capability, or ability of a particular step to remove impurities, to set specification for critical quality attributes, it is important to assess process capability accurately. One approach is to develop a mechanistic model of a specific process, and leverage this model to predict the input specification based on the final output specification. However, this approach is very process specific, and would involve significant amount of time and effort for model development. Hence, this approach is rarely used for acceptance criteria setting.

Another way to understand process capability is to do a worst case scenario study. In such study, we challenge the chromatography column or filter with a significant higher than typical amount of impurity, and find out the maximum impurity reduction this step can achieve. A
challenge with this approach is that sometimes it could be difficult to find a feed stream with such a high impurity level while ensuring these impurities are representative. In addition, a lot of experiments need to be conducted before we are able to reach the saturation point.

Some other simpler approach used in the industry to understand process capability is to compare the average impurity levels of the feed and the product, and the average reduction is used to calculate the in-process acceptance criteria. While this is relatively straightforward, one caveat is that it assumes that the impurity clearance is independent of the feed impurity level. For instance, two feeds with different impurity levels are assumed to undergo the same degree of reduction; however, this is not always the case, as shown later in our study. This analysis also represents a need for a better tool to characterization process capability in monoclonal antibody manufacturing.

1.3.3.2. Process Characterization

As discussed earlier, process characterization is one approach that the industry uses widely to establish critical process parameters that have significant impact on product quality. Figure 1.7 shows an overview of the different steps in process characterization. An initial risk assessment is performed on all process parameters in each unit operation. Based on development history
and previous process knowledge, each process parameter is assigned a risk score. Based on the internal score cutoff for high risk parameters, those that have a high risk score are categorized as potential critical process parameters, which are then carried onto further studies.

![Figure 1.7 Overview of Process Characterization](image)

During the second phase of process characterization, experimental studies, designed using various statistical tools, such as OFAT (one factor at a time), DoE (design of experiment), PCA (principal component analysis), are carried out to understand the relationship between each potential process parameter and critical quality attributes. These analyses, combined with process knowledge and experience, determine which parameters are both statistically and practically significant. For those that have both significance, we categorize them as critical
process parameters, or CPPs. During manufacturing, it is crucial to implement stringent control on these parameters to ensure that all process intermediates or drug substance meet the specification to achieve efficacy and safety.

1.3.3.3. Process Validation

The definition of ‘process validation,’ also known as process qualification, is to gather statistically sufficient evidence that the process is empirically understood and under control [31]. Process validation is required to perform prior to obtaining product approval.

The most recent ICH and FDA guidance endorse a new paradigm of process validation, based more on process understanding and control of critical parameters and less on product testing [30]. Table 1.5 lists the steps to test the robustness of a process. Steps 1-3 are part of the process characterization. After identifying the CPPs and quantifying their impact on product quality, process validation, which is step 4, is conducted to demonstrate the critical parameters can be monitored and controlled at commercial scale batches. Typically, FDA requires manufacturers to demonstrate that their process is able to perform consistently, without any major deviation, in at least three consecutive batches. At the end of each batch, the drug substance and drug products need to meet all the specification in order to successfully pass process validation.

1 Identification of critical variables relating to the reproducibility of the process
Determination of the ranges for these variables based on a sound empirical understanding of the process

Quantification of variable effects and interactions at the extremes of their ranges

Demonstration that critical variables can be monitored and controlled during manufacturing runs

Table 1.5 Steps in testing the robustness of a process [30]

In addition to demonstrate process consistency and robustness in three consecutive batches, the new FDA guidance points to a lifecycle approach to process validation. As shown in Figure 1.8, the lifecycle approach consists of three stages: process design, process qualification and process monitoring [30]. While the first two stages have been part of the traditional approach, continuous process monitoring (1.3.3.4) is a new added stage in the lifecycle approach.

Figure 1.8 Lifecycle approach to process validation [30]

1.3.3.4. Process Monitoring
As discussed in 1.3.3.3, continuous process monitoring, which monitors critical process parameters as part of annual product review and other monitoring programs, has now been part of the new process validation process. Further, continuous monitoring is beneficial to the manufacturers, because if the process trends suggest some potential deviation, mitigation strategies can be developed in advance to minimize the risks.

Statistical tools, such as Shewart control chart, are used to monitor critical process parameters and critical quality attributes, and to ultimately ensure that they are within the predetermined process control limits, or specification. In addition to monitoring past batches performance, the ability to leverage historical knowledge to predict the performance of future batches is very critical.

Although advanced quantitative methods, mathematical and statistical models have been in used in various aspects of pharmaceutical drug development and manufacturing, such as clinical [32] and product quality risk quantification [33], application of such tools in predicting drug manufacturing performance is yet to be developed. The conventional approach, or the approach that is widely used in the industry, for predicting batch performance usually assumes the mean of the upcoming batch is equal to the mean of the historical batches. While this approach can give a reliable estimation when the all batches perform very consistently, it might not be sensitive to subtle changes in certain batches, especially when the sample size of the historical data is much larger than that of the recent data. Hence, it is important to develop a more reliable and accurate tool to predict future performance of manufacturing batches, by leveraging
historical information. This way, we can minimize process deviation, product failure and product recalls.

1.3.4. Process Optimization to reduce cost of goods

To meet the increasing demand for monoclonal antibody products, and the increasing competition within the industry, biopharmaceutical companies been constantly looking for ways to improve manufacturing productivity while trying to reduce the overall cost, or cost of goods (COGS). As discussed earlier, bioprocesses have to be carefully fine-tuned to ensure the consistent quality of the material produced, and initiatives such as Quality by Design (QbD) should result in the development of robust and flexible processes [4].

In addition, pressure from competition, such as molecules being approved for the same indications, patent expiry, and the arrival of generics, will put increased pressure on sale prices. Hence, minimizing the production cost will give a manufacturer a competitive edge in maintaining economically viable products [4,34,35,36,37].

Monoclonal antibody production cost can be often broken down to five categories: raw materials, consumables, labor, facility and other (Table 1.6). While facility and other costs are fixed once the manufacturing facility is set, costs, such as raw materials, consumables and labor, can be further reduced upon further process optimization.

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>

25
With the recent advancement in process simulation software, there are a number of software tools that the industry often uses to analyze cost for pharmaceutical manufacturing. Specifically, for monoclonal antibody production, SuperPro Designer has been one of the most dominant process simulation tools used by the industry [4]. SuperPro Designer takes into account all the process steps (figure 1.9), including process parameters (flow rate, number of chromatography cycles, size of equipment, volume of buffer charged), types of consumable used, and all the associated costs.

Additionally, fixed costs related to the infrastructure, such as building and equipment, can also be incorporated in the calculation. The output of the process model includes overall throughput and cost, throughput and cost for each step, and the cost breakdown in terms of the five categories shown above. These results are useful in pinpointing steps, which have the high cost, low utilization, or low throughput, for further process optimization.
Owing to the improvement in the cell culture process, and the advance in expression technology, the cell culture titer coming out of the bioreactor has increased from below 1 g/L to 1-5 g/L, with some companies reporting 10-13 g/L for extended culture duration [17]. The increase in cell culture titer has shifted the manufacturing and economic bottleneck from upstream processing (USP) to downstream processing (DSP).

Sommerfeld and Strube have shown that as cell culture titer increases from 0.1 to 1 g/L, the ratio of USP to DSP costs drops from 55:45 to 30:70 [38]. At higher product titer, larger chromatography columns, membrane areas, buffer consumption and/or additional chromatography or filtration cycles would be required to purify the cell culture harvest from upstream. Experience in the industry shows [39] that the overall cost per gram of drug

Figure 1.9 Example of SuperPro Design Model of Monoclonal Antibody Production Process [4].
substance purified initially decreases as titer increases. However, the cost plateaus as the titer increases further. In a higher titer process, the increase in downstream cost can potentially negate the gains in titer, or overall process throughput. Hence, reducing downstream production cost through process optimization is an effective strategy to alleviate process bottleneck, and make the production process more cost effective.

A Design of Experiment (DoE) approach has been used widely in the industry for process optimization [4]. DoE is very efficient in understanding the effect of parameters, their interactions, and generating a predictive model with a minimum number of experiments. As discussed previously, results from DoE can help us identify a list of statistically significant parameters; in this specific case, parameters that have significant impact on cost of goods. As a result, process development teams can develop strategies to further optimize these parameters to reduce downstream cost of goods.

However, sometimes multiple statistically significant parameters are identified through the DoE study, but the resources available and timeline can only allow us to optimize a selected number of parameters. In this case, DoE might not be able to identify the relative importance of each parameter [39], or which parameters are more effective in reducing cost of goods. Hence, a new optimization approach, and an additional optimization tool, needs to develop to further elucidate the relative contribution of each parameter to the downstream cost of goods.
1.4 Aims & Organization of Thesis

The preceding sections of this chapter have provided an introduction to biopharmaceutical drug development process, and highlighted the impact of monoclonal antibodies as an important class of therapeutic agents. With the intense competition within the industry, and the stringent product approval guidelines from the FDA, biopharmaceutical companies not only have to ensure their products show superior efficacy, safety, but also need to develop a manufacturing process that is robust, consistent and cost effective. In this section, we discussed the various ways that the biopharmaceutical industry practices to ensure product quality and consistency, and reduce costs. However, the presented literature review and industry survey highlighted that at present there is still a need to develop better tools to characterize process capability, predict biopharmaceutical manufacturing performance and optimize manufacturing processes to reduce cost of goods.

Consequently, the aim of this thesis is the development of a systematic framework to optimize and control monoclonal antibody manufacturing process. This will facilitate more informed decision-making when identifying and evaluating process optimization strategies, leveraging historical manufacturing and development data to predict future manufacturing performance, and understanding process capability to enable in-process specification setting given the final product specification. In order to realize this aim, a set of objectives was established and these form the basis of each of the proceeding chapters.
In Chapter 2, we present a framework to prioritize optimization strategies during monoclonal antibody process development. Design of Experiment (DoE), in conjunction with Principal Component Analysis (PCA), is employed to identify process parameters that have the most impact on downstream production cost. Statistically significant parameters are identified through a DoE study, while the PCA characterization is applied as an independent tool to further elucidate the relative importance of these parameters. A stochastic approach incorporating process uncertainties is used to illustrate the distribution of downstream cost of goods under different process conditions. Overall, this systematic approach is able to prioritize development strategies under compressed timeline, and enable biopharmaceutical companies to achieve a competitive advantage in today’s market.

Chapter 3 presents a process prediction tool that is capable of leveraging available manufacturing and development data to predict the performance of future monoclonal antibody production batches. Hence, if a potential process deviation is suspected in an upcoming manufacturing batch, strategies can be developed in advance for risk mitigation. This chapter tries to estimate future process performance using both a conventional approach, or an approach that is widely used in the industry, and a Bayesian approach, which is proposed in our research. While both approaches arrive at a similar prediction when a process performs consistently, the Bayesian approach is better at capturing heterogeneity when there is inconsistency emerging in recent batches. Hence, in such case, the Bayesian approach enables a more accurate prediction of future batch performance.
Chapter 4 highlights the importance of setting appropriate acceptance criteria, or process specification, for process intermediates to increase the likelihood of the final drug substance and drug product meeting the final product release specification. When setting acceptance criteria for process intermediates, it is important to first understand process capability, or quantify the impurity clearance of each process step. However, this process involves either challenging experimentation or an estimation method that might not be comprehensive.

Chapter 4 proposes the use of neural network to understand process capability. This approach not only is able to delineate the relationship between the feed and product impurity level for a specific step, but is also able to define the acceptance criteria for the feed impurity level based on a predetermined product impurity level. These acceptance criteria enable us to determine whether or not to forward process the step based on the feed impurity level.

In Chapter 5 a summary of the main conclusions of this thesis is presented and some future directions of research to augment this work are presented. Finally, papers by the author, published during the course of this work are shown in the appendices.
2 Integrating Design of Experiment and Principal Component Analysis to Reduce Downstream Cost of Goods in Monoclonal Antibody Production

2.1 Introduction

Monoclonal antibodies (mAb) have become an increasingly important class of agents that contribute to several major advances in pharmacotherapy. In the past few years, FDA has approved over 15 new therapeutic antibody products, and granted breakthrough designation and accelerated the approval of several oncology mAbs such as Keytruda (Merck) and Opdivo (Bristol-Myers Squibbs). Indeed, the market for monoclonal antibodies is rapidly expanding, and it represents the industry segment with the highest growth rate over the last decade [4].

To respond to the increasing market demand for mAbs, biotech and pharmaceutical industry, in collaboration with academia, have enhanced their strategies to increase bioprocessing productivity. Owing to the improvement in the cell culture process, and the advance in expression technology, mAbs product titer has increased from below 1 g/L to 1-5 g/L, with some companies reporting 10-13 g/L for extended culture duration [17]. The increase in cell culture titer has shifted the manufacturing and economic bottleneck from upstream processing (USP) to downstream processing (DSP).

Sommerfeld and Strube have shown that as cell culture titer increases from 0.1 to 1g/L, the ratio of USP to DSP costs drops from 55:45 to 30:70 [38]. At higher product titer, larger chromatography columns, membrane areas, buffer consumption and/or additional chromatography
or filtration cycles would be required to purify the cell culture harvest from upstream. Experience in the industry shows (which is simulated in our case study later in this chapter) that the overall cost per gram of drug substance purified initially decreases as titer increases; however, the cost plateaus as the titer increases further. In a higher titer process, the increase in downstream cost can potentially negate the gains in titer, or overall process throughput.

Given the strong competition in the pharmaceutical industry, the entry of biosimilars into the market, combined with the recent changes in the U.S. healthcare system, the ultimate success for monoclonal antibody process development will be driven by the ability to produce a high throughput and cost-effective process [34, 40]. While there have been a number of efforts trying to increase production capacity by increasing cell culture titer, the industry has also been exploring strategies to reduce the cost of goods by optimizing downstream processing. Strategies such as increasing downstream yield, membrane or resin reuse, chromatography or membrane loading capacity, as well as decreasing cycle time and buffer consumption have been utilized to improve throughput, reduce raw material and consumable usage, shorten processing time, and ultimately achieve a lower downstream cost of goods. Under compressed drug development time scales, it is important for companies to effectively prioritize their strategies to accelerate the process development timeline, and thus achieving a competitive advantage.

This chapter specifically focuses on reducing the downstream cost of goods, which is defined by the downstream cost per unit of drug substance, through optimizing the purification process. Building on our prior modeling work in pharmaceutical manufacturing [41 – 44], we propose a systematic decision-making framework that (i) utilizes Design of Experiments (DoE) in
conjunction with Principal Component Analysis (PCA) to identify and verify downstream process parameters that impact the downstream cost of goods, and (ii) predicts the economic outcome of selected strategies through a stochastic analysis (Figure 2.1).

![Systematic approach to facilitate decision making during monoclonal antibody process development to reduce cost of goods](image)

**Fig 2.1** Systematic approach to facilitate decision making during monoclonal antibody process development to reduce cost of goods

2.2. Method

2.2.1. Monoclonal Antibody Production Process

An example of a typical monoclonal antibody production process is depicted in Figure 2.2. The process consists of an upstream process section (USP) and a downstream process section (DSP). While the USP includes inoculation, cell culture and primary recovery, the DSP consists of Protein A chromatography, followed by two ion exchange steps (flowthrough and bind-and-elute), virus
filtration and ultrafiltration and diafiltration. The downstream purification train is designed to capture the antibody, reduce impurities, such as aggregates, host cell protein, DNA, leached protein A and adventitious virus, and finally concentrate and formulate the product.

**Fig 2.2 Typical monoclonal antibody manufacturing process**

2.2.2. Process Simulation Software – SuperPro

A process simulation tool such as SuperPro Designer Version 9.0, Build 9, Special Build 2200 (Intelligen, Scotch Plains, NJ http://www.intelligen.com/) has the capability of integrating both process and business modeling functions to facilitate strategic decision making during process
development and large scale manufacturing. Some might attempt to model the cost of goods using excel. However, since changing in one parameter will cascade the impact on multiple parameters downstream, and SuperPro is more efficient to capture this impact compared to excel. Input to this tool includes a detailed description of the process steps, and their related costs, such as capital investment, total spending in infrastructure, labor and raw material costs.

Figure 2.3 shows the SuperPro model that we use in this study. The model consists of the major process steps shown in Fig 2.2, and the associated steps, such as sterile filtration, buffer exchange and media prep. The parameter values, raw material and facility costs used in building this model derived from literature and process experience. The resulting throughput and cost models generated can then be utilized to estimate the final unit cost of drug substance or drug product generated at manufacturing scale [4].
Fig 2.3 Monoclonal Antibody Production Process Flowsheet
2.2.3. Design of Experiment (DoE)

A design of experiments approach was taken to explore the impact of selected parameters on the downstream cost of goods. Compared to a One Factor at A Time (OFAT) approach, which can become very cumbersome when there are more parameters (typically > 2), DoE is more efficient in understanding the effect of parameters, their interactions, and generating a predictive model with a minimum number of experiments.

Table 2.1 shows the six parameters studied in our DoE. These parameters can potentially reduce the downstream cost of goods by increasing the throughputs (yield) or decrease the consumable cost (loading). The current process ranges are ranges typically experienced in early phase process development, while the optimized range represent the reasonable target ranges after further optimization. This DoE study encompasses both the current and optimized ranges and is designed to explore whether the changes in parameters within these ranges have a statistical significant impact on downstream COGs. A central composite design was utilized in this DoE study and a total of 300 simulations of downstream COGs were performed for titer set point at 0.5, 2, 4 and 7 g/L.
Table 2.1 Parameters and associated ranges for DoE Study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Current Process Range</th>
<th>Optimized Process Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Throughput</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein A Yield (%)</td>
<td>75 ± 5</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>IEX I Yield (%)</td>
<td>85 ± 5</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>IEX II Yield (%)</td>
<td>80 ± 5</td>
<td>90 ± 5</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein A Loading (g mAb/g resin)</td>
<td>30 ± 3</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>IEX I Loading (g mAb/g resin)</td>
<td>200 ± 10</td>
<td>250 ± 10</td>
</tr>
<tr>
<td>IEX II Loading (g mAb/g resin)</td>
<td>30 ± 3</td>
<td>50 ± 3</td>
</tr>
</tbody>
</table>

An R-sq value of greater than 95% is required to establish the validity of the DoE model. A P-value of < 0.05 is required to indicate statistical significance.

2.2.4. Principal Component Analysis

To further verify the conclusions derived from the DoE analysis, the same set of data were processed using principal component analysis (PCA) [45]. PCA is a statistical tool that converts a set of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (PC). The first PC captures the largest part of variance in the data, the second PC the next largest, and so on. The result of PCA is displayed as loading plots, showing contributions from original variables [45].
2.2.5. Monte Carlo Simulation

Since there are inherent uncertainties in large scale bioprocess production, it is important to account for these uncertainties during process modeling for representative results. The key sources of technical uncertainties affecting the biomanufacturing often arise from cell culture titer, chromatography loading, yields and process cycle time [46]. Stochastic modeling with Monte Carlo simulation was utilized in this case study to understand the output distribution of downstream COGs, by incorporating the effect of uncertainties in process parameters. The details of our Monte Carlo approach are described below.

2.3. Case Study

2.3.1. Overview of the study

A simulation case study is set up to compare the different DSP optimization strategies for high titer (1 - 7g/L) process. Typically, during 2nd generation process development, or post approval process optimization, efforts are devoted to improve cell culture titer, and ultimately to increase production capacity, and improve process economic.

In this case study, we first examine the impact of titer on the overall cost of drug substance through Monte Carlo simulation. Previous research has suggested that the high purification cost can sometimes negate the benefits gained from the increase in titer and throughput [17]. Hence, to
reduce the downstream purification cost for higher titer process, we first identify a list of parameters that may have potential impact on downstream cost of goods, then rank them based on previous process knowledge. Finally, we have identified 6 parameters to carry forward in our study (Table 2.1). These parameters, such as chromatographic yield and loading, could reduce the downstream COGs by increasing throughput and reducing the raw material and consumable consumption.

When prioritizing development efforts under compressed timelines, it is advantageous to select strategies that can maximize the reduction of downstream COGs. A Design of Experiments (DoE) approach is utilized to explore the impact of selected parameters on downstream COGs and identify statistically significant parameters on COGs. To further confirm and refine our conclusions from the DoE study, Principal Component Analysis (PCA) is used as an alternative method to understand the contribution of each parameter to the change in downstream COGs. Finally, for the top parameters selected to pursue forward, Monte Carlo simulation is performed to evaluate the frequency distribution of downstream COGs by incorporating process uncertainties and variability. This systematic approach help prioritize strategies to reduce downstream COGs.

2.3.2. Key Assumptions

There are several key assumptions made in the DoE and Monte Carlo simulations:

(1) The cost of upstream process is assumed to be independent of titer, although the upstream cost can sometimes vary depend on the feeding strategy and culture duration at different titers. In this
paper, we focus on identifying strategies to reduce the downstream cost, which is unlikely to be impacted by the upstream cost.

(2) The number of chromatography cycle is fixed in this case study, and the facility is assumed to have the flexibility to adjust the column size depending on the incoming streams.

(3) While some companies continue to manufacture the 2\textsuperscript{nd} generation process at the original commercial site, other might move to a different site due to facility fit, cost and other business and strategic reasons. Here, we are assuming that 2\textsuperscript{nd} generation process is manufactured at a new commercial site, and hence, the capital cost is relevant in our case.

2.3.3. Results

2.3.3.1. Monte Carlo Simulation Results at Current Downstream Operating Conditions

In order to account for the uncertainties inherent in large scale biomanufacturing, representative triangle distributions of the current operating range indicated in Table 1 are assigned to the six parameters: Protein A loading, IEX I loading, IEX II loading, Protein A yield, IEX I yield and IEX II yield. The base value represents the most likely value, bounded by the maximum and minimum values, which are formed by taking into account process fluctuations.

The triangular distribution is derived based on data from manufacturing, pilot and laboratory scale. Triangular distribution is also what we typically experienced across various monoclonal antibody programs, and is used in an antibody cost analysis by Farid et al [34]. The range for cell culture
titer in this study ranges from 0.5g/L to 7g/L, and a uniform distribution is used to ensure that all titer set points within the range were simulated with equal probabilities. A total of 300 simulations are performed and the simulated cost per gram of drug substance at different titers is shown in Figure 2.4.

![Figure 2.4 Impact of titer on the overall cost per gram of drug substance](image)

Initially as titer increases from 0.5g/L to 2g/L, the cost per gram of drug substance drops from $700g/L to $ 200g/L, or a 3.5 fold reduction. However, as the titer increases further beyond 2g/L, the cost per gram of drug substance plateaus – minimal to no change is observed with the increase in titer. At lower titers of 0.5 – 2g/L, the production cost of antibody is more dominant and
therefore maximizing the throughput by increasing the titer is generally an efficient strategy to decrease cost of goods [4].

However, at titers greater than 2g/L, larger chromatography columns, which translate into larger resin and buffer consumption, larger membrane areas or possibly longer processing time, would be required to accommodate for the increased amount of antibody coming from upstream. Although the throughput continues to increase with titer, the purification cost increases at a much faster rate that it negates the gains from the increase in throughput. As a result, improvement in downstream efficiency, either by increase throughput, decrease operation cost or a combination of both, could further reduce the downstream COGs.

2.3.3.2. Design of Experiments to explore the impact of selected DSP parameters on downstream cost of goods

A central composite response surface is used to explore the effect of selected chromatographic parameters on downstream COGs at a titer range of 0.5 – 7g/L. For all DoE models generated, the R-sq values are greater than 95%, indicating the validity of the model. Table 2.2 displays the p-values of each parameter at the selected titer (0.5g/L, 2.0g/L, 4.0g/L and 7.0g/L). At 0.5g/L, all parameters have been shown to be statistically significant, as they all have p-values of less than the alpha cut-off (0.05).
Compared to the IEX I loading, all other parameters have more significant effect as they have much lower p-values. At higher titers of 2g/L, 4g/L and 7g/L, the IEX I loading is no longer statistically significant while the other parameters continue to have p-values of < 0.0001 and thus statistically significant. IEX I loading has the least to none statistical significance because ion exchange resin is relatively inexpensive compared to Protein A resin. In addition, in this case study, since the IEX I step is operated in flowthrough mode, the resin already has a high resin utilization rate of ~ 200 g/L loading, any further increase in IEX I loading would only result in a marginal impact on downstream COGs.

Although the DoE analysis in this study is able to screen statistically significant parameters, it doesn’t give insight into the relative importance of these parameters as they all have the same p-values of < 0.0001. Additional analysis, such as PCA, would be helpful to elucidate the relative importance of each parameter on reducing downstream cost of goods. Since PCA is commonly used to reduce dimensionality of dataset, the results from the PCA analysis would enable us to rank the statistically significant parameters from the DoE study, and thus to identify the top parameters for further optimization. Theoretically, we could have optimized all parameters to the optimized range, or to some extend beyond the current range, instead of selecting only the top parameters for further optimization. However, the former approach is rather time and resource intensive, as it would take a series of experiments to identify the appropriate ranges for the associated parameters to accompany the change of our main parameters. In addition, every change in the process would require subsequent process characterization and validation to justify the change, per the FDA guidance.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.5</th>
<th>2.0</th>
<th>4.0</th>
<th>7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein A Yield</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IEX I Yield</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IEX II Yield</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Protein A Loading</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IEX I Loading</td>
<td>0.0303</td>
<td>0.5921</td>
<td>0.4748</td>
<td>0.5131</td>
</tr>
<tr>
<td>IEX II Loading</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Table 2.2 P-values from DoE Analysis**

2.3.3.3. Principal Component Analysis to confirm or refine DoE results

In order to verify and further refine the conclusions derived from the DoE analysis, the DoE data are processed through PCA. The first principal component captures ~ 30% of the total variance in the downstream cost of goods. The loading plot (Figure 2.5) from the first principal component confirmed that at all titers analyzed, the IEX loadings have lower contributions to the downstream cost of goods compared to chromatographic yields or protein A loading. As discussed in previously, IEX resin in general is less costly, therefore increasing the resin utilization is unlikely to have a significant impact on the downstream COGs.
The loading plot shows that at 0.5g/L, IEX II yield has the biggest contribution to the total variance. Furthermore, the chromatographic yields (ProA yield, IEX I yield and IEX II yield) have higher loading scores, or bigger contribution, compared to chromatographic loading (ProA loading, IEX I loading and IEX II loading). This trend indeed aligns with what have been shown in previous literature research that at a lower titer, especially at < 1.0g/L, the purification cost is relatively lower [17]. Thus, increasing the overall throughput is a more competitive strategy to reduce cost.

At higher titers of > 2g/L, protein A loading has the most influence on the downstream cost of goods; more importantly, its contribution to the total variance increases as the titer increases. The
cost of Protein A resin becomes more significant as titer increases, because more resin would be needed to purify the incoming stream, given the number of chromatography cycle is fixed in this study. The cost of this highly expensive resin is indeed ~ 85% of the total consumable cost at 7g/L titer. Hence, an increase in Protein A loading would increase the resin utilization and therefore decrease the total amount of Protein A resin consumption.

In addition, the loading plot shows the intra-relationship of parameters. For instance, as titer increases, the contribution of chromatographic yields (ProA yields, IEX I and II yield) decreases, while the contribution of chromatographic loading (ProA loading and IEX II loading) increases. This implies that the purification cost becomes more dominant at higher titer. Reduction in resin consumption, especially the protein A resin, is likely to decrease the downstream COGs significantly.

This case study, along with scientific justification, suggests that both Protein A loading and IEX II yield have the most influence on the downstream cost of goods. Monte Carlo simulation is utilized to generate the frequency distribution of downstream COGs by optimizing either the Protein A loading or the IEX II yield and the results are compared with the average downstream COGs generated by deterministic approach.

2.3.3.4. Monte Carlo simulation results at optimized process conditions
Similar to the Monte Carlo simulation set-up in the previous section, representative triangular distributions indicated in Table 2.3 are assigned to the six key downstream parameters and titer. The fluctuations in these parameters are typically observed in large scale manufacturing.

Three hundred simulations are performed and Figure 2.6 shows the downstream COGs frequency distributions for (1) base case, in which all parameters are set at current process conditions, (2) Optimized Protein A Loading, in which the Protein A loading is operated at target condition while the other parameters remain constant, and (3) Optimized IEX II Yield, in which the IEX II yield is operated at target condition while the other parameters remained constant. Protein A loading and IEX II yield are chosen for further study because of their strong contribution to the variance in downstream COGs through the PCA analysis.

At a lower titer of 0.5g/L, the frequency distribution of the downstream COGs is positively skewed at both optimized conditions, although the mean is slightly lower when the IEX II yield is optimized. At 2g/L, in the base case, the downstream COGs span from $47.5/g to $70/g, whereas the cost distribution is much narrower in the optimized cases: with a range of $40/g – $57.5/g when the Protein A loading is optimized, and $42.5/g - $60/g when the optimized IEX II yield is optimized.
In addition, compared to the base case, increase in IEX II yield reduces the mean downstream COGs by 11%, while a 14% reduction is achieved by increasing the Protein A loading. This observation confirms our previous conclusion that Protein A loading is more significant than IEX II yield in terms of their impact on downstream COGs.

As the titer increases to 7g/L, the mean downstream COGs is reduced by almost 20% at the optimized Protein A loading condition while 15% reduction is achieved at optimized IEX II yield. Moreover, the “risk,” which is measured by the standard deviation, is lower at the optimized Protein A condition as compared to the base case or to the optimized IEX II yield condition.
While the more traditional approach to estimate the average downstream COGs at optimized conditions is often a deterministic estimation, a stochastic approach with Monte Carlo simulation can sometimes offer a more accurate estimation for processes that are inherently random [42]. Figure 2.7 shows the cumulative probability plots of the downstream COGs for different process conditions, and the black line indicates the average downstream COGs obtained by deterministic approach. At 0.5g/L, the deterministic estimates are close to the expected mean from the Monte Carlo simulation in all three conditions. However, as titer increases to 2.0g/L, about 70% of the population is below the deterministic estimate with optimized Protein A loading, while 80% of the population is above the with optimized IEX II yield. Hence, predicting the downstream COGs using a deterministic approach can sometimes overestimate or underestimate the mean, consequently distorting the ranking of parameters. The comparison between the two approaches at 7g/L titer further emphasizes the limitation of the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Current Process Range</th>
<th>Optimized Process Range</th>
<th>Titer (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Throughput</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein A Yield (%)</td>
<td>75 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IEX I Yield (%)</td>
<td>85 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IEX II Yield (%)</td>
<td>80 ± 5</td>
<td>90 ± 5</td>
<td></td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein A Loading (g mAb/g resin)</td>
<td>30 ± 3</td>
<td>42 ± 3</td>
<td>0.5 ± 0.2; 2.0 ± 0.5; 7.0 ± 0.5</td>
</tr>
<tr>
<td>IEX I Loading (g mAb/g resin)</td>
<td>200 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IEX II Loading (g mAb/g resin)</td>
<td>30 ± 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3 Parameters and associated ranges for Monte Carlo Simulation
deterministic approach. The deterministic analysis suggests that optimizing IEX II yield is more effective, as indicated by lower expected downstream COGs, contradicting the conclusion from the PCA analysis.

Monte Carlos simulation results suggest that the most of the population is below the deterministic mean in the case of optimized Protein A loading, whereas most of the population is above the deterministic mean in the case of optimized IEX II yield. These results highlight the drawback of using a deterministic approach in decision making and not accounting for process uncertainties.

Fig 2.7 Cumulative distribution function of downstream COGs at base case and optimized condition
2.4. Conclusion

This chapter provides a systematic framework to identify parameters that have the most impact on downstream costs of goods, and ultimately facilitate strategic decision making during process optimization for monoclonal antibody production. A design of experiments approach enables a screening of statistically significant parameters, based on the p-values.

Further analyzing the DoE data through principal component analysis confirms the conclusion from the DoE study and, more importantly, offers insights on the relative contribution of each parameter to downstream of goods. Finally, a stochastic simulation is utilized to generate the frequency distribution of the downstream cost of goods by incorporating the process uncertainty. Although there are only 6 parameters in our study, this is intended to serve as an example of how we can implement DoE-PCA and Monte Carlo simulation to enable decision making during 2nd generation process development. In other cases, there might be more parameters, and this approach can systematically identify strategies that can maximize the cost reduction under a compressed process development timeline.

Process optimization has been utilized in various stages of drug development, such as pre and post drug approval, to increase flexibility and decrease production cost. In addition to control the cost through process optimization, another approach the industry uses is to minimize product failure through process monitoring, ensuring the consistency and quality of products.
3 Leveraging Bayesian Approach to Predict Drug Manufacturing Performance

3.1. Introduction

The pharmaceutical and biotechnology industry face unprecedented challenges and competition due to the expiration of blockbuster drugs, shorter time-to-market, declining in R&D productivity and stringent regulatory requirements. The entry of generic drugs accounts for substantial loss of market shares for brand-name manufacturers, significantly impacting the financial resources for R&D activities [47]. Zantac, a brand-name product, experienced a price erosion of 90% within 2 years after the generic version entered to the market [48]. While the industry is striving to increase its pipeline to recoup the loss from patent expiration, many companies are also restructuring their R&D to increase efficiency and reduce operational cost.

Merger and acquisition is one strategy that larger companies use to acquire the pipeline of the other companies. For example, Sanofi expanded its biologics pipeline by acquiring Genzyme. Similarly, the acquisition of Pharmasset accelerated Gilead’s development of the Hepatitis C virus (HCV) franchise. One of the products from Pharmasset has become a blockbuster drug, Sovaldi, which represents a large portion of Gilead’s revenue. In addition to merger and acquisition, pharmaceutical and biotech companies have started to outsource part of their basic discovery, preclinical animal studies or clinical pharmacology studies to contract research organization [48].

While R&D is undergoing restructuring, a lot of attention also focuses on manufacturing. According to estimates, the manufacturing costs can be as high as 27-30% of sales for manufacturers of brand name pharmaceuticals [35, 36, 49], and more than double the share of costs for research and development [35, 36]. In an effort to reduce manufacturing cost, some approaches
aim at optimizing and streamlining the manufacturing process to reduce the cost of goods, while other approaches focus on ensuring process and quality consistency, and ultimately minimizing process deviation and batch failures.

For the latter, routine, ongoing assessment of process performance and product quality is critical to ensure process consistency, and most importantly, to ensure high quality pharmaceuticals reach patients in timeline manner [50]. Prior to transferring a process to commercial scale, critical process attributes (CPA) and critical quality attributes (CQA) are selected through a quality by design (QbD) process. During the production of each batch, statistical tools such as Shewart control chart, are used to monitor these CPAs and CQAs, ensuring that they are within the predetermined process control limits.

In addition to monitoring the current performance, the ability to predict the performance of future batches is very critical. If we suspect any systemic deviation or shortage of drug production, mitigation strategies can be developed in advance to minimize the risk. Although advanced quantitative methods, mathematical and statistical models have been in used in various aspects of pharmaceutical drug development and manufacturing, such as clinical [8] and product quality risk quantification [9], application of such tools in predicting drug manufacturing performance is yet to be developed.

The conventional approach, or the approach that is widely used in the industry, for predicting batch performance usually assumes the mean of the upcoming batch is equal to the mean of the historical batches. While this approach can give a reliable estimation when the all batches perform very
consistently, it might not be sensitive to subtle changes in certain batches, especially when the sample size of the historical data is much larger than that of the recent data. The Bayesian approach, however, offers a rigorous way to quantify prior information and combine it with recent data to obtain a prediction for upcoming batches.

Building on our prior modeling work in pharmaceutical manufacturing [41, 42, 43, 44], we propose a framework that compares the application of the conventional and Bayesian approach to predict drug manufacturing performance. Although Bayesian statistics has been often used in clinical studies and simulation, there is little development of such application in the pharmaceutical manufacturing setting. Furthermore, this chapter also discusses tools to determine when Bayesian is more appropriate than the conventional approach, and finally discuss the limitation for the Bayesian approach.

3.2. Methodology

This chapter discusses the use of a conventional and Bayesian approach for parameter prediction in the context of monoclonal antibody (mAb) manufacturing. Prior to transferring the production process to commercial scale, CPAs and CQAs are identified through process characterization studies.

According to the ICH (International Council on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human Use) guideline, CPAs are process related attributes,
physical, and CQAs are physical, chemical, biological or microbiological property or characteristics that should be within an appropriate limit, range, or distribution to ensure process consistency and desired product quality, respectively.

Typically, CPAs and CQAs can be identified based on the severity of harm to process consistency or to a patient (safety and efficacy) resulting from failure to meet that attributes. In a biologics manufacturing process, typical CPAs include titer and yield, and typical CQAs include impurity level and potency. In our case study, we focus on two attributes in a downstream purification step, specifically the Protein A chromatography yield, IEX chromatography yield and Host Cell Protein (HCP) impurity level in protein A product, as these have been identified as CPA and CQA for the step.

3.2.1. Data Collection

The data from the case study are actual data collected from a monoclonal antibody manufacturing process. Both the yield and HCP impurity level data are collected at every cycle, and there are about 5–6 cycles per batch in this process. All data have been validated by internal quality control group. In addition, the datasets are screened for potential outliers, and they would be excluded only if there is sufficient rationale to support the exclusion of such data points. These data are scaled for confidentiality purposes, but the general trends are preserved.

3.2.2. Conventional and Bayesian Approach for Mean Prediction
Figure 3.1 shows the set-up of the conventional and Bayesian approaches to predict the mean of the upcoming batch. The older and recent batches are used as a training dataset, while the most recent batch is treated as a test dataset. The goal was to evaluate how comparable the predicted means are to the actual test mean.

For the conventional approach, or the approach that’s widely practiced in the industry, the predicted mean is believed to be approximately the mean of all the batches conducted thus far, or the overall mean of the training dataset, and the standard deviation of the mean is computed as the observed standard deviation divided by the sample size. While this approach is relatively straightforward, there are several incidences where this approach might not be able to offer a reliable prediction of future batch performance.
For example, if the historical dataset is much larger than the recent dataset, any subtle changes or systemic deviation, caused by shift in operation, raw material or other factors, in the recent batches would not be able to be reflected in the mean. The Bayesian approach, however, offers a more reliable way of estimating the mean by leveraging information from historical batches and capturing changes from recent batches.

The Bayesian posterior probability is defined as the follows:

\[
p(\theta \mid x) = \frac{(p(x \mid \theta)p(\theta))/(p(x))}{p(x)}
\] (3.1)

The posterior probability given the evidence \( x \), \( p(\theta \mid x) \), is equal to the likelihood function, or the probability of the evidence given the parameters, \( p(x \mid \theta) \), times the prior probability, \( p(\theta) \), normalized by the probability of evidence \( x \), \( p(x) \). In our case, prior distribution is derived from the historical data and the likelihood was derived from the recent dataset, and we use the new information from the recent dataset to update our prior knowledge of the process.

Figure 3.2 shows the process of generating the different distributions. We first gather the appropriate data, and then determine the distribution based on the data collected. Usually a biological process follows a normal distribution because the process is tightly controlled at a manufacturing setting, and the data tend to center at the mean. After identifying a distribution, it is crucial that we validate it. For instance, if we hypothesize that a dataset follows a normal distribution, a normality test should be conducted to verify it. After the validation, we would then extract the key parameters, such as mean and standard distribution in the case of a normal distribution, and perform simulation against typical manufacturing set points.
In most cases, we derive the prior distribution from historical manufacturing data. Based on previous process experience and consultation with subject matter experts, we derive the prior distribution from a minimum of 25 cycles, or 5 batches. This should be sufficient to give a reliable and representative prior distribution, unless the data are highly variable, in which case more data would be needed to generate the prior distribution. In the case where there aren’t enough historical data, we would need to gather data from experiments performed at the laboratory or pilot scale to have a large enough dataset to derive a prior distribution.

However, since the latter dataset are generated at a smaller scale compared to the manufacturing scale, poolability test such as one way analysis of variance (ANOVA) needs to be conducted to ensure the smaller scale data are representative of the manufacturing scale data, since our ultimate objective is to predict the batch performance at manufacturing scale.

The likelihood distribution is typically generated from the data of the most recent batch, because we believe that any systematic change occurs in this batch is likely to be carried onto the subsequent batch. Although there are numerous ways to leverage the manufacturing data to generate the likelihood distribution, we chose only the most recent batch because we wanted to capture any change from this recent batch to predict the performance of the subsequent batch. In
our case study, since we use the most recent batch as the test dataset, we form the likelihood distribution from the 2nd to the most recent batch.

With the process knowledge and consultation with subject matter experts in the field, the CPA and CQA of this process, similar to other manufacturing processes, follow a normal distribution. To verify the validity of this assumption, a normality test, such as Anderson–Darling statistics, which measures how well the data follow a normal distribution, was conducted using Minitab. For a normal distribution, the two key parameters are mean and standard deviation. In terms of the likelihood distribution, the mean and standard deviation are simply those of the most recent batch. For the prior distribution, the prior mean is initially calculated as the mean of the historical batches and the standard deviation of the mean is calculated as the observed standard deviation divided by the sample size. As a result, the standard deviation decreases as the sample size of the historical data increases.

The posterior mean is a weighted average of the prior mean and the likelihood mean, with the weight inversely proportional to the standard deviation of the prior mean and the standard deviation of the likelihood data. Hence, as the standard deviation or the uncertainty of the prior mean decreases, the posterior mean would be heavily dominated by the prior mean. Similar to the results from the conventional approach, the heterogeneity between the prior and likelihood became diminished in the posterior mean.

To address this issue, Ibrahim and Chen [51] proposed the concept of the power prior, based on the notion of the availability of historical or prior data. The power parameter, with \(0 \leq \delta \leq 1\), will dictate how much historical data information are to be used in the analysis. However, in their
approach, the power parameter has a tendency to be close to zero. In other words, much of the historical data would not be used in predicting the mean [52].

Here, we propose a different approach in deriving the prior standard deviation, such that the predicted mean is not overwhelmed by the historical data due to the large sample size. Instead of using the overall observed standard deviation divided by the square root of the sample size of the historical data, we first calculate the standard deviation of the mean for each batch, and the prior standard deviation of the mean is an average of the standard deviation from all these batches, as shown below:

$$
Standard\ deviation\ of\ mean_{Prior} = \frac{\sum_{i=1}^{N_{batch}} standard\ deviation\ of\ mean_{batch\ i}}{N_{total\ number\ of\ batches}}
$$

(3.2)

Based on this formula, the standard deviation of mean is indeed the average standard deviation with respect to the number of batches, instead of number of cycles. Since our goal is to estimate the mean for the upcoming batch, it is reasonable to calculate the average standard deviation of the mean per batch instead of per cycle. With this approach, the standard deviation of prior distribution would be less likely to be affected by the size of the historical dataset. Hence, the prediction would be less likely to be overwhelmed by the prior mean.

After establishing the key parameters, a simulation is conducted to derive the posterior probability (Eq. 3.1) against a range of relevant process set points, which are typically the minimum and the maximum of the manufacturing range. In addition, the predicted mean and their range from both
approaches are compared to the actual mean. The difference between the predicted and the actual mean would inform how accurate each approach is.

3.3. Results

3.3.1. Conventional and Bayesian Approaches Result in Different Predicted Means

With the emergence of process analytical tools (PAT) as well as other data acquisition tools, process and product quality data become readily available. Therewith, it is critical to have a reliable computational tool to monitor and predict process performance, by leveraging available data.

Figure 3.3 is a statistical process control diagram of Protein A chromatography yields. The older batches, which consist of 34 cycles, have higher yield than the recent batch, which has 6 cycles. The change in the yield is due to the replacement of chromatography resins. Ideally, a predicting tool would be able to incorporate information from both the older and recent batches when estimating the trend of the upcoming batches.
Figure 3.4 shows the probability distribution of the mean for different datasets, prior, likelihood, predicted means by the conventional and Bayesian approach. Table 3.1(dataset 1) also shows the predicted mean, their respective confidence interval and the actual mean.

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Mean Ratio</th>
<th>Predicted Mean</th>
<th>Actual Mean:</th>
<th>Predicted Mean</th>
<th>Actual Mean:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bayesian mean (95% CI)</td>
<td>Conventional mean (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.24</td>
<td>78.6 (75.1 – 82.0)</td>
<td>83.6 (81.0 – 86.2)</td>
<td>77.3 (74.2 – 80.4)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.08</td>
<td>86.4 (82.9 – 90.0)</td>
<td>86.7 (84.1 – 89.4)</td>
<td>84.2 (82.1 – 86.3)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1 Impact of the Mean Ratio on the Predicted Means
The conventional predicted mean is dominated by the prior mean, and overestimates the actual mean for the upcoming batch. In fact, with the conventional approach, as the number of historical batches increased, the historical data would dominate the mean and the heterogeneity between the historical and recent batch would have less impact on the predicted mean. Consequently, this approach may result in misleading conclusions, especially where there is a systemic deviation from historical batches.

However, the Bayesian predicted mean falls between the prior and the likelihood mean, and more importantly, it accurately predicts the test mean. Hence, if a systemic deviation is suspected in a
process, this modified Bayesian approach would be likely to offer a more accurate estimate than the conventional approach, as the Bayesian approach is better at capturing the heterogeneity between the prior and likelihood and less sensitive to the sample size of the prior dataset.

To test the robustness of this conclusion, we have applied both approaches in other attributes, in other processes, such as IEX yield (figures 3.5 and 3.6). Similar to the conclusions that we arrive with the Protein A yield, the Bayesian approach offers a more reliable estimate than the conventional approach in cases whether there are subtle changes in recent batches.

![Statistical Process Control Diagram of IEX Chromatography Yield](image)

**Fig 3.5 Statistical Process Control Diagram of IEX Chromatography Yield.** Blue diamonds represent older batches, red diamonds represent recent batches and black diamonds represent most recent batches.
3.3.2. Prior and likelihood mean ratio vs Bayesian predicted mean

To quantify the heterogeneity between the prior and likelihood mean, ratio between the prior and likelihood mean is determined [51]. Here, we analyze two sets of protein A chromatography yield data. In one of them, the prior and likelihood come from the same population, whereas in the other one (as shown in the example above), there is heterogeneity between the prior and likelihood distribution.
Table 3.1 shows the summary of these two sets of data: their mean ratio, predicted mean, the corresponding confidence interval and the actual mean. In the case where both the likelihood and prior come from the same population, or the mean ratio is close to unity, both the conventional and Bayesian results in similar predicted mean.

Although the Bayesian predicted mean is a weighted average between the prior and the likelihood, when both of these distributions have similar means, the Bayesian predicted mean is similar to the overall mean. Hence, the two approaches result in a similar estimation.

However, as the heterogeneity between the prior and likelihood distributions increases, or the ratio of the two means moves away from unity, the predicted means and their 95% confidence interval by the two approaches start to deviate. As mentioned in previous sections, in the case where there is more prior data compared to the likelihood data, the Bayesian approach is more sensitive at capturing the heterogeneity between the two distributions. Therefore, while the conventional predicted mean is dominated by the prior mean, the Bayesian predicted mean would have the contributions from both the prior and likelihood distributions.

Based on the results from this study, the mean ratio can be used as a tool to determine whether Bayesian is needed for a particular analysis. If the mean ratio is close to unity, or the prior and likelihood distributions come from the same population, then the two approaches should result in similar predicted means. However, as the mean ratio deviates from unity, or there is heterogeneity between the prior and likelihood mean, then Bayesian would be better at capturing this
heterogeneity, especially when the prior sample size is much larger than the likelihood sample size.

3.3.3. Impact of uncertainty in likelihood data on Bayesian predicted mean

Our previous analysis suggests that when there is heterogeneity between the historical and current data, Bayesian, compared to the conventional approach, is better at capturing the heterogeneity and thus offering a more accurate prediction of the future batch. However, it is important to note that the Bayesian posterior mean is a weighted average of the prior mean and the observed data, with the weighting corresponding to the relative precisions of the prior and the likelihood. Therefore, when the prior is highly precise compared to the likelihood, the prior is weighted heavily and the posterior mean is dominated by the prior mean.
Figure 3.7 shows the host cell protein impurity level in the Protein A chromatography eluate. Similar to the Bayesian analysis conducted for the Protein A chromatography yield, the older batches are used to form the prior distribution, the more recent batch is used to form the likelihood distribution and the most recent batch is used as a test dataset.

As shown in Figure 3.8, since we switched the testing assay, the prior distribution appears to be much tighter than the likelihood distribution, and hence the posterior distribution of the mean is dominated by the prior mean. In this case, although there is heterogeneity (mean ratio = 0.66) between the historical and the current data, the Bayesian approach was not able to capture it and the predicted mean underestimates the actual mean.
The results from this analysis suggest that a low precision of the observed data can be a limitation to the Bayesian approach. One way to overcome this limitation includes using the same or similar testing method across all batches to ensure consistent precision from batch to batch.

**Fig 3.8** Probability Distribution of Prior, Likelihood and Predicted Means of Protein A Eluate HCP Level
3.4. Summary and Conclusion

In this chapter, both the conventional and the Bayesian approaches are used to predict the performance of an upcoming manufacturing batch, by leveraging the data from historical batches. Although the conventional approach is easier to implement, it might not be sensitive enough to capture the heterogeneity between historical and current batches. For instance, when the sample size of the historical data is much larger than the current data, the heterogeneity between these two is diminished, because the predicted mean is largely dominated by the historical mean. Therefore, in the case where there is a systemic deviation, this approach is less likely to result in an accurate prediction of the upcoming batch.

Compared to the conventional method, Bayesian is shown to be often better at capturing the difference between the historical and current data, and is therefore able to make better predictions. When using the Bayesian approach, it is important to note that the posterior mean is a weighted average of the prior mean and the likelihood mean, with the weight inversely proportional to the standard deviations of the prior and likelihood data. Hence, as the standard deviation of the prior mean decreases, due to large sample size, the posterior mean would be heavily dominated by the prior mean.

Therefore, in the calculation of the prior standard deviation, we do not just take the overall standard deviation of all data, which can be heavily affected by sample size. Instead, we first calculate the standard deviation of the mean for each batch, and the prior standard deviation of the mean would be the average of the standard deviation per batch. As shown in our analysis, this approach
circumvents the issue of the predicted man being overpowered by the historical mean. The predicted mean is indeed comparable to the actual mean.

While Bayesian is better at capturing the heterogeneity than the conventional approach, there are occasions that both approaches offer the same prediction, especially in the case where the batches perform consistently. Here, we present a tool to determine whether a Bayesian approach is necessary for a particular analysis. The ratio between the historical and current mean can be used to measure process consistency. For instance, as the ratio deviates more and more from unity, then Bayesian is likely to be a better approach, as there is inconsistency between historical and current batch performance.

As mentioned previously, the Bayesian posterior mean is a weighted average between the historical mean and observed data, weighting by the certainty of each distribution. As a result, if the current data is much more spread out, or less informative than the prior mean, due to the change in assay precision or other factors, then the prior mean would have a larger contribution to the posterior mean. This is indeed a limitation to the Bayesian method. To overcome this limitation, it is critical to control the precision from batch to batch to ensure a reliable estimation.

In addition, Bayesian can capture the heterogeneity between the historical and observed data when there is a systemic change in the manufacturing process. In other words, the subsequent batch should have a similar trend to the current batch and the change in the current batch is systemic but not sporadic. Both the uncertainty of the distribution and the nature of process change in the current data can limit the accuracy of the Bayesian approach.
As the FDA regulations become more stringent, and more data become available with the development of advanced data acquisition tools, it is important to leverage available information to monitor and predict process performance. Although the Bayesian approach, which is widely used in clinical settings, is less frequently used in pharmaceutical manufacturing, it is better at capturing process heterogeneity compared to the conventional approach. Therefore, when there is a systemic change in the manufacturing process, the Bayesian approach can enable a more accurate prediction of future manufacturing performance.

In addition to minimizing batch failure through reliable process monitoring and trending, biopharmaceutical companies need to ensure their final drug product meet the predefined acceptance criteria, for product safety and efficacy. To meet this goal, biopharmaceutical companies need to conduct a series of process characterization and validation studies, to fully understand the nature of the manufacturing process, identify critical process parameters and their operating range. Furthermore, it is paramount that appropriate immediate product specification is set based on process capability and final drug product specification. These intermediate specifications will serve as forward processing criteria for each step and ensure a higher likelihood for meeting the final specification, and successfully releasing the batch.
4 Neural Network to Understand Process Capability and Process Intermediates Acceptance Criteria in Monoclonal Antibody Production Process

4.1. Introduction

Monoclonal antibodies are typically produced by mammalian cell culture in bioreactors that range in scale of 1-20 m³. Regardless of scale, the production and purification of mAb present important challenges [18]. Such a complex multistep process, which encompasses several seed and production bioreactors, as well as a number of chromatography and filtration steps, needs to be carefully monitored and controlled as many of the in-process variables can impact the product quality. Recent developments at the FDA recommend the application of risk-based approaches to control product quality based on an enhanced process and product understanding [53].

In our previous studies, we established a framework that integrates design of experiments (DoE) and principal component analysis (PCA) to identify process parameters that have significant impact on critical process attributes [39]. Hence, we can now control the process by ensuring that these parameters are operated within the acceptable range, and thus improving process robustness and consistency.

Furthermore, since any quality issues in manufacturing will delay the release of a batch and can sometimes disrupt the supply chain, we developed a systematic framework to predict future manufacturing performance or product quality using a Bayesian approach [54]. Therefore, in cases where we suspect a potential deviation in the future manufacturing batches, appropriate mitigation strategies can be developed in advance to reduce risks or batch failures.
In addition to identifying critical process parameters, and developing a framework to predict drug manufacturing process performance, it is very important to have appropriate impurity acceptance criteria for process intermediates. In fact, defining the in-process acceptance criteria is part of the control strategy, which will eventually go into the drug validation regulatory package.

While there is considerable literature describing the different approaches to define acceptance criteria or specification for the final drug substance, which directly links to the clinical efficacy and safety [53], the industry is still learning the different ways to set process intermediates acceptance criteria. These acceptance criteria lead to a tradeoff between the likelihood a consumer will acquire a suboptimal dose and the likelihood a lot will fail release [20]. Although narrower acceptance criteria are more likely to ensure a successful batch release, and to achieve pre-defined safety and efficacy, it could potentially reject batches that would otherwise have met the final drug specification. Hence, it is critical to establish accurate and appropriate acceptance criteria.

While the end goal of drug manufacturing is to meet the final drug specification by having stringent control of process intermediates, there are several ways of establishing in-process acceptance criteria. A widely used approach is to use the final drug substance or product specification as a reference, and deduce the specification for products from previous steps based on the process capability of those steps.

For instance, if the final process step has a constant 3% impurity reduction, and if the specification for the final product is 2%, then the product of the step before the final step, or the input to the
final process step should have impurity level no greater than 5%. However, the difficulty here is in knowing what the impurity reduction for each step is.

One approach is to develop a mechanistic model of a specific process, and leverage this model to predict the input specification based on the final output specification. However, this approach is very process specific, and would involve significant amount of time and effort for model development. Hence, this approach is rarely used for acceptance criteria setting.

Another way to understand process capability is to do a worst case scenario study. In such study, we challenge the chromatography column or filter with a significant higher than typical amount of impurity, and find out the maximum impurity reduction this step can achieve. A challenge with this approach is that sometimes it could be difficult to find a feed stream with such a high impurity level while ensuring these impurities are representative. In addition, a lot of experiments need to be conducted before we are able to reach the saturation point.

Some other simpler approach used in the industry to understand process capability is to compare the average impurity levels of the feed and the product, and the average reduction is used to calculate the in-process acceptance criteria. While this is relatively straightforward, one caveat is that it assumes that the impurity clearance is independent of the feed impurity level. For instance, two feeds with different impurity levels are assumed to undergo the same degree of reduction; however, this is not always the case, as shown later in our study.

Building on our prior modeling work in pharmaceutical manufacturing [41, 42, 43 and 44], we propose an alternative way to calculate process capability, by using neural network to characterize
the relationship between the feed and the product quality attributes of a particular process step. It is a promising modeling technique especially for datasets having the kind of non-linear relationships that are frequently encountered in pharmaceutical processes.

Contrast to what’s commonly practiced in the industry, we do not have to assume that the impurity reduction is independent of the feed. The neural network model can identify and learn correlative patterns between feed and product data pairs. Once trained, it may be used to predict the product impurity level based on the feed impurity level, or vice versa. One of the most useful properties of neural networks is their ability to generalize.

Diagnostic plots will be used to evaluate the goodness of fit before arriving at the final neural network model. In addition, we will compare this approach with the conventional approach practiced in the industry, and determine if there is any difference in model prediction accuracy. Finally, we will discuss how we can use both approaches in setting acceptance criteria for process intermediates.

4.2. Methodology

4.2.1. Overview of Monoclonal Antibody Production Process

A typical monoclonal antibody production process consists of an upstream process (USP) section and a downstream (DSP) process section, as shown in Figure 4.1. While the USP includes
inoculation, cell culture and primary recovery, the DSP consists of Protein A chromatography, followed by two ion exchange steps (follow-through and bind-and-elute), virus filtration and ultrafiltration. In general, biological molecules are much more complex changes in the production process. Therefore, it is imperative to closely monitor the critical quality attributes for process intermediates, such as Protein A product (or IEX I feed), or IEX I product (or IEX II feed), to ensure the final drug substance and product meets the predetermined specification.

In our case study, we focus on the clearance of protein aggregate through various downstream chromatography steps. Protein aggregation is a key challenge in the development of protein
biotherapeutics. Aggregates are defined as any physically associated or chemically linked non-native species of two or more protein monomers, as shown in Figure 4.2 [55]. It is widely accepted in the scientific literature that protein aggregation can augment a protein-specific immune response, and therefore lead to the formation of anti-drug antibodies (ADA) [56,57], which can cause adverse events, such as neutralization of endogenous protein or reduce efficacy of a biotherapeutic [58]. Hence, it is critical to reduce protein aggregate level, to ensure maximum drug efficacy.

![Fig 4.2 Overview of Protein Aggregate Formation](image)

4.2.2. Data Collection
The data from the case study are a combination of actual data from several monoclonal antibody manufacturing processes and simulated data. All data and simulated models have been validated by internal quality control group. These data have been scaled for confidentiality purposes but the general trends are preserved.

4.2.3. Approach to estimate process capability

4.2.3.1 Conventional Approach

The conventional approach, or what is commonly practiced in the industry, to understand process capability, or the impurity reduction of a particular step, is as follow:

\[
\hat{Y}_{prod,i} = Y_{feed,i} - \bar{Y}_{red}
\]

where the product aggregate level \(\hat{Y}_{prod,i}\) is estimated as the difference between the feed aggregate level \(Y_{feed,i}\) and the average aggregate reduction \(\bar{Y}_{red}\).

4.2.3.2 Alternative Approach – Neural Network
Although the conventional method is relatively straightforward, it assumes the impurity reduction is constant regardless of the feed impurity level. However, this is not true in all cases. For example, protein aggregates usually consist of a mixture of multi-mers, such as dimer, trimer, and other higher order multi-mers. The higher order aggregates are usually easier, and the first to be purified while dimers are much more challenged to be reduced.

Hence, it is often the case that if the feed stream has a higher level of aggregates, we observe a higher percent of aggregate reduction, since most of the higher order aggregates are being removed. On the other hand, if the feed stream has very low level of aggregate, which is most likely comprised of dimers, the impurity reduction might be much smaller since the dimers are difficult to remove. Hence, for the same purification step, impurity clearance could vary based on the feed impurity level, and using the conventional estimation method would not be able to reflect this phenomenon.

In addition to conventional approach, one might attempt to model the data using higher order of polynomials, such as quadratic regression. However, this approach might not be able to describe the data appropriately (Appendix Table A7.3.1.1). Furthermore, this approach can involve in a lot of trial and errors in order to find the best fit. Hence, here we propose the use of neural network to relate the feed impurity level to the product impurity level, such that the product impurity level is a function of the feed impurity level. The model is built and simulated using MATLAB®.
The neural network models are composed of simple elements operating in parallel. The connection between elements largely determines the network function. A neural network can be trained to perform a particular function by adjusting the value of the connections (weights) between elements [59]. Hence, neural networks are able to recognize patterns even from noisy and complex data in which there is a considerable degree of variation and to estimate non-linear relationships [60].

The use of neural networks is an expanding area in the field of pharmaceutical research. Some of the important applications are in molecular modeling, pharmacokinetic and pharmacodynamics modeling, and *in vitro/in vivo* correlation [60, 61,62,63,64]. However, the application of neural networks in understanding process capability and define process specification is still limited. In our study, we develop neural network models to correlate the feed aggregate level with the corresponding product aggregate of several chromatography steps.

4.2.3.2.1 Neural Network Model Selection and Evaluation

Typically, neural networks are adjusted, or trained, so that a particular input leads to a specific output [59]. As shown in Figure 4.3, the network is trained, by comparing the model predicted output with the target, until most of the network outputs match the targets. Many such input-target pairs are needed to train a network [59].
In our study, the neural network is trained by backpropagation algorithm, which is the most widely used neural network learning method [65]. As shown in Figure 4.4, the input layer communicates with the external environment and presents the pattern to the neural network [66]. The hidden layer is the collection of neurons which has transfer function (usually sigmoidal function), applied on it, as well as provides an intermediate layer between input and output layer [67]. In order to ensure the performance of the neural network, it is critical to select an appropriate topology, and two of the important governing elements are the training algorithm within the backpropagation family, as well as the number of hidden layer and nodes.
4.2.3.2.1.1 Training Algorithm

The training algorithm depends on whether the network is being used for pattern classification or function approximation. Since we are trying to approximate the correlation between the feed and product aggregate, some of the common algorithms are Levenberg-Marquardt (LM), Scaled Conjugate Gradient (SCG) and BFGS Quasi-Newton (BFG).

For function approximation problems, LM has the fastest convergence. The performance of BFG is similar to that of LM, but it does not require as much storage as LM. However, the computation required for BFG increases geometrically with the size of the network. SCG algorithm seems to perform well over a wide variety of problems, particularly for networks with a large number of weights [59].
4.2.3.2.1.2 Hidden Layer and Nodes

The standard network that is used for function fitting is a two-layer feedforward network, with a sigmoid transfer function in the hidden layer and a linear transfer function in the outer layer. Since in our study, we have a continuous mapping from one finite space (feed aggregate level) to another (product aggregate level), we have chosen a single hidden layer [66]. The number of nodes in the hidden layer is set to a default of 10. This number can be increased to enable a better model fit.

To select an appropriate algorithm for a dataset, we divide the data into 3 groups, 70% of the data is used for training, while 15% of the data is used for validation and the remaining 15% is used for testing. The number of nodes in the hidden layer is initially set to default. Each dataset is run using all three algorithms (LM, SCG and BFG), and the sum of square error (SSE) and number of epoch, or iteration, are recorded. The algorithm that yields a low SSE with a reasonable epoch is selected to carry forward.

Once the training algorithm is established, we adjust the number of nodes to possibly improve the SSE and epoch. The number of nodes that yields a low SSE with a reasonable epoch is selected.

To ensure we have a good model, we evaluate the regression plots of network outputs with respect to target for training, validation and test sets. For a good model fit, most of the data should fall along a 45 degree line, where the network outputs are equal to the targets.
In addition, an error (errors = targets – output) histogram is plotted to obtain additional verification of network performance. For a good model fit, the error distribution should be roughly normal centers around 0.

4.2.4 Comparison between Conventional and Neural Network Approaches

To evaluate how accurately each approach characterizes the data, the following criteria are used [68]:

1) **Observed vs Predicted**: After an appropriate model is established for each approach, product aggregate level ($Y_{prod}$) is estimated using the models and the feed aggregate ($Y_{feed}$) data. The predicted product aggregate ($\hat{Y}_{prod}$) is then compared with the actual product aggregate ($Y_{prod}$). Deviation from the diagonal and the presence of any significant trend indicate a potential lack of fit.

2) **Residual Plot**: Ordinary residuals are the difference between the observed ($Y_{prod}$) and model-predicted ($\hat{Y}_{prod}$) values

\[ e_i = \hat{Y}_{prod,i} - Y_{prod,i} \]
where \( i = 1,2,3,4 \ldots n \). Positive residuals indicate that the model overpredicts the observation, whereas negative residuals indicate that model underpredicts the observation. The presence of any obvious trend indicates a potential lack of fit.

(3) **SSE:** Another metric of goodness of fit is the square difference between observed and predicted values, or the sum of square error (SSE)

\[
SSE = \sum_{i=1}^{n} (y_{prod,i} - \hat{y}_{prod,i})^2 = \sum_{i} e_i^2
\]

where a small SSE indicates a better model fit.

4.2.5 Simulation to Define Acceptance Criteria

During process development, it is critical to define appropriate acceptance criteria for process intermediate aggregate level. Based on the safety and efficacy testing, we are often given a quality specification for the product. Subsequently, we have to derive an appropriate specification for the feed, which is used to determine whether or not to proceed with the step.

If the feed aggregate level is outside of the acceptance limit, then we might not want to proceed with the step, since the product is less likely to meet the pre-defined specification. Although a narrower in-process specification increases the probability of meeting the product specification, it
could potentially reject batches that would have otherwise met the final drug specification. Hence, it is critical to establish an accurate in-process specification.

4.2.5.1 Conventional Approach

To capture the variability of aggregate reduction($Y_{red}$), Monte Carlo simulation is performed using a normal distribution, with the mean and standard deviation of the aggregate reduction from the original dataset. The predicted product aggregate level is calculated by subtracting the simulated($Y_{red}$) from the feed. This process is repeated 300 times, and the mean and 90% confidence interval are generated for each feed aggregate level. Based on the pre-specified product aggregate level, we can predict what the corresponding feed aggregate level is from the mean curve.

4.2.5.2 Neural Network Approach

Each time a neural network is trained, it can result in slightly different solution due to different initial weight and bias values and different divisions of data into training, validation and test sets. To account for this variability, the neural model is run on each of the dataset for 300 times, and the mean and 90% confidence interval are computed. Based on the pre-specified product aggregate level, we can predict what the corresponding feed (or product from previous step) aggregate level is from the mean curve.
If we need to adopt a more conservative approach, we can use the 90% confidence interval curve, instead of the mean curve, to predict the feed aggregate acceptance limit. To evaluate the acceptance limit set by each approach, we compare the mis-specification (type I and type II error) in each case. In addition, we compare the acceptance limits with the limits generated by the internal validated models.

4.3. Results

In this study, we try to understand the process capability of a chromatography step, or the aggregate reduction across the step. Figure 4.5 shows the correlation between the chromatography feed and product aggregate level from monoclonal antibody purification processes of four different products. Figure 4.6 shows the aggregate reduction with respect to the feed aggregate level, respectively. The aggregate reduction increases at higher feed aggregate level, as most of the higher-order aggregates are cleared by the chromatography step.

In process 4, there seems to be an initial decrease in aggregate level at the feed level of 1.5 – 2.5%. This could be attributed to the composition of the aggregate in the feedstream. For instance, there might be a larger percentage of dimers, which are harder to be removed, present in the feed that has a 2.5% total aggregate. Hence, the reduction of aggregate for this particular feedstream is relatively lower compared to the feed that has a 1.5% total aggregate.
4.3.1. Neural Network Training Algorithm and Architecture

To select an appropriate training algorithm for each of the process, all datasets are run with three approximation algorithm (LM, SCG and BFG), with the initial number of hidden neurons set at default. SSE and number of epoch are used as the screening criteria.

Table 4.1 shows the algorithm and number of nodes for each process. For all four processes, Levenberg-Marquardt (LM) is selected as the best training algorithm, which results in a low SSE and epoch. LM is indeed the most common algorithm for function approximation in neural network modeling [59].
Fig 4.5 Chromatography feed vs. product aggregate levels in purification processes from four different products. The x-axis presents the feed aggregate level and the y-axis represents the product aggregate level. Each plot contains 1002 datapoints.
Fig 4.6 Chromatography feed vs. product aggregate reduction levels in all four processes. The x-axis shows the feed aggregate level, and the y-axis shows the aggregate reduction level.
After we establish the training algorithm, different numbers of nodes (8, 10, 12, 14, 16, 18, 20 and 22) are tested, and the selected numbers of nodes result in low SSE with a reasonable number of epoch.

<table>
<thead>
<tr>
<th>Process</th>
<th>Algorithm</th>
<th>Number of Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LM</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>LM</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>LM</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>LM</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 4.1 Neural Network Algorithm and Number of Nodes in Each Process

4.3.2 Conventional Approach Model

For the conventional approach, we assume that aggregate reduction is constant, irrespective of the feed aggregate level. Table 4.2 shows the modeling equation for each of the process, where $\hat{Y}_{prod,i}$ is the predicted product aggregate level, $Y_{feed,i}$ is the feed aggregate level, and $\bar{Y}_{red}$ is the average aggregate reduction.
Equation: \( \hat{Y}_{prod,i} = Y_{feed,i} - \bar{Y}_{red} \)

<table>
<thead>
<tr>
<th>Process</th>
<th>( \bar{Y}_{red} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>4.11</td>
</tr>
<tr>
<td>3</td>
<td>2.94</td>
</tr>
<tr>
<td>4</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Table 4.2 Conventional Model Equations

4.3.3 Comparison between Conventional and Neural Network Approach

To evaluate the accuracy of the model that we establish using the conventional and neural network approaches, we analyze the observed vs. predicted plot, residual plot and SSE. Figure 4.7 shows the predicted product aggregate level compared to the actual aggregate level, using either the conventional or neural network approach. The red line represents the 45° line, and the green line is the least squares regression (LSR) line. With the neural network approach, most of the data fall on the 45° line, and the LSR line overlaps with the 45° line, suggesting a near-perfect fit. However, with the conventional approach, some of the data, as well as the LSR line, deviate from the 45° line, suggesting a lack of fit.

In addition to analyzing the obs. vs. pred plot, we evaluate the residual plot. As shown in Figure 4.8, the conventional approach seems to underestimate the product aggregate level at low feed
aggregate level, and overestimate at high feed aggregate level. This is because the conventional approach assumes constant aggregate reduction throughout. In fact, aggregate reduction tends to be lower at low feed aggregate level, and higher at high feed aggregate level, as shown in Fig 4.6. Conversely, with the neural network approach, the residuals scatter around the zero reference line (red line), without any significant trend, suggesting a better fit than the conventional approach.

Finally, another important metrics to evaluate model accuracy is the SSE. As seen in Table 3, in all four processes, the neural network model predictions result in much lower SSE compared to the conventional method.

<table>
<thead>
<tr>
<th>Process</th>
<th>Conventional</th>
<th>Neural Network</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91.00</td>
<td>34.37</td>
</tr>
<tr>
<td>2</td>
<td>1068.48</td>
<td>52.22</td>
</tr>
<tr>
<td>3</td>
<td>1777.49</td>
<td>54.56</td>
</tr>
<tr>
<td>4</td>
<td>884.61</td>
<td>14.87</td>
</tr>
</tbody>
</table>

Table 4.3 SSE of Model using Conventional and Neural Network Approaches

As a result, prediction using the neural network approach seems to be superior to the conventional approach, as suggested by the observed vs. predicted plot, residual plot and SSE. Rather than
assuming the aggregate reduction is constant across all feed aggregate level, the neural network analyzes each pair of the feed and product aggregate level, and generates a model with appropriate weight to capture the correlation between the feed and product [60]. In addition, the neural network model is able to capture the dynamic of aggregate reduction with respect to feed aggregate level.
Fig 4.7 Observed vs. Model Predicted Aggregate Level calculated using Conventional and Neural Network Approaches. The x-axis shows the predicted product aggregate level, and the y-axis shows the actual product aggregate level. The top panel of each process shows the conventional approach results, whereas the bottom panel shows the neural network approach results. The red line is the 45° reference line, in which the predicted outputs are equal to the actual aggregate level, and the green line is the least squared regression (LSR) line based on the relationship between the predicted and the actual data. With the neural network approach, the LSR overlap with the 45° reference line.
Fig 4.8 Model Predicted Aggregate level vs. Residuals from Conventional and Neural Network Approaches. The x-axis represents the predicted product aggregate level, and the y-axis represents the residual level, which is the difference between the predicted and the actual product aggregate level. The red line represents a reference line where the residuals are zero, or the predicted product aggregate levels are equal to the actual aggregate level.
4.3.4 Definition of Process Acceptance Criteria

Figure 4.9 shows the simulated feed and product aggregate levels. The red horizontal line indicates the pre-defined product specification, and the red, blue and green vertical lines represent the acceptance limits for the feed aggregate level, estimated by the internal mechanistic model, neural network and conventional approach, respectively. Compare to the conventional approach, the neural network approach has a lower Type I error, or is less likely to reject batches that in fact meet the product specification. The neural network estimates are also closer to the internal simulated model estimates, as shown in Table 4.4.

<table>
<thead>
<tr>
<th>Process</th>
<th>Internal Model</th>
<th>Conventional</th>
<th>Neural Network</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.46</td>
<td>3.27</td>
<td>3.42</td>
</tr>
<tr>
<td>2</td>
<td>6.71</td>
<td>6.83</td>
<td>6.70</td>
</tr>
<tr>
<td>3</td>
<td>8.15</td>
<td>8.02</td>
<td>8.22</td>
</tr>
<tr>
<td>4</td>
<td>6.19</td>
<td>5.01</td>
<td>6.27</td>
</tr>
</tbody>
</table>

Table 4.4 Feed Acceptance Limit calculated using the Internal Model, Conventional and Neural Network Model

<table>
<thead>
<tr>
<th>Process</th>
<th>Conventional</th>
<th>Neural Network</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.46%</td>
<td>5.77%</td>
</tr>
<tr>
<td>2</td>
<td>10.17%</td>
<td>8.77%</td>
</tr>
<tr>
<td>3</td>
<td>2.16%</td>
<td>3.69%</td>
</tr>
<tr>
<td>4</td>
<td>0%</td>
<td>4.27%</td>
</tr>
</tbody>
</table>

Table 4.5 Comparison of Error % between Conventional and Neural Network Models
Figure 4.9 Feed and Product Aggregate Level, with product specification (red horizontal line), and associated feed acceptance limits derived from internal model (red dashed vertical line), conventional model (green dashed line) and Neural Network model (blue dashed line).
Table 4.5 shows the error %, or the percentage of batches that meets the feed acceptance limit but fails the product specification. With the neural network approach, although we have a slightly higher error %, we are able to capture more batches that meet the product specification, and less likely to have Type I error. Since the error% with the neural network approach is less than 10%, which is our internal standard, we believe that the neural network is a better approach since the cost of falsely rejecting a manufacturing batch can be consequential.

In addition, depending on what data are available for the feed and aggregate level, using the conventional, or simply taking the average of the aggregate reduction of the available data can create some type of bias. For example, if mostly high feed aggregate data are available, then the average reduction might be overestimated, and the product aggregate level would be underestimated using the conventional approach. Hence, the distribution of the available data can affect the error% of the conventional approach. However, neural network can learn from both high and low feed aggregate data, and derive the relationship between the feed and product aggregate level. Therefore, the neural network model is less likely to be impacted by the distribution of data available.

4.4. Conclusion

Setting appropriate in-process acceptance criteria is important in ensuring success in drug manufacturing, and increasing the likelihood that drug substance or product will meet the final release specification. In order to establish accurate feed acceptance criteria, it is critical to
understand process capability, and accurately characterize the relationship between the feed and product aggregate level.

In this chapter, we compare the performance of the conventional approach, which is widely practiced in industry, and the neural network approach. While the conventional approach is much simpler, it assumes that the impurity reduction is constant, and thus might not be able to provide a good estimate of process capability in cases where impurity reduction is feed dependent. In contrast, neural network model is better able to characterize the relationship between the feed and product aggregate level, and to estimate the feed aggregate acceptance limit based on the product aggregate specification. Our study suggests that it is beneficial to consider using neural network models for improving manufacturing of biopharmaceuticals.
5 Conclusions & Future Work

Monoclonal antibody has become a major therapeutic agent for various diseases, such as oncology, immunology and infectious diseases. As compared to small molecules drugs, monoclonal antibody drugs tend to be more efficacious, with less off target effects. Hence, the demand for monoclonal antibody drugs have increased significantly over the last decade, with the sales of monoclonal antibody drugs reached $75 billion in 2013.

To meet the increased demand in monoclonal antibody drugs, biopharmaceutical companies have developed new process technologies, such as extended cell culture, continuous chromatography, tangential flow filtration, to improve the productivity and output of upstream and downstream production. In addition, the ensure the continuously supply of drug products, as well as the quality of the drug products, FDA has mandated process characterization and validation as part of the drug approval package. The process characterization and validation activities should enable an identification of critical quality attributes (CQA), critical process parameters (CPP) and the corresponding acceptance criteria, and operating ranges, respectively.

To minimize product failure, and increase competitiveness of their products, biopharmaceutical companies have developed significant efforts to (1) monitor and understand the trend of drug production performance, specifically focusing on the yield, and critical product attribute; and (2) develop appropriate in process specification, which also serves as forward processing criteria to determine whether or not the process a specific step. In addition, with the intense competition within the industry, it is important for biopharmaceutical companies to develop drugs that not only show superior efficacy and safety, but also are cost effective.
This chapter summarizes the efforts made in this thesis to develop decision framework and tools to identify development strategies for manufacturing cost reduction, leverage historical manufacturing batches to predict future production performance, and finally develop in process specification based on process capability. Furthermore, it also points out a number of future developments that will increase the robustness, consistency and economy of the monoclonal antibody production process.

5.1. Overall Conclusions

The primary aim of this thesis has been the design and development of decision – support framework and quantitative tools that is capable of optimizing and simulating monoclonal antibody manufacturing process, predicting process performance and setting in process specification for critical quality attributes. The resulting tool described throughout this thesis is capable of facilitating more informed decision making when evaluating which optimization strategies to adopt, if a potential process deviation will arise based on past and recent process performance, and whether or not to process a step forward based on the intermediate specification. The aim of this body of work was realized through a number of objectives that formed the basis of each of the preceding chapters. These chapters clearly demonstrate that the framework is powerful, and more reliable for process optimization, monitoring and control.

Chapter 2 details the development approach to establish a decision – support framework to support process optimization to reduce manufacturing cost of goods. This is achieved through a
combination of analyses, such as risk assessment, Design of Experiment (DoE) and Principal Component Analysis (PCA). The risk assessment, of Failure Mode Effects Analysis (FMEA), enables the identification of a list of potential critical parameter that may have significant impact on the attribute we are evaluating, in this case cost of goods. These parameters are then carried onto the DoE study, through which parameters that have statistically significant impacts on the cost of goods are identified, and these parameters are now call critical process parameters, or CPPs. This is what has been done frequently in the biopharmaceutical industry.

While the DoE studies enable the identification of critical process parameters, sometimes it doesn’t give further insights into the relative importance of each parameter. Consequently, this makes the process development very challenging, because there isn’t much guidance on how we should prioritize the process development strategies, or which parameters to optimize first given the limited resources available. Hence, we introduce another tool, principal component analysis, which can give the relative contribution of each critical parameter to the cost of goods. In our case study, the combination of DoE and PCA has effectively identified and prioritized parameters for further optimization to reduce cost of goods.

Monoclonal antibody production process is inherently more variable than small molecule drug production process, mostly due to the large variability in the cell culture process. Hence, when we are analyzing the monoclonal antibody process, it is important to capture this variability, specifically variability in titer, and in chromatography and filtration yields. Rather than using a deterministic approach, we have shown that using a stochastic approach, or Monte Carlo simulation, is more effective in understanding the impact on cost of goods when a specific process
parameter is optimized. The framework illustrated in chapter 2 not only enables us to identify a list of critical process parameters, but also enables us to understand what the impact is when some of these parameters are optimized.

In addition to control the cost of monoclonal antibody manufacturing, biopharmaceutical companies closely monitor and understand the trend of their manufacturing batches, to minimize potential batch failures. Chapter 3 illustrates the use of Bayesian approach, and also historical manufacturing data to predict the future trend of batch performance, such as process yield, and critical quality attributes.

The conventional method, or the method that’s typically practiced in the industry, is to take the average of the historical batches and assume the future batches have the same average performance. However, as shown in our case study, while the conventional method is simpler or more straightforward, in cases where there is inconsistency between the historical batches and the recent batches, the conventional approach might not be able to reflect the changes in the recent batches, especially when the size of the historical dataset is much larger.

To address this gap, we have introduced the Bayesian approach. In contrast to the conventional approach, the Bayesian approach calculates the posterior mean, or the average performance for the future batches, by using the historical batches to form a prior belief or trend, and updating it with the recent batch data. This way, we can capture both trends from historical batches and recent batches.
In our case study, when there is inconsistency between the historical and recent batches, the Bayesian method is better at predicting the mean of future batches compared to the conventional approaches. Conversely, when the manufacturing batches perform consistently, both approaches yield similar estimates, simply because there is no new information from the recent batches to update the historical trend.

Hence, to determine which approach we should use to estimate the mean of the future batch performance, we need to calculate the ratio of the means between the historical and recent batches. As the ratio deviates from unity, it suggests there is inconsistency between the batches, and in this case, Bayesian approach might be able to give a more accurate estimate.

Finally, in order to successfully release a manufacturing batch, the drug substance and drug product must pass the acceptance criteria for all critical quality attributes, to ensure safety and efficacy of the drug. Thus, it is important to set process intermediate specification, to ensure every step in the monoclonal antibody manufacturing process meet the specification, and ultimately increase the probability of the final drug substance and drug product meet the acceptance criteria.

To accurately set the in process specification, we first understand the capability of each step to remove impurities, such as aggregates, HCP, DNA and leached Protein A. Subsequently, process intermediate specification, or acceptance criteria, can then be set based on the process capability and the final drug substance specification. One of the most challenging parts is to have an accurate understanding of the process capability, and Chapter 4 illustrates the use of neural network to estimate process capability in monoclonal antibody downstream production process.
Based on the literature survey and conversations with industry experts, there are multiple approaches to estimate process capability. While some approaches are more complicated, such as conducting a series of experimentation to challenge the chromatography resin to understand the maximum impurity clearance capability, the most typical approach, or the conventional approach, used to find the average impurity reduction is by calculating the difference between the average feed and product impurity level. This approach assumes that impurity removal is constant at all time.

However, as shown in our analyses, impurity clearance sometimes depends on the feed impurity level. Especially in the case of aggregates, the aggregate removal is much higher when the feed aggregate level is higher, because the feedstream has mostly higher order aggregates and they are much easier to be removed. Using the conventional approach in this case would not be able to accurately estimate the impurity clearance level of a particular step.

Hence, we propose to use neural network to estimate process capability. Unlike the conventional approach, the neural network approach does not assume a constant impurity removal across all feed impurity level. Rather, it can identify and learn correlative patterns between feed and product data pairs. Once trained, it may be used to predict the product impurity level based on the feed impurity level, or vice versa. One of the most useful properties of neural networks is its ability to generalize. In our study, the neural network approach has been shown to be better at estimating process capability and ultimately setting intermediate process specification.
5.2 Future Work

The objective of this body of work has been the design and development of a systematic framework to optimize and control monoclonal antibody manufacturing process. The preceding chapters have demonstrated how the framework has successfully fulfilled this objective. The framework is clearly a powerful tool for evaluating process optimization strategies, predicting the trend of monoclonal antibody manufacturing performance and setting in process acceptance criteria, and can therefore act as a strong foundation from which future work can build; several examples are highlighted and discussed below.

In addition to evaluating the different parameters for further optimization in a mAb manufacturing process, the DoE-PCA and Monte Carlo simulation framework can be used to evaluate the different modes of chromatography, cell culture and filtration technologies in the production process. For example, during process development, there are multiple instances that we have to decide what modes of chromatography, such as mixed mode, cation exchange, hydrophobic interaction chromatography, to use in a process. In addition to consider process fit and flexibility, we need to evaluate how each mode impact the different attributes, such as overall throughput, product quality and cost. The DoE-PCA and Monte Carlo simulation framework can easily enable us to visualize the impact.

Furthermore, many biopharmaceutical companies have started to adopt new technologies, such as continuous cell-culture techniques [69, 70], single pass tangential flow filtration (SPTFF) [71], and continuous precipitation [72]. Again, the DoE-PCA and Monte Carlo simulation can be used
here to evaluate each of these technologies, compare it with the traditional manufacturing platform and facilitate decision making on whether or not to invest in a new technology.

In our analyses, we have illustrated how neural network was used to understand impurity clearance capability of each step, and therefore using such information to help setting process intermediate specification. In addition to the application in setting specification, the neural network approach can be used to evaluate the different emerging technologies mentioned above. Impurity clearance capability is often one of the important criteria during technology evaluation.

Finally, in our case study, we have used Bayesian approach to predict future manufacturing batch performance. The same approach can also be used to forecast the demand for a particular drug, as well as the supply, or the annual throughput. This information will be critical in accurate supply chain planning, and avoid disruption or shortage in drug supply.

In conclusion, the future work that has been outlined looks to increase the scope and application of the systematic framework not only in monoclonal antibody manufacturing, but also in technology evaluation and supply chain planning. Over the next decade, more and more new technologies will be developed to facilitate drug manufacturing, for better process fit, productivity, flexibility and improved process economics. Before making significant investments in these new technologies, it is very important for biopharmaceutical companies to conduct an in-depth risk and benefit analysis, compare the existing with the emerging technologies, and leverage appropriate decision – support framework to identify the best suited strategies.
6. References

15. Smith BT. Introduction to Diagnostic and Therapeutic Monoclonal Antibodies. University of New Mexico Health Sciences Center, Pharmacy Continuing Education. 2012.
22. Genentech, A Study of rhuMab VEGF (Bevacizumab) in Combination with Chemotherapy in Patients with Previously Treated Breast Cancer: rhuMAb VEGF-CSR AVF2119g Final, Clinical Study Report Synopsis, 2013.


### 7. Appendix

7.1 Chapter 2 Appendix

7.1.1 Tables

**Table A7.1.1.1 Typical Parameters and Setpoints in Upstream Steps**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Setpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Culture (Upstream)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N-2 Bioreactor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>L</td>
<td>200</td>
</tr>
<tr>
<td>Medium Fill Volume</td>
<td>L</td>
<td>175</td>
</tr>
<tr>
<td>Medium Transfer in Rate</td>
<td>L/h</td>
<td>117</td>
</tr>
<tr>
<td>Temp</td>
<td>°C</td>
<td>37</td>
</tr>
<tr>
<td><strong>N-1 Bioreactor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>L</td>
<td>1300</td>
</tr>
<tr>
<td>Medium Transfer in Rate</td>
<td>L/h</td>
<td>708</td>
</tr>
<tr>
<td>Temp</td>
<td>°C</td>
<td>37</td>
</tr>
<tr>
<td><strong>Production Bioreactor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>L</td>
<td>20000</td>
</tr>
<tr>
<td>Feed Amount</td>
<td>L</td>
<td>1200</td>
</tr>
<tr>
<td>Feed Flow Rate</td>
<td>L/h</td>
<td>3.6</td>
</tr>
<tr>
<td>Temp</td>
<td>°C</td>
<td>37</td>
</tr>
<tr>
<td>Reaction Time</td>
<td>day</td>
<td>14</td>
</tr>
<tr>
<td>Target Concentration</td>
<td>g/L</td>
<td>depends on experimental condition</td>
</tr>
<tr>
<td>Monoclonal Antibody Cost</td>
<td>$/mg</td>
<td>3</td>
</tr>
<tr>
<td><strong>Primary Recovery (Upstream)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Centrifugation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biomass Removal</td>
<td>%</td>
<td>98</td>
</tr>
<tr>
<td>Sedimentation Efficiency</td>
<td>%</td>
<td>30</td>
</tr>
<tr>
<td>Volumetric Throughput per Unit</td>
<td>L/h</td>
<td>1800</td>
</tr>
<tr>
<td>Number of Cycle per Batch</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Depth Filtration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flux</td>
<td>L/m²·min</td>
<td>4.2</td>
</tr>
<tr>
<td>Biomass Removal</td>
<td>%</td>
<td>100</td>
</tr>
<tr>
<td>Filter Pore Size</td>
<td>μm</td>
<td>0.2</td>
</tr>
<tr>
<td>Filter Area</td>
<td>m²</td>
<td>10</td>
</tr>
<tr>
<td>Unit Cost</td>
<td>$</td>
<td>1000</td>
</tr>
<tr>
<td>Reuse</td>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>
Table A7.1.1.2 Typical Parameters and Setpoints in Downstream Steps

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Setpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purification (Downstream)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column Volume</td>
<td>L</td>
<td>143</td>
</tr>
<tr>
<td>Resin Cost</td>
<td>$/L</td>
<td>14000</td>
</tr>
<tr>
<td>Resin Replacement Frequency</td>
<td>Cycles</td>
<td>65</td>
</tr>
<tr>
<td>Resin Binding Capacity</td>
<td>g/L</td>
<td>28.6</td>
</tr>
<tr>
<td>Eluant Volume in Product Stream</td>
<td>Bed Volume</td>
<td>2</td>
</tr>
<tr>
<td>Elution Linear Velocity</td>
<td>cm/min</td>
<td>5</td>
</tr>
<tr>
<td>Number of Cycles per Batch</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td><strong>Virus Inactivation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pH Hold Time</td>
<td>min</td>
<td>60</td>
</tr>
<tr>
<td>Filter Flux</td>
<td>L/m²·min</td>
<td>6.8</td>
</tr>
<tr>
<td>Filter Area</td>
<td>m²</td>
<td>10</td>
</tr>
<tr>
<td><strong>IEX I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column Volume</td>
<td>L</td>
<td>76</td>
</tr>
<tr>
<td>Resin Binding Capacity</td>
<td>g/L</td>
<td>199</td>
</tr>
<tr>
<td>Resin Cost</td>
<td>$/L</td>
<td>2445</td>
</tr>
<tr>
<td>Replacement Frequency</td>
<td>Cycles</td>
<td>40</td>
</tr>
<tr>
<td>Number of Cycles per Batch</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>IEX II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column Volume</td>
<td>L</td>
<td>105</td>
</tr>
<tr>
<td>Resin Binding Capacity</td>
<td>g/L</td>
<td>31.7</td>
</tr>
<tr>
<td>Eluant Volume in Product Stream</td>
<td>Bed Volume</td>
<td>5</td>
</tr>
<tr>
<td>Resin Cost</td>
<td>$/L</td>
<td>2290</td>
</tr>
<tr>
<td>Replacement Frequency</td>
<td>Cycles</td>
<td>40</td>
</tr>
<tr>
<td>Number of Cycles per Batch</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td><strong>Viral Filtration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filter Cost Per Unit</td>
<td>$</td>
<td>1000</td>
</tr>
<tr>
<td>Filter Flux</td>
<td>L/m²·min</td>
<td>0.7</td>
</tr>
<tr>
<td>Filter Area</td>
<td>m²</td>
<td>10</td>
</tr>
<tr>
<td><strong>UFDF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane area</td>
<td>m²</td>
<td>2.95</td>
</tr>
<tr>
<td>Replacement Frequency</td>
<td>Cycles</td>
<td>30</td>
</tr>
<tr>
<td>Number of Diavolume</td>
<td>Diavolume</td>
<td>6</td>
</tr>
<tr>
<td>Concentration Factor</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Filtrate Flux</td>
<td>L/m²·h</td>
<td>35</td>
</tr>
</tbody>
</table>
7.1 Chapter 4 Appendix

Table A7.3.1.1 SSE of Model using Quadratic Regression and Neural Network Approaches

<table>
<thead>
<tr>
<th>Process</th>
<th>SSE</th>
<th>Neural Network</th>
<th>Quadratic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.37</td>
<td>8103.14</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>52.22</td>
<td>53.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>54.56</td>
<td>66.67</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14.87</td>
<td>21.25</td>
<td></td>
</tr>
</tbody>
</table>
7.1.2 Figures

Figure A7.1.2.1 Overview of key parameters in SuperPro

**Input**
- Equipment
- Raw Materials
- Unit Operation
- Yield
- Process condition (flowrate, number of cycles)
- Cost (RM, Product selling price, facility, labor etc)

**Process**

**Output**
- Cycle time
- Bottleneck /Gantt Chart
- Cost analysis
- Throughput
- Sensitivity analysis
- Technology evaluation (disposable, continuous, membrane vs column)
- Waste generation
Figure A7.1.2.2 Material Cost Break Down by Unit Operation
Figure A7.1.2.3 Consumable Cost Break Down by Unit Operation
Figure A7.1.2.4. Annual Operating Cost Break Down by Unit Operation
Figure A7.1.2.5. Overview of Central Composite DoE Design
### Table 7.2.1.1 Host Cell Protein (HCP) level at Different Manufacturing Batches

<table>
<thead>
<tr>
<th>ID</th>
<th>HCP Level (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Batch 1</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>48.015</td>
</tr>
<tr>
<td>2</td>
<td>44.352</td>
</tr>
<tr>
<td>3</td>
<td>50.589</td>
</tr>
<tr>
<td>4</td>
<td>54.054</td>
</tr>
<tr>
<td>5</td>
<td>55.341</td>
</tr>
<tr>
<td>6</td>
<td>60.984</td>
</tr>
<tr>
<td><strong>Batch 2</strong></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>174.24</td>
</tr>
<tr>
<td>8</td>
<td>189.09</td>
</tr>
<tr>
<td>9</td>
<td>205.92</td>
</tr>
<tr>
<td>10</td>
<td>168.3</td>
</tr>
<tr>
<td>11</td>
<td>174.24</td>
</tr>
<tr>
<td>12</td>
<td>172.26</td>
</tr>
<tr>
<td><strong>Batch 3</strong></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>51.48</td>
</tr>
<tr>
<td>14</td>
<td>45.045</td>
</tr>
<tr>
<td>15</td>
<td>54.945</td>
</tr>
<tr>
<td>16</td>
<td>56.925</td>
</tr>
<tr>
<td>17</td>
<td>62.469</td>
</tr>
<tr>
<td>18</td>
<td>65.34</td>
</tr>
<tr>
<td><strong>Batch 4</strong></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>38.61</td>
</tr>
<tr>
<td>20</td>
<td>46.827</td>
</tr>
<tr>
<td>21</td>
<td>63.954</td>
</tr>
<tr>
<td>22</td>
<td>72.567</td>
</tr>
<tr>
<td>23</td>
<td>53.658</td>
</tr>
<tr>
<td>24</td>
<td>39.105</td>
</tr>
<tr>
<td><strong>Batch 5</strong></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>118.8</td>
</tr>
<tr>
<td>26</td>
<td>116.82</td>
</tr>
<tr>
<td>27</td>
<td>114.84</td>
</tr>
<tr>
<td>28</td>
<td>146.52</td>
</tr>
<tr>
<td>29</td>
<td>145.53</td>
</tr>
<tr>
<td>30</td>
<td>133.65</td>
</tr>
<tr>
<td><strong>Batch 6</strong></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>102.96</td>
</tr>
<tr>
<td>32</td>
<td>94.446</td>
</tr>
<tr>
<td>33</td>
<td>109.89</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>34</td>
<td>124.74</td>
</tr>
<tr>
<td>35</td>
<td>124.74</td>
</tr>
<tr>
<td>36</td>
<td>138.6</td>
</tr>
<tr>
<td><strong>Batch 7</strong></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>104.94</td>
</tr>
<tr>
<td>38</td>
<td>90.882</td>
</tr>
<tr>
<td>39</td>
<td>97.218</td>
</tr>
<tr>
<td>40</td>
<td>100.98</td>
</tr>
<tr>
<td>41</td>
<td>93.852</td>
</tr>
<tr>
<td>42</td>
<td>106.92</td>
</tr>
</tbody>
</table>

Note: all the data were rescaled for confidentiality purposes
Table 7.2.1.2 Summary of Host Cell Protein (HCP) level at Different Manufacturing Batches

<table>
<thead>
<tr>
<th>Batch #</th>
<th>mean</th>
<th>std</th>
<th>std of mean</th>
<th># runs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>52.225</td>
<td>5.86787</td>
<td>2.395547714</td>
<td>6</td>
</tr>
<tr>
<td>Batch 2</td>
<td>180.675</td>
<td>14.24706</td>
<td>5.816337765</td>
<td>6</td>
</tr>
<tr>
<td>Batch 3</td>
<td>56.034</td>
<td>7.368686</td>
<td>3.008253646</td>
<td>6</td>
</tr>
<tr>
<td>Batch 4</td>
<td>52.4535</td>
<td>13.70947</td>
<td>5.596867128</td>
<td>6</td>
</tr>
<tr>
<td>Batch 5</td>
<td>129.36</td>
<td>14.5185</td>
<td>5.927152773</td>
<td>6</td>
</tr>
<tr>
<td>Batch 6</td>
<td>115.896</td>
<td>16.3427</td>
<td>6.671878596</td>
<td>6</td>
</tr>
<tr>
<td>Batch 7</td>
<td>99.132</td>
<td>6.283394</td>
<td>2.565184906</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 7.2.1.3 Protein A Yield level at Different Manufacturing Batches

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch 1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>80.75</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>83.6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>84.55</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>97.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>78.85</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>82.65</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>92.15</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>85.5</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>82.65</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>87.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td></td>
<td>86.45</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>95.95</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>91.2</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>92.15</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>93.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch 4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td></td>
<td>81.7</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>87.4</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>69.35</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>82.65</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>82.65</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>86.45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch 5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td></td>
<td>78.85</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>102.6</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>80.75</td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>84.55</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>85.5</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>84.55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch 6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td></td>
<td>83.6</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>83.6</td>
</tr>
<tr>
<td>31</td>
<td></td>
<td>84.55</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>83.6</td>
</tr>
<tr>
<td>33</td>
<td></td>
<td>85.5</td>
</tr>
<tr>
<td>34</td>
<td></td>
<td>90.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID</th>
<th>Batch 7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td></td>
<td>66.5</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>69.35</td>
</tr>
<tr>
<td>37</td>
<td></td>
<td>69.35</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td>66.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>73.15</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>71.25</td>
<td></td>
</tr>
<tr>
<td>Batch 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>73.15</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>75.05</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>74.1</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>78.85</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>78.85</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>83.6</td>
<td></td>
</tr>
<tr>
<td>Batch 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>74.1</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>79.8</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>79.8</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>78.85</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>78.85</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>88.35</td>
<td></td>
</tr>
</tbody>
</table>

Note: all the data were rescaled for confidentiality purposes
Table 7.2.1.4 Summary of Protein A level at Different Manufacturing Batches

<table>
<thead>
<tr>
<th>Batch #</th>
<th>mean</th>
<th>std</th>
<th>std of mean</th>
<th># runs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>86.6875</td>
<td>7.614829</td>
<td>3.807414641</td>
<td>4</td>
</tr>
<tr>
<td>Batch 2</td>
<td>84.8667</td>
<td>4.602029</td>
<td>1.878770283</td>
<td>6</td>
</tr>
<tr>
<td>Batch 3</td>
<td>92.3083</td>
<td>3.367702</td>
<td>1.374858579</td>
<td>6</td>
</tr>
<tr>
<td>Batch 4</td>
<td>81.7</td>
<td>6.471167</td>
<td>2.641842791</td>
<td>6</td>
</tr>
<tr>
<td>Batch 5</td>
<td>86.1333</td>
<td>8.468687</td>
<td>3.457327163</td>
<td>6</td>
</tr>
<tr>
<td>Batch 6</td>
<td>85.1833</td>
<td>2.595894</td>
<td>1.059769367</td>
<td>6</td>
</tr>
<tr>
<td>Batch 7</td>
<td>69.35</td>
<td>2.618969</td>
<td>1.069189724</td>
<td>6</td>
</tr>
<tr>
<td>Batch 8</td>
<td>77.2667</td>
<td>3.924623</td>
<td>1.602220681</td>
<td>6</td>
</tr>
<tr>
<td>Batch 9</td>
<td>79.9583</td>
<td>4.631351</td>
<td>1.89074133</td>
<td>6</td>
</tr>
</tbody>
</table>
7.3 Chapter 4 Appendix

7.3.1 Figures

Figure 7.3.1.1. Process 1 Algorithm Search – BFG (10 nodes) Error Histogram

![Error Histogram with 20 Bins](image-url)
Figure 7.3.1.2. Process 1 Algorithm Search – BFG (10 nodes) Regression Analysis
Figure 7.3.1.3. Process 1 Algorithm Search – LM (10 nodes) Error Histogram
Figure 7.3.1.4. Process 1 Algorithm Search – LM (10 nodes) Regression Analysis
Figure 7.3.1.5. Process 1 Algorithm Search – SCG (10 nodes) Error Histogram
Figure 7.3.1.6. Process 1 Algorithm Search – SCG (10 nodes) Regression Analysis

Training: $R=0.85733$

Validation: $R=0.91541$

Test: $R=0.89777$

All: $R=0.87311$
Figure 7.3.1.7. Process 1 Node Optimization – LM (8 nodes) Error Histogram
Figure 7.3.1.8. Process 1 Node Optimization – LM (12 nodes) Error Histogram
Figure 7.3.1.9. Process 1 Node Optimization – LM (14 nodes) Error Histogram

Error Histogram with 20 Bins

Errors = Targets - Outputs

Instances

-0.7114 -0.6365 -0.5605 -0.4851 -0.4096 -0.3342 -0.2587 -0.1833 -0.1078 -0.03239 -0.04306 -0.1185 -0.194 -0.2694 -0.3449 -0.4203 -0.4958 -0.5712 -0.6467 -0.7221
Figure 7.3.1.10. Process 1 Node Optimization – LM (16 nodes) Error Histogram

Error Histogram with 20 Bins

Instances

Errors = Targets - Outputs

-0.7459 -0.6707 -0.5955 -0.5202 -0.445 -0.3697 -0.2945 -0.2192 -0.144 -0.06872 -0.006523 0.08177 0.157 0.2323 0.3075 0.3828 0.458 0.5333 0.6085 0.6837

Training
Validation
Test
Zero Error
Figure 7.3.1.11. Process 1 Node Optimization – LM (18 nodes) Error Histogram
Figure 7.3.1.12. Process 1 Node Optimization – LM (20 nodes) Error Histogram
Figure 7.3.1.13. Process 1 Node Optimization – LM (22 nodes) Error Histogram
Figure 7.3.1.14. Process 1 Node Optimization – LM (8 nodes) Regression Analysis
Figure 7.3.1.15. Process 1 Node Optimization – LM (12 nodes) Regression Analysis
Figure 7.3.1.15. Process 1 Node Optimization – LM (14 nodes) Regression Analysis
Figure 7.3.1.15. Process 1 Node Optimization – LM (16 nodes) Regression Analysis
Figure 7.3.1.16. Process 1 Node Optimization – LM (18 nodes) Regression Analysis
Figure 7.3.1.17. Process 1 Node Optimization – LM (20 nodes) Regression Analysis

Training: R=0.88787

Validation: R=0.88858

Test: R=0.85237

All: R=0.88304
Figure 7.3.18. Process 1 Node Optimization – LM (22 nodes) Regression Analysis
Figure 7.3.1.19. Process 2 Algorithm Search – BFG (10 nodes) Error Histogram
Figure 7.3.1.20. Process 2 Algorithm Search – BFG (10 nodes) Regression Analysis
Figure 7.3.1.21. Process 2 Algorithm Search – LM (10 nodes) Error Histogram
Figure 7.3.122. Process 2 Algorithm Search – LM (10 nodes) Regression Analysis

Training: $R=0.90076$

Validation: $R=0.87982$

Test: $R=0.8863$

All: $R=0.89493$
Figure 7.3.1.23. Process 2 Algorithm Search – SCG (10 nodes) Error Histogram
Figure 7.3.1.24. Process 2 Algorithm Search – SCG (10 nodes) Regression Analysis

- **Training**: $R = 0.89688$
  - Output $\sim 0.81 \times \text{Target} + 0.31$

- **Validation**: $R = 0.88022$
  - Output $\sim 0.76 \times \text{Target} + 0.41$

- **Test**: $R = 0.88765$
  - Output $\sim 0.84 \times \text{Target} + 0.24$

- **All**: $R = 0.89381$
  - Output $\sim 0.8 \times \text{Target} + 0.32$
Figure 7.3.1.25. Process 2 Node Optimization – LM (8 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.26. Process 2 Node Optimization – LM (12 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

-0.7049  -0.6211  -0.5373  -0.4535  -0.3697  -0.2859  -0.2021  -0.1183  -0.03456  0.04923  0.133  0.2168  0.3005  0.3844  0.4682  0.552  0.6358  0.7196  0.8034  0.871

Errors = Targets - Outputs

Instances

-0  20  40  60  80  100  120  140  160

Training
Validation
Test
Zero Error
Figure 7.3.1.26. Process 2 Node Optimization – LM (14 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.27. Process 2 Node Optimization – LM (16 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.28. Process 2 Node Optimization – LM (18 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.29. Process 2 Node Optimization – LM (20 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.30. Process 2 Node Optimization – LM (22 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.31. Process 3 Algorithm Search – BFG (10 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

- Training
- Validation
- Test
- Zero Error

Errors = Targets - Outputs
Figure 7.3.1.32. Process 3 Algorithm Search – LM (10 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

Instances

Errors = Targets - Outputs

Training
Validation
Test
Zero Error
Figure 7.3.1.33. Process 3 Algorithm Search – SCG (10 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.34. Process 3 Node Optimization – LM (8 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

Instances

Errors = Targets - Outputs

-0.7641 -0.644 -0.5239 -0.4039 -0.2838 -0.1637 -0.04362 0.07646 0.1965 0.3166 0.4367 0.5568 0.6769 0.7969 0.917 1.037 1.157 1.277 1.397 1.517
Figure 7.3.1.34. Process 3 Node Optimization – LM (12 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

Instances

Errors = Targets - Outputs

-0.7287 -0.6883 -0.4887 -0.2473 -0.1271 -0.00665 0.1137 0.2344 0.3544 0.4747 0.5958 0.7154 0.8357 0.9561 1.0761 1.1971 1.3171 1.4371 1.5581

Training
Validation
Test
Zero Error
Figure 7.3.1.35. Process 3 Node Optimization – LM (14 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

Errors = Targets - Outputs
Figure 7.3.1.36. Process 3 Node Optimization – LM (16 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.37. Process 3 Node Optimization – LM (18 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.38. Process 3 Node Optimization – LM (20 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.39. Process 3 Node Optimization – LM (22 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.40. Process 4 Algorithm Search – BFG (10 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.41. Process 4 Algorithm Search – LM (10 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.41. Process 4 Algorithm Search – SCG (10 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.42. Process 4 Node Optimization – LM (8 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.43. Process 4 Node Optimization – LM (12 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.44. Process 4 Node Optimization – LM (14 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

Errors = Targets - Outputs

Instances

-0.6077 -0.5535 -0.4992 -0.445 -0.3908 -0.3366 -0.2824 -0.2281 -0.1739 -0.1197 -0.06547 -0.01125 0.004297 0.09719 0.1514 0.2056 0.2599 0.3141 0.3683 0.4225
Figure 7.3.145. Process 4 Node Optimization – LM (16 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.46. Process 4 Node Optimization – LM (18 nodes) Error Histogram and Regression Analysis
Figure 7.3.1.47. Process 4 Node Optimization – LM (20 nodes) Error Histogram and Regression Analysis
Training: $R=0.98746$

Validation: $R=0.98578$

Test: $R=0.98688$

All: $R=0.98696$
Figure 7.3.1.47. Process 4 Node Optimization – LM (22 nodes) Error Histogram and Regression Analysis

Error Histogram with 20 Bins

Errors = Targets - Outputs
7.4 Papers by the Author

The following papers are included in this appendix are listed below:

