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Developing integrated downstream processes for next-generation biologics

ANTON JOHANNES LÖFGREN | CHEMICAL ENGINEERING | LUND UNIVERSITY

Developing integrated downstream processes for next-generation biologics

Anton Johannes Löfgren

Department of Chemical Engineering
Lund University, Sweden
2020

ACADEMIC THESIS

which, by due permission of the Faculty of Engineering of Lund University, will be publicly defended on the 4th of December 2020 at 9:15 in lecture hall KC:G at the Center for Chemistry and Chemical Engineering, Naturvetarvägen 14, Lund, for the degree of Doctor of Philosophy in Engineering.

The faculty opponent is Professor Anurag Rathore from the Department of Chemical Engineering at IIT Delhi, New Delhi, India.

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Developing integrated downstream processes for next-generation biologics

Abstract

Manufacturing of biopharmaceuticals is a costly and time consuming process due to its inherent complexity and variability. The downstream process is the most expensive part and it involves multiple non-trivial steps. Continuous integrated manufacturing is a new concept that is encouraged by authorities. The advantages include uninterrupted operation, automation, consistent product quality, lower residence times and decreased capital and operational costs.

Novel techniques and concepts require a holistic process understanding as well as a technical competence to implement them, and to do that, there are multiple practical challenges that must be overcome. There is a growing need to rapidly adjust to market demands and also to decrease costs as the industry gets increasingly competitive. The ability to adjust a facility or production line, to manufacture a variety of biopharmaceuticals, must be taken into consideration when developing integrated systems. It is also important to be aware of how to monitor critical quality attributes to properly ensure product quality. Another important attribute of integrated systems is intuitive and how easy to use they are.

The aim of the thesis is to demonstrate, based on a selection of case studies, how some of the presented challenges can be overcome. The case studies have integrated systems that are flexible and have advanced process analytical technology. One of the papers in this thesis also focuses on the optimization of coupled columns sequences with the purpose of reducing costs. Overall, the results of this thesis shows the potential of continuous integrated designs for purification of biopharmaceuticals while simultaneously increasing both performance and product quality.

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I am not an optimist.

I'm a very serious possibilist.

– Hans Rosling

Abstract

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Populärvetenskaplig Sammanfattning

Att ta ett läkemedel till marknaden är extremt tidskrävande och kostsamt. Processen medför stora kostnader för att hantera patent, arkivering till myndigheter samt de kliniska prövningar som måste gås igenom för att bevisa att läkemedlet har en effekt och är säkert att använda. Men det finns både tid och pengar att spara på en effektivare tillverkningsmetod, där det dessutom finns potential för avancerad styrning och autonomt beslutsfattande.

Biofarmaceutika är biologiska läkemedel som odlas i mikroorganismer, såsom jäst eller bakterier. När ett biologiskt läkemedel har odlats i en mikroorganism kan du inte direkt injicera det i en patient utan det måste först renas från små mikroorganismer och deras komponenter. Denna process, att ta bort allt utom din mycket specifika läkemedelsprodukt, är oerhört dyr och kräver mycket manuellt arbete på grund av de många olika uppreningsmetoderna som krävs.

Denna avhandling handlar om ny teknik som kan användas för att automatisera stora delar av reningsprocessen vilket tillåter att experiment kan köras dygnet runt så att personal kan använda sin tid mer effektivt. Det öppnar också möjligheter för smartare beslutsfattande under produktionen. Forskare kan till exempel snabbt reagera på resultaten av sina experiment och göra förändringar i ett tidigt skede. Den nya tekniken är också byggd för att vara flexibel, vilket innebär att olika reningsmetoder enkelt kan kombineras för att producera en mängd olika biologiska läkemedel. Detta är särskilt användbart för laboratorier som producerar och testar olika biofarmaceutika, där en kombination av olika metoder krävs för varje produkt, vilket är vanligt i tidiga skeden av produktutveckling.

En typisk minifabrik, där tillverkningsprocesserna har skalats ner och körs autonomt, består av pumpar som driver läkemedlet genom en sekvens av reningsmetoder. Antalet enhetsoperationer som kan användas är i teorin obegränsat med detta tillvägagångssättet. Styrningen av all utrustning är en av de komplicerade delarna och av denna anledning utvecklas en helt ny programvara som vi kallar Orbit. Programmet möjliggör även avancerat beslutsfattande över hela tillverkningen.

Delar av denna teknik har redan använts i vissa laboratorier med goda resultat. Utvecklingen av smarta minifabriker kommer att förändra industrin så att kostnaderna minskar, samt effektivisera forskningen så att framtida medicin når oss snabbare.

Acknowledgements

Throughout this PhD journey, there are many who have given a great deal of support and assistance these last years. First and foremost, my fantastic supervisors, Bernt Nilsson and Niklas Andersson, who are always available with genuine enthusiasm. In regards to general insightfulness, work enjoyability and expertise, the bar you set is incredibly high. To Bernt, I want to specifically thank you for all the encouragement and appreciation I have received from you these years. And to Niklas for always being able to solve all problems I come to you with, then humbly (and incorrectly) framing it as it was thanks to me that you solved it.

I am grateful to Peter Tiainen at Novo Nordisk for being a visionary and for showing me how to see solutions instead of impossibilities. Everyone at Sobi are also gratefully acknowledged for being invaluable in their support, both technical and encouraging. A special thanks to Lotta Berghard for being a fantastic collaborator and organizing everything up in Stockholm, making it possible for us to publish.

I cannot begin to express my thanks to everyone at the Department of Chemical Engineering for making every day at work enjoyable and fun. All the fikas, with the fruitful, and also not so fruitful, discussions have been a constant source of energy. There are so many of you that have cared for me and for that I am extremely grateful. Particularly Michael Cimbritz who has given much appreciated advice in multiple disciplines, Maria Messer and Gity Yahoo for doing your magic and keeping me sane, Gertrud Persson for always listening and providing unwavering support, Leif Stanley for your relentless patience and ability to fix anything, Åsa Davidsson for your helpfulness and guidance in all matters, Herje Schagerlöf for your excellent chemical and chromatography knowledge, and Paula Leckius for your perfectionist attitude towards making this thesis look as good as possible.

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multifaceted expertise in all projects and endeavours that we have shared. Madelène Isaksson, for being a source of wisdom and for the excellent proof-reading. Daniel Espinoza, for being an awesome colleague the short time we have shared together.

In addition, I would like to thank former close colleagues and master thesis students that have made this time more than enjoyable, including Anders Bojsen, Hans-Kristian Knutson, Karolina Arkell, Marianne Olofsson, Oliver Persson, Johan Olsson, Simon Svensson, Hanna Danielsson, Viktor Geraldsson and Linnéa Petersson, as well as Daniel Lundell who provided valuable input for organizing Chapter 6.

Finally, I would like to offer my most heartfelt thanks to my friends and family. This thesis would not see the light of day were it not for you. This applies especially to Lovisa, who provides me with endless encouragement and love.

October 2020, Lund
Anton Löfgren

Contents

Preface

Contents and Contributions of the Thesis

This thesis consists of seven introductory chapters and six papers. This section describes the five chapters, the contributions of each paper and the contributions made by the author, which will be referred to in the text by their roman numerals. It also gives a brief description of the appendices and provides a list of additional publications by the author of this thesis. The papers are appended in the end of the thesis.

Chapter 1 — Introduction

The first chapter describes the aim and purpose of the thesis. A brief history of biopharmaceuticals is presented. The research performed in this thesis is also put in a philosophical perspective.

Chapter 2 — Biopharmaceutical production

Biopharmaceutical production techniques are introduced in this chapter with a focus on chromatography and its principles.

Chapter 3 — Process development

This chapter focuses on the monitoring and risk minimization of biopharmaceutical production techniques. This is combined with perspectives on reducing costs through platform approaches and an analysis of where production costs are in the process.

Chapter 4 — Integrated design

The design of integrated systems, used in most of the papers in this thesis, is explored in this chapter. The concept of periodic counter-current chromatography (PCC) is also introduced here.

Chapter 5 — Optimization

Optimization of directly connected column sequences are presented in this chapter. It also features some aspects of PCC optimization and a multi-flowrate loading approach of a capture column.

Chapter 6 — Supervisory control

In this chapter, the software that has been used for control of all case studies in this thesis is introduced. Some more advanced techniques, such as iterative learning control for the loading of a PCC, is also explored.

Chapter 7 — Concluding Remarks

This chapter concludes the thesis with a brief summary of the results and some suggestions for future work.

Appendix A — Peak detection

A peak detection algorithm based on derivate analysis.

Paper I — Design and control of integrated chromatography column sequences

Andersson, N., Löfgren, A., Olofsson, M., Sellberg, A., Nilsson, B., and Tiainen, P. (2017). Design and control of integrated chromatography column sequences. *Biotechnology progress*, 33(4):923–930

The first published paper explaining the concept of an early version of *Orbit*, as well as the methodology of the integration. A case study, with the three common proteins, ribonuclease A, cytochrome C and lysozyme, was performed. Four chromatography steps were used in this purification: capture, buffer exchange, ion exchange and finally a buffer exchange.

I developed part of the software used in the experiment as well as took part in planning and execution.

Paper II — Designing an Autonomous Integrated Downstream Sequence From a Batch Separation Process, An Industrial Case Study

Löfgren, A., Andersson, N., Sellberg, A., Nilsson, B., Löfgren, M., and Wood, S. (2018). Designing an autonomous integrated downstream sequence from a batch separation process- an industrial case study. *Biotechnology journal*, 13(4):1700691

The second published paper with *Orbit*. Batch protocols from an industrial application were used for the design of the integrated sequence, purifying a recombinant protein. The integraed sequence consisted of an anion exchange step, a virus inactivation reactor followed by a hydrophobic interaction step.

I performed the experiments, analysed the results and wrote the article.

Paper III — Integration of a complete downstream process for the automated lab-scale production of a recombinant protein

Gomis-Fons, J., Löfgren, A., Andersson, N., Nilsson, B., Berghard, L., and Wood, S. (2019). Integration of a complete downstream process for the automated lab-scale production of a recombinant protein. *Journal of biotechnology*, 301:45–51

Another publication with *Orbit*, where a recombinant protein was purified using an integrated sequence of three bind-and-elute chromatography columns and a flow-through membrane chromatography step. An ultrafiltration-diafiltration step for final up-concentration and formulation was also implemented on the same setup.

I performed the first batch and integration experiments and assisted in writing the article.

Paper IV — Optimization of Integrated Chromatography Sequences for Purification of Biopharmaceuticals

Löfgren, A., Yamanee-Nolin, M., Tallvod, S., Fons, J. G., Andersson, N., and Nilsson, B. (2019). Optimization of integrated chromatography sequences for purification of biopharmaceuticals. *Biotechnology progress*

This paper introduces a new method for non-linear optimizations of integrated column sequences. The method takes into account column sizes, flow rates and scheduling aspects. It is demonstrated with two case studies in the paper.

I developed the new method, performed the optimization case studies and wrote the article.

Paper V — Multi-flowrate Optimization of the Loading Phase of a Preparative Chromatographic Separation

Sellberg, A., Nolin, M., Löfgren, A., Andersson, N., and Nilsson, B. (2018). Multi-flowrate optimization of the loading phase of a preparative chromatographic separation. In *Computer Aided Chemical Engineering*, volume 43, pages 1619–1624. Elsevier

This paper presents a study of the optimal loading of a capture step using a time-variant flow rate. The varying flow rate was found through modeling of breakthrough curves and optimization with an objective function that consisted of a weighted sum of three key process indicators: degree of utilization, production rate and process yield.

I contributed towards planning of the paper, the experiment, as well as writing of the paper.

Paper VI — An integrated continuous downstream process with real-time control: A case study with periodic countercurrent chromatography and continuous virus inactivation

Löfgren, A., Gomis-Fons, J., Andersson, N., Nilsson, B., Berghard, L., and Lagerquist Hägglund, C. (submitted september 2020). An integrated continuous downstream process with real-time control: A case study with periodic countercurrent chromatography and continuous virus inactivation

This paper is an intensification of paper II, with a continuous solvent/detergent virus inactivation before the process, a PCC system for continuous capture and the polishing step on another machine that handles the pools from the PCC. The setup is also equipped with both an adaptive pooling algorithm and an iterative learning control scheme so that

the PCC cycle can adapt to concentration changes in the feed.

I planned the study, performed the experiments, analyzed the results and co-wrote the article.

Related Oral Presentations (Lead Author/Presenter)

Below is a list of conference presentations by the author related to the work described in the above papers.

Integrated Continuous Biomanufacturing III, Cascais, Portugal (2017) — Conversion of an industrial batch separation process to an autonomous integrated downstream process A case study, Anton Löfgren, Lund University, Sweden

Other related publications

Single-shooting optimization of an industrial process through co-simulation of a modularized Aspen Plus Dynamics model

Yamanee-Nolin, M., Löfgren, A., Andersson, N., Nilsson, B., Max-Hansen, M., and Pajalic, O. (2019). Single-shooting optimization of an industrial process through co-simulation of a modularized aspen plus dynamics model. In *Computer Aided Chemical Engineering*, volume 46, pages 721–726. Elsevier

1

Introduction

The production of biopharmaceuticals is an exciting field. There are, however, pressing issues for the industry which need to be addressed. Research and development for new pharmaceuticals is getting increasingly expensive whilst many active patents are about to expire which will open up the market. In summary, there is a need for more effective production systems that are also flexible enough for research and development purposes.

This opening chapter introduces the concept of biopharmaceuticals, a perspective of what research in applied sciences is and concludes with the aims and scope of the thesis.

1.1 Biopharmaceuticals

A new DNA species was first created in 1973 with the purpose of insertion into bacterial cells. The new DNA replicates itself inside the bacteria, producing proteins that could serve as pharmaceuticals (Cohen et al., 1973). This laid the foundation for the biotech industry as it is today. The first biopharmaceutical introduced to the market was human insulin in 1982, produced by Eli Lilly, licensed from Genentech. Novo (today Novo Nordisk) launched their human insulin product in 1987 that instead was produced in yeast, the common *Saccharomyces cerevisiae*, replacing their porcine platform (Nielsen, 2013). The market has grown rapidly since then. In 2018, there were 316 active licenses of biopharmaceutical products, and of the 6000 products that were in clinical development globally, around 40 % of were them are biopharmaceuticals (Walsh, 2018).

Biopharmaceuticals, or therapeutic proteins, are used in practically all medical branches and have become one of the most effective treatment modalities (Kesik-Brodacka, 2018). They are very different from synthetic or chemical-based drugs. Synthetic drugs are generally small molecules composed of a few atoms, manufactured through standardized chemical reactions. Biopharmaceuticals, on the other hand, are much larger molecules comprised of typically 100-1000 times more atoms. They are also produced in living organisms with inherently complex reactions. Due to their source and complexity, there is often an element of variability involved in the manufacturing of biopharmaceuticals (Rader, 2008). This is one of the reasons

why biopharmaceuticals are not classified by their composition, but instead by their manufacturing process (Dimitrov, 2012; Carter, 2011).

Included in the concept biopharmaceuticals are recombinant proteins, including antibodies, nucleic acids and genetically engineered cell-based products (Walsh, 2018). However, monoclonal antibodies are by far the largest class of biopharmaceutical. They are proteins used by the immune system for specific purposes, often to neutralize pathogens. Between 2015 and 2018, 53% of all first time approvals were antibodies (Walsh, 2018). Some of the reasons for the growing popularity of antibodies are their high specificity, they are generally well tolerated, which means that the risks are lower, and there is an efficient production platform that requires little modification (Ecker et al., 2015).

Biosimilars are therapeutic proteins that are similar to older approved products which have lost their patent protection. They can change the market drivers, from speed to market, to quality and efficiency in production (Dimitrov, 2012). Biosimilars will continue to offer new market opportunities as more and more patents will expire, which will open up for increasingly competitive production facilities and techniques.

1.2 Applied science

In this section an attempt is made at putting this thesis in a greater perspective. Historically, philosophers have focused on sciences that try to describe the world, which can be called basic research. In a paper called *The aim and structure of applied research* (Niiniluoto, 1993), the author tries to define different types of research and more specifically outline what applied sciences is, which historically have been neglected by philosophers.

Different research areas, ranging from basic to technological development, can be placed on a spectrum: Basic research - predictive science - design science - technology development, where the former are more basic and the latter are more applied.

Basic research is defined as a "systematic pursuit of new scientific knowledge without the aim of specific practical application". The results are purely cognitive and there is a degree of truthfulness attached to them. This stands in contrast to the definition of applied research as "the pursuit of knowledge with the aim of obtaining a specific goal". Design sciences obtain knowledge from experiments of crucial variables or from a theoretical background, whatever works to find optimal methods to produce the desired goals. Instead of law like rules in basic research, design sciences make use of "rational inference", an example being "if you want to heal a patient with these symptoms, use this treatment." Technology is design and use of material and social artefacts that can interact and transform reality. These artefacts cannot be true or false. However, they create new possibilities and utilities. It is instead interesting to talk about effectiveness which is relative to the intended use in a specific technology. Niiniluoto states that technologies should be assessed in terms of their economic, ergonomical, ecological, aesthetic, ethical and social aspects.

Regarding the papers in this thesis, the optimization paper (Paper IV) could be seen as design science, "if you want to not break your columns but want quick results, use this flow rate". This also applies for the multi-flow rate optimization paper (Paper V).

The other case studies are mainly technology development, which are assessed on their effectiveness.

1.3 Aim and scope

The main work in this thesis is focused on exploring helpful tools in the quest for more effective downstream processes, and especially continuous and integrated downstream processes. A few different setups for purification of biopharmaceuticals are studied and assessed mainly in terms of their automative abilities. Optimization studies are also performed to increase the effectiveness of continuous and integrated designs, as well as a capture step with a multi-flow rate strategy.

Chapter 2 briefly introduces the concept of downstream processes with a focus on chromatography. Chapter 3 provides a more multi-faceted background in regards to continuous and integrated systems and some regulatory, design, analytical and economic aspects. The case and optimization studies are introduced in Chapter 4 where the physical designs of the setups are explored. Chapter 5 is focused on the optimal design characteristics of these systems. Finally, the foundational aspects of the software used with the systems is laid out in Chapter 6, together with the algorithms for control.

2

Biopharmaceutical production

This chapter briefly introduces the most important steps for production of biopharmaceutical products. The background knowledge described here is an important basis for the next chapters since most of the techniques are built on top of these concepts.

2.1 Biopharmaceutical processing

The entire process for the manufacturing of a biopharmaceutical is commonly divided into upstream and downstream. The upstream part normally consists of the fermentation of cells which produces the protein product. A small sample of cells, called the inoculum is added to a reactor to grow. The reactor is then scaled up as seen in Figure 2.1. The downstream part is the rest of the remaining steps which follow after the reactors. This example process of a monoclonal antibody purification scheme consists of centrifugation, three chromatography steps, five filtration steps and some intermediate temporary storage steps.

The fermentation media from the bioreactor contains numerous unwanted impurities, including large amounts of water, proteases, DNA, endotoxins, culture media components and cellular debris, as well as unwanted modifications of the target product. To purify the target product from this complex mixture, it is common to perform three different chromatography steps, in addition to the filters and membranes. The purpose of the first step, often called the *capture* step, is to reduce the amount of water and get the product in a stable environment. There is usually an intermediate step called the *purification* step with the purpose of removing most of the impurities. In some cases the product may be so pure after the capture step that this step is unnecessary. The purpose with the final *polishing* step is to remove impurities that are similar to the product, including aggregated, incorrectly glycosylated and oxidized variants of the product (Jagschies et al., 2007).

Downstream processing is often referred to as the “bottleneck” in production due to great improvements in cell-cultures and product concentrations (titers), combined with the many and expensive steps in downstream processing (Gronemeyer et al., 2014).

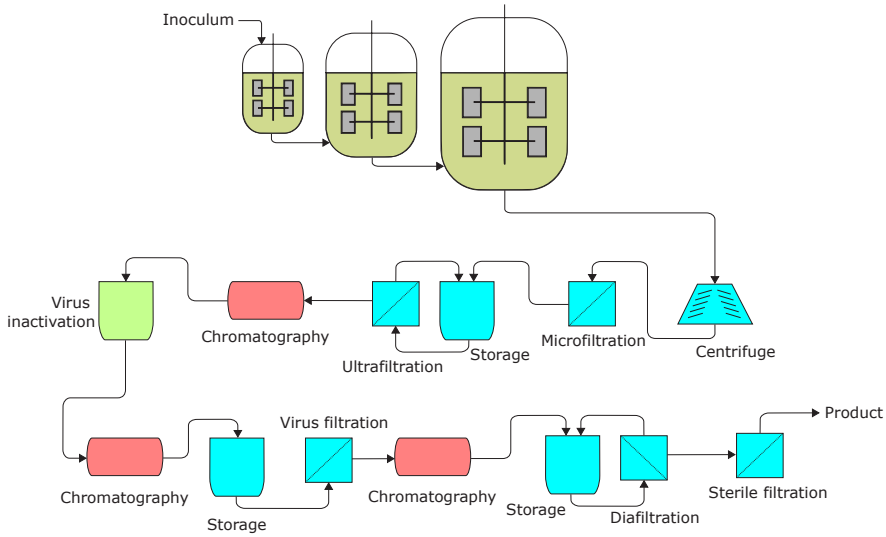


Figure 2.1: An example of a complete production process of monoclonal antibodies (Sommerfeld and Strube, 2005). The product is grown in the reactors in the top, followed by several downstream processing steps to get a viable and pure product.

2.2 Chromatography

Chromatography has been used for over a century for isolation of various substances. Today, pharmaceutical manufacturing is unthinkable without it due to its separation power. Chromatography is used both for analysis, by separating substances, often under high pressure aiming at a high resolution, and as a preparative tool for isolation and purification of a target substance (Schmidt-Traub et al., 2012). The characteristics and dynamics are well known today, illustrated with advanced modelling techniques (Kempe et al., 1999; Bellot and Condoret, 1991). There is both liquid (LC) and gas chromatography (GC), where the former is the only technique used and discussed in this thesis.

The mixture to be separated is fed to a prepared column. This phase is shown to the left in Figure 2.2. After a time the mixture is replaced by another mobile phase, allowing the present mixture to be separated. The output is monitored and separated peaks are detected if the experiment was successful. The separation occur due to small beads which the column is packed with, also called resin or stationary phase. The beads are porous, have a size of around 10-100 μm and usually have an active chemical group attached to the walls inside the beads which the feed mixture can interact with. The active group together with the mobile phase (and some other variables such as temperature and flow rate) together determine how the mixture will traverse the column and the effectiveness of the separation. The active group is what determines the type of the column: ion exchange based, hydrophobic, affinity or size exclusion. Size exclusion, or buffer exchange, is a special type since it has has no active group and separates based only on

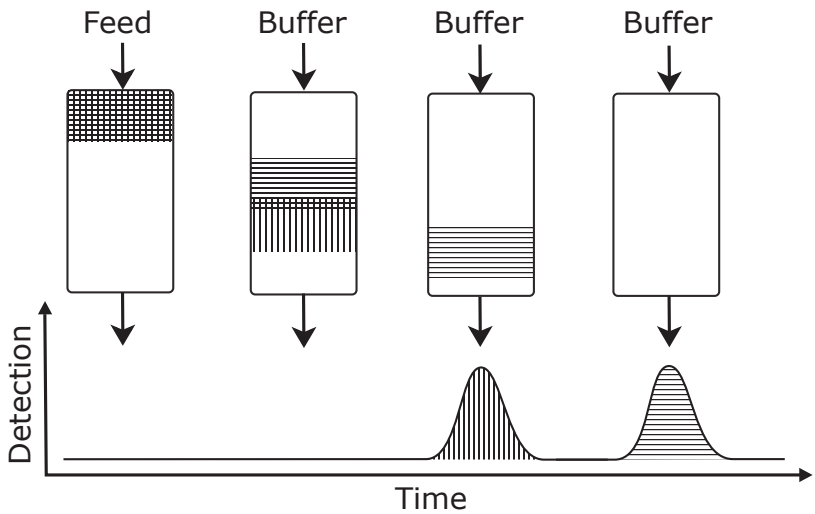


Figure 2.2: An example of how a mixture with two components is separated over time when passing through a packed column. The two compounds are detected ideally in a bell-shaped curve.

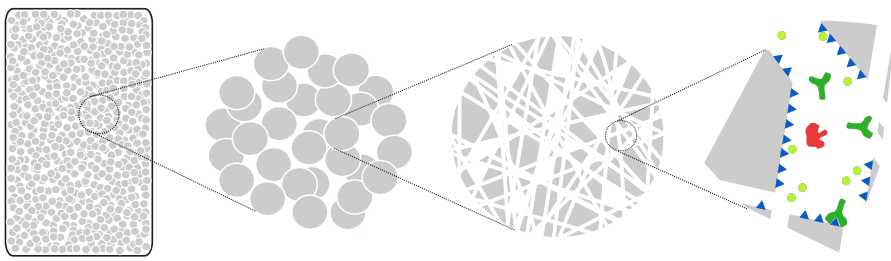


Figure 2.3: Four scales of a chromatography column. It is packed with very small beads which often are very porous. The active groups are located in the inside of the beads at the walls of the resin, where the product molecules diffuses in and possibly interacts.

size differences. Large molecules which are too big to enter the beads will experience the column as having less volume and thus pass through faster.

Figure 2.3 is a representation of a column packed with small porous beads. When molecules travels through the column, they might wander into the beads or just pass by it. If they go inside the bead they might then bind in to an active group inside the bead. These possibilities are determined by factors such as the type of interaction between the target molecule and the active group, flow rate, concentration of protein, the mobile phase, the stationary phase and many other factors. A lower flow rate, for example, increases the likelihood of a protein entering the bead since it has more time available for diffusion. This is utilized in Paper V. Another way to increase the likelihood to enter the bead is to connect columns after each other, performed in Paper VI.

An important concept in preparative chromatography, which will be used in later chapters, is the Dynamic Binding Capacity, *DBC*. This is applicable when product adsorbs to the to active groups. At a defined flow rate, preferably the same that will be used in actual operation, the *DBC* is the amount of product that you can load before product breakthrough occurs (Fahrner et al., 1999). It is called the $DBC_{1\%}$ when breakthrough of the target protein is measured at 1% of the feed level.

2.3 Chromatographic system

A chromatographic setup is a complex system which requires several synchronized components. Nowadays, the pressure requirement is significant and largely determines the type of chromatography, analytical or preparative (Carta and Jungbauer, 2020). A typical setup for a single column is seen in Figure 2.4. From the left side it starts with the buffers connected to the inlets to the pumps, BP and AP. Two pumps allows for a gradient shift of the elution buffers. The paths from the pumps merge and are mixed before reaching the injection valve (InjV) where a sample can be placed. The sample can be injected into the column in a load phase by turning the injection valve. The column valve (ColV) has slots for several columns and can also operate in a by-pass mode. The sensors are placed directly after the column valve, starting with the ultraviolet (UV) monitor and then followed by conductivity and pH monitors. At the end, an outlet valve is placed to direct the flow to be collected or discarded, depending on the phase.

The UV sensor is often set to monitor at 280 nm because amino acids with aromatic rings have a maximum absorbance there. This makes it a useful tool to monitor the concentration of proteins. The signals from the conductivity and pH sensors are also important, since they provide information on mobile phase composition, which determines the quality of the separation and hence the quality of the product.

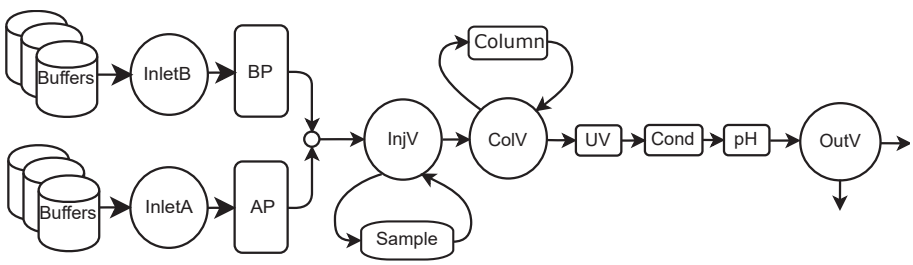


Figure 2.4: A schematic of chromatographic system for a single chromatography column, starting with the buffers on the left and the outlet valve to the right. Two pumps (BP and AP) are required for gradient elution of the product.

3

Process development

This chapter starts with an introduction to the concept of continuous and integrated systems, founding a basis for the rest of the chapter. Throughout this chapter, it is important to keep in mind that risk minimization is still a very high priority in the pharmaceutical field (Kannt and Wieland, 2016), and must continue to be so during exploration of innovative processes.

3.1 Continuous and integrated systems

Continuous manufacturing has been successfully implemented and disruptive in the steel, petrochemical, chemical, food, and pharmaceutical industries (Reay et al., 2013; Anderson, 2001; Laird, 2007). For example, large US steel companies had made huge investments in batch processing, and were therefore unwilling or unable to adapt, which led to that they were outcompeted (Zydney, 2016).

Continuous and integrated operations are defined in a white paper by Konstantinov and Cooney (2015). Unit operations are continuous if they can process a continuous flow and has minimal internal hold volume. Output of continuous unit operations can be continuous or cyclic (semicontinuous). Processes are continuous if they consist of connected continuous unit operations with minimal hold volume in between. Processes are integrated if they are physically connected. Processes are fully continuous or end-to-end continuous if all unit operations are continuous. Hybrid processes are a mix of batch and continuous.

Upstream processes have evolved significantly, refining expression parameters for optimal growth, viability and product titer (Shukla and Thömmes, 2010). Continuous output with perfusion (Kunert and Reinhart, 2016) or fed-batch (Spens and Häggström, 2007) reactors puts even more pressure on downstream processes to keep up. The Food and Drug Administration (FDA) has even approved continuous upstream products (Lee, 2017), demonstrating their willingness to be part of the change.

Regardless of the economic benefits, there are challenges in implementing continuous downstream processes, including process robustness, characterization and regulatory concerns (Somasundaram et al., 2018). A demonstrated robustness and consistent quality is even more important when steps are operated together and potential failure affects the full sequence. Another barrier is the high initial capital investment connected

to new technology, replacing traditional stainless steel tanks.

A recommendation by Konstantinov and Cooney (2015), when realizing continuous manufacturing, is to not only connect batch steps but also think about redesigning individual unit operations, which works best when vendors are involved. An example of this is the integration of a continuous capture step with a perfusion reactor, performed with an ÄKTA Periodic Counter-Current 75 fourcolumn PCC system (Cytiva, formerly GE Healthcare, Uppsala, Sweden), demonstrated by (Warikoo et al., 2012). This technique is further explained in the following chapters. Another product example is the Cadence™ BioSMB (Bisschops and Brower, 2013) for a simulated moving bed technology, used mainly to separate similar products from each other.

3.2 Regulatory control

For a drug to be allowed to be marketed to the general public, it first needs to be approved in the United States by the Food and Drug Administration (FDA), and in Europe by the European Medicines Agency (EMA).

After an application is sent to the authorities, the drug is tested rigorously for effectiveness and potential side effects in what is called the clinical trials (Junod, 2008). The later stages involve hundreds or thousands of people and are very costly. Figure 3.1 is a representation of potential products going through approval and clinical trials. Cost estimations are based on DiMasi et al. (2016). Since most of the potential candidates do not even go through the clinical trials (approximately 11% (Paul et al., 2010)), and even more are scrapped before, the biggest cost for a company is in the form of non-approved products. This demonstrates the benefit of introducing integrated and automated processes in early development.

Since biopharmaceutical products are defined by their manufacturing process, it is costly to change anything after application and regulatory approval. Risk avoidance, combined with a race to market and relatively small cost benefits with an effective production, are reasons as to why the field has been quite conservative in the past. The rise of biosimilars, combined with FDA support of continuous manufacturing (Chatterjee, 2012) can possibly push manufacturers to readjust.

The FDA sees several advantages with continuous manufacturing, including no manual handling which increases safety, shorter processing time with more consistent quality, increased efficiency, more flexible operation, lower capital costs and reduced inventory and an amenability to real-time release testing (Chatterjee, 2012; Fisher et al., 2019). The definition of batches for documentation and tracing is important, but the regulators see no problem with this since they define it as a ‘lot’ which can be produced in a unit of time or quantity (Chatterjee, 2012; Jungbauer, 2013).

3.3 Platform design

A platform is a templated approach as a starting point for developing production processes. It streamlines and greatly reduces the process development time. The regulatory

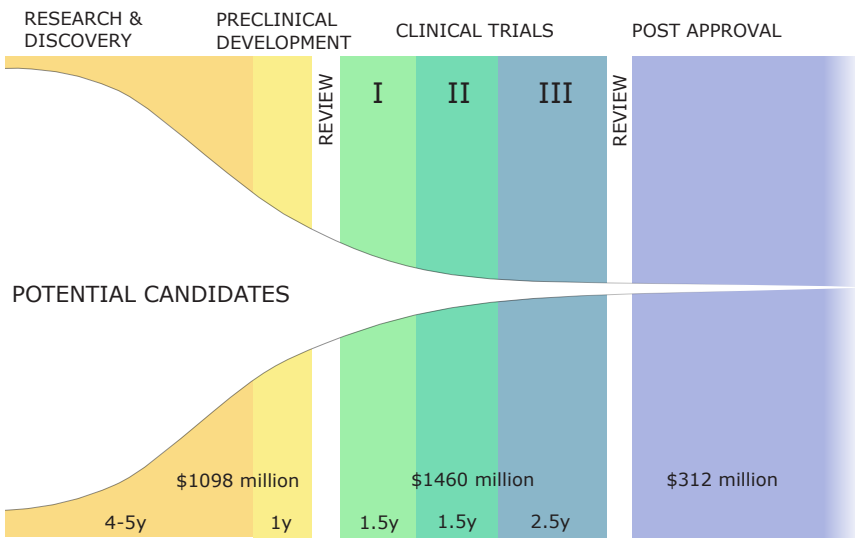


Figure 3.1: An overview of the cost and time commitment to take biopharmaceutical products to the market. The cost is for producing a single biopharmaceutical and is based on calculations from DiMasi et al. (2016). The number of potential candidates decreases in all phases, but the curve is especially steep in the early stages.

processing is also greatly facilitated (Farid, 2008; Shukla et al., 2007). This can have significant effect on the time and cost to get a product to market.

A well-known platform is the mAb-platform (Kelley, 2007; Shukla et al., 2007). The template is not a strict process but rather offers a common philosophy and alignment as to which unit operations to include. It is common to have an affinity capture column (with Protein A as the active group, which antibodies specifically bind in to), followed by viral inactivation, two polishing steps, viral filtration and finally dia-filtration (Shukla et al., 2007). With a lot of variability in mAbs there is a lot of research needed in finding alternative approaches and possibilities in tweaking the template (Shukla and Thömmes, 2010).

A non-mAb-platform is evaluated by Walther et al. (2015), which contains a general continuous capture followed by batch intermediate and polishing purification. Since the capture step cannot be Protein A for a general platform, the downstream design becomes a bit more complicated. A general strategy in integrated systems is to redesign the chromatography steps to avoid extra buffer exchange steps so that the pool can be directly transferred to the next column (Carson, 2005; Werner, 2004).

A complementing approach in reducing R&D costs is to implement a ballroom-like facility, defined as “a large manufacturing area that has no fixed equipment and minimal segregation due to the use of functionally closed systems” (Wolton and Rayner, 2014), which is possible since continuous processes often are smaller and can thus fit. They are also enclosed which reduces the need for a clean-room classification. The principle

is to have flexible unit operations with single-use equipment, enclosed in a single production room. The unit operations include media and buffer preparation, fermentation and downstream processing (Hsu et al., 2012; Klutz et al., 2015).

3.4 Quality by Design

The International Conference on Harmonisation (ICH) is a committee that brings together regulatory authorities and industry to provide guidelines for pharmaceutical production. The committee promotes Quality by Design (QbD), and especially the Q8(R2) document (Guideline, ICH, 2009). QbD is a response to the need for a more holistic, scientific and proactive approach to pharmaceutical development, and is defined in Q8(R2) as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". The purpose is to build it into the manufacturing process, through a thorough understanding of critical quality attributes (CQAs) of the product - as opposed to being tested afterwards (Rathore and Winkle, 2009). A benefit of this approach is that changes can be made within an approved process design without a regulatory approval.

From a QbD perspective, the dynamics of startup and shutdown, with regards to quality, is important to consider when designing integrated continuous processes. Proper measurement systems are required for monitoring and control, e.g. to ensure proper quality after drifting or uncontrolled changes. Furthermore, possibilities for product rejection have to be implemented and criteria established beforehand (Allison et al., 2015; Lee et al., 2015).

It can be valuable to understand how material flows through a system. An approach to characterize these dynamics is to perform a tracer experiment or through process modelling. This knowledge is helpful when backtracking errors, provides understanding of the system and affects the design of control systems (Lee et al., 2015). Continuous and integrated processes essentially require a systems approach with regards to design, control and optimization (Badman and Trout, 2015). However, there are often many critical quality attributes spread out over multiple different unit operations combined with the impact of raw material attributes. All these parameters makes for a complex picture of related variables and resulting product properties.

3.5 Analytics and PAT

To effectively monitor CQAs, a proper analytical system is required. Process Analytical Technology (PAT) was first discussed by FDA in 2001, and in 2004 a document was released with guidelines defining it as 'a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality' (FDA, US and others, 2004). It has since been part in several of the ICH quality guidelines.

Biopharmaceutical downstream processing is challenging to control using PAT as many of the impurities are closely related to the product. Related to this reason is that qualitative at-line analysis generally takes longer time than the available time for decision making. There has, however, been demonstrations of real-time measurements capable of providing rough quality quantification (Rathore et al., 2010). Where direct measurements are not possible, soft-sensor technologies can provide a substitute in some cases (Kroll et al., 2017; Sommeregger et al., 2017). Several other techniques have also been explored, such as pool-based decision making and principal component analysis (Rathore and Kapoor, 2015).

While continuous and integrated techniques certainly offer advantages, they also introduce potential risks that are important to be aware of. In integrated systems, typically with very precise synchronization, the entire process would stop if only one of the steps would fail. The likelihood of failure increases with the number of integrated steps since the risks of individual steps are multiplied with each other. Another problem would occur if the pool from a faulty column would be mixed with pools from properly functioning columns, such as in periodic counter-current chromatography. Redundancy can be built into a system to counteract some of the problems, for example having extra columns to replace defective ones. Another measure is to have the possibility to pause the process or divert defective pools. For these options to be possible it is necessary to have a real-time monitoring system in place.

The biopharmaceutical field is quite problematic for data scientists due to several reasons: the lack of proper data formats, standardized interfaces, low number of experiments, conservative information/operation technology environments as well as regulatory restrictions (Steinwandter et al., 2019). Knowledge management is a discipline that has emerged to guide knowledge flows in a system to remedy some of these concerns (Herwig et al., 2015).

3.6 Economic considerations

There are several issues that makes cost estimations of biopharmaceutical production complicated. Excluding reasons for manufacturers to withhold data, some examples include which steps are included in the analysis, whether cost of goods are for a single product or for a multi-purpose facility, or which scale the product is manufactured in (Farid, 2007).

Both demand and upstream technology have increased dramatically, further making the downstream portion of production a bottleneck in economic terms (Gronemeyer et al., 2014). Resin technology will remedy some of this imbalance, but downstream processes still need to be more intensified (Farid, 2008).

Modeled processes show that for clinical trials, material consumption (media, buffers, precipitants) is essentially the same when comparing batch and continuous operation, but costs for labor (process, quality assurance/quality control), consumables (filters, resins, bags) and facility (capital expenses and other facility-related costs) are all reduced by around 40% (Xenopoulos, 2015). Another simulated assessment of an integrated continuous biomanufacturing platform found that costs could be reduced by 55%, compared to conventional manufacturing (Walther et al., 2015)

Around 2010, R&D costs were estimated to be around $1.2-1.8 billion (Paul et al., 2010). In contrast to 'Moore's law', which is an observation that the number of transistors in a dense integrated circuit doubles about every two years, there is an observation of R&D efficiency, in the pharmaceutical industry, which has declined steadily. It is called 'Eroom's law' ('Moore's law' backwards). The efficiency is measured in the number of drugs brought to the market per spent US dollar (Scannell et al., 2012).

30% of costs in clinical trials can be derived to process development and manufacture (Bogdan and Villiger, 2010). Since most biopharmaceuticals do not get past the clinical trials, there is a great incentive in reducing these costs, especially for early phases as they constitute the major part. Smaller, flexible, semi-continuous and integrated systems will generally reduce costs in all phases (Pollock et al., 2013). These techniques are further explored in the next chapters.

4

Integrated design

In this chapter, which is a central part of this thesis, the design of integrated sequences is explored. A natural path to a fully continuous downstream process is to focus on integrated processes operated in batch mode (Rathore et al., 2015), which is the main part of this chapter. It contains various unit operations, including chromatography, ultrafiltration-diafiltration (UFDF), reactors and scales. The design aspect of papers I, II, III and VI will be featured, where the studies in the first three are in batch mode and the last is a hybrid continuous. The evolution and incremental approach of the design throughout the papers will be demonstrated in combination with the increased complexity and necessary considerations.

I will refer to *purification steps* or simply *steps* as the unit operations, e.g. a chromatography step, ultra-/diafiltration or reactor step, in this chapter, and *phases* will be referred to as the operations within the steps, e.g. loading, washing or elution.

An example of a Gantt chart containing three chromatography steps is seen in figure 4.1. The time of retention of the product is shorter in an integrated setup because all phases directly involved with the product, i.e. not regeneration and equilibration, are put sequentially over the purification steps.

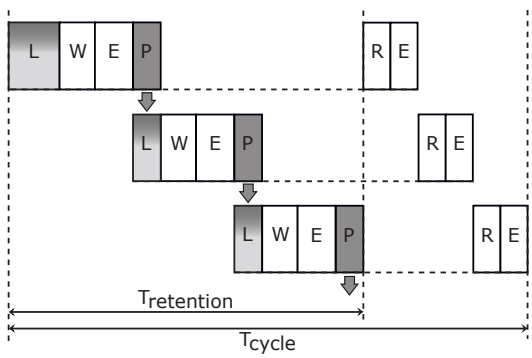


Figure 4.1: A gantt chart with three chromatography steps on a single unit. The product retention time in total is shorter since the equilibration and regeneration phases can be performed later. The cycle time is the total time of all steps and phases that has to be performed before a new cycle can be started.

4.1 Universal design

The aim with having a design suitable for multiple purposes is similar to the platform approach discussed in Section 3.3: it is a useful starting point which also simplifies regulatory aspects. Figure 4.2 is a representation of how the basic integrated setup can be implemented on an ÄKTA Pure. From the top, the buffers are connected to an inlet valve and then its corresponding pump, the A pump (AP)or the B pump (BP). The two pumps then merge into a mixer valve before going to the injection valve, InjV in the figure. The injection valve can be switched so that the sample is loaded onto the capture column. When the flow is directed to either the loop valve, LV, or the column valve, CV, the two versatile valves, VV1 and VV2, are switched to direct the flow path coming from the pumps. In the setup in the figure the flow path is directed to the loop valve and then through the sensors to the outlet valve. If only one of the versatile valves would be switched in this case the flow path would be directed to the waste.

The blue path in figure 4.2 is the path that the pool takes when it is eluted from column 1 and directed to column 2. Two things are noteworthy, the first is that the path goes through the first versatile valve a second time but in a separate chamber. The second thing to note is that any potential product that is not bound in to the second column goes directly to waste, on the same principle as above. Auxiliary sensors can be put on the waste stream to account for potential product losses.

The general principles of this setup was used in papers I, II and III. In II the pool in between the steps was also diluted with a stream from the sample pump. The dilution connection was put between the outlet valve and the versatile valve.

4.1.1 UFDF implementation

An ultra-/diafiltration (UFDF) step was implemented in paper III. The outline for the integrated design is represented in Figure 4.4. The universal design from Figure 4.2 is still used as a base, but some changes had to be made to the design with the inclusion of UFDF due to multiple components.

The UFDF design, built around the UF module, is demonstrated in Figure 4.3. The pump at the feed stream pushes the product through the UV-sensor, to estimate the concentration, to the UF module, where the product does not permeate and goes to the retentate stream and back to the flask. The pump at the permeate stream accurately measures the flow rate so that the amount of discarded buffer is known. This operation continually increases the product concentration in the flask since the volume is reduced. The pressure is monitored on both sides of the membrane to keep it in balance, too low pressure and too little will permeate and too much pressure and the membrane will break. The UFDF setup can also be operated in a DF mode with the purpose of replacing the buffer that the product is in. During DF, the amount of buffer that is discarded in the permeate stream is replaced sequentially.

The column valve has a built in pressure sensor so the feed and permeate pressures can easily be measured, corresponding to a pre- and post-column pressure. A manual valve was put on the retentate stream to set a standard back pressure. Since the pressure varies depending on flow rate and concentration a relationship was calibrated offline so that it could be estimated at all times.

The UFDC is incorporated as seen in Figure 4.4. The membrane is put on the col-

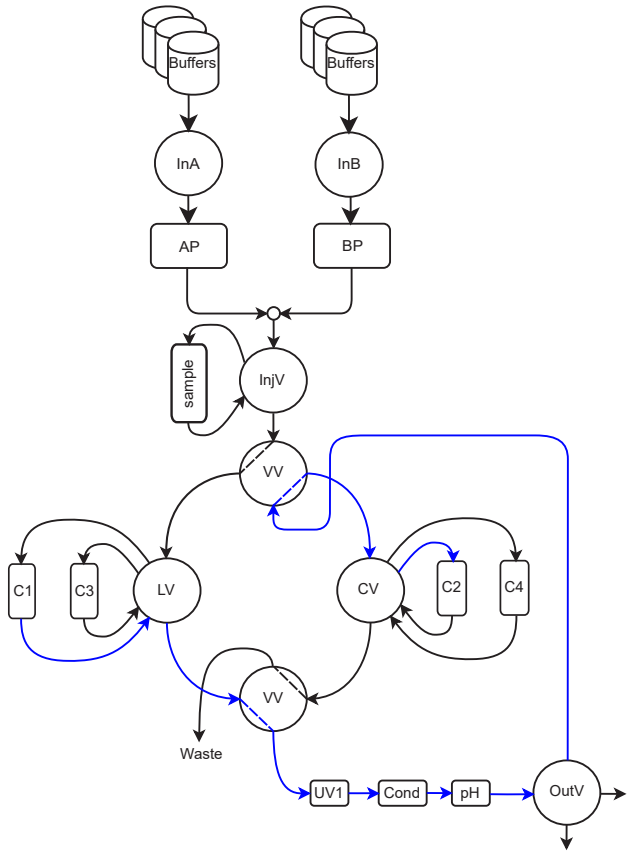


Figure 4.2: A single integrated chromatography sequence setup for 4 columns. In the elution phase of the first column, valves are set for the blue path so that the pool is transferred from column one, through loop valve (LV) and the versatile valve (VV), through the sensors, the outlet valve back to the first versatile valve and finally to the column two on the column valve (CV).

umn valve with the pressure sensors and the flask directly at the retentate with the manual valve. The permeate is pumped through the sensors and the outlet valve before reaching the feed pump and then discarded.

The column named C3, located on the left-hand side of figure 4.4, is a low-volume membrane chromatography column that is run in a flow-through mode. Since it has a low volume and is of type flow-through it can not be placed on either the loop valve or the column valve, which is why it is placed on a versatile valve on the recycling loop. This concept is further expanded on in Section 4.3.

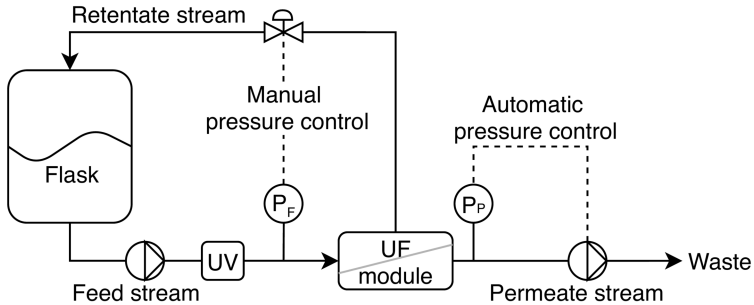


Figure 4.3: Block diagram of the UFDF process from Paper III. There are pressure sensors both on the permeate side and on the feed stream. The retentate is returned to the flask where it will be pumped around again until the product is at a desired concentration.

4.2 Continuous feed

The purpose of the first chromatography step in a downstream process is to concentrate and isolate the product (Jagschies et al., 2007). Affinity columns or ion exchange columns are well suited for the task, preferably with a high capacity. Columns for batch processes have usually been designed to have long residence times to increase capacity utilization. This approach unfortunately results in a low throughput and large columns (Godawat et al., 2012). However, there are for continuous chromatography demonstrated solutions that only have one-column. This solution works for low titers and requires a surge tank between the reactor and the column which buffers the load during regeneration (Kamga et al., 2018).

With titers over 2 mg/ml, multi-column continuous techniques are found to have a higher productivity and consume less buffer (Baur et al., 2016). Multiple columns can be run in parallel or concurrently to handle a continuous stream from a bioreactor (Jungbauer, 2013). A simple solution is to have two columns that alternatingly receive the feed. While one is loading, the other performs the remaining steps, see figure 4.5. Before a column starts bleeding product, they alternate roles and the valves switch. This concept is easily incorporated into the integrated systems described above.

A more complex method is the so-called twin-column captureSMB (Angarita et al., 2015), where one column first is loaded when the other goes through the other phases. When the first column starts bleeding product, a connection switches so that the second column receives the flow through. This interconnected state continues until a breakthrough concentration of X% of the feed, through the first column, is reached. With interconnected states it is possible to go well over the $DBC_{1\%}$ since the breakthrough is captured by the second column, and thus, more resin can be utilized (Pollock et al., 2013; Mahajan et al., 2012).

After the interconnected state, the columns alternate roles and the loading of the second column continues and the first column can be eluted and regenerated. This concept utilizes more binding sites of the columns and is therefore more efficient than the twin-capture method.

An even more complex system of capturing a continuous feed is what is called peri-

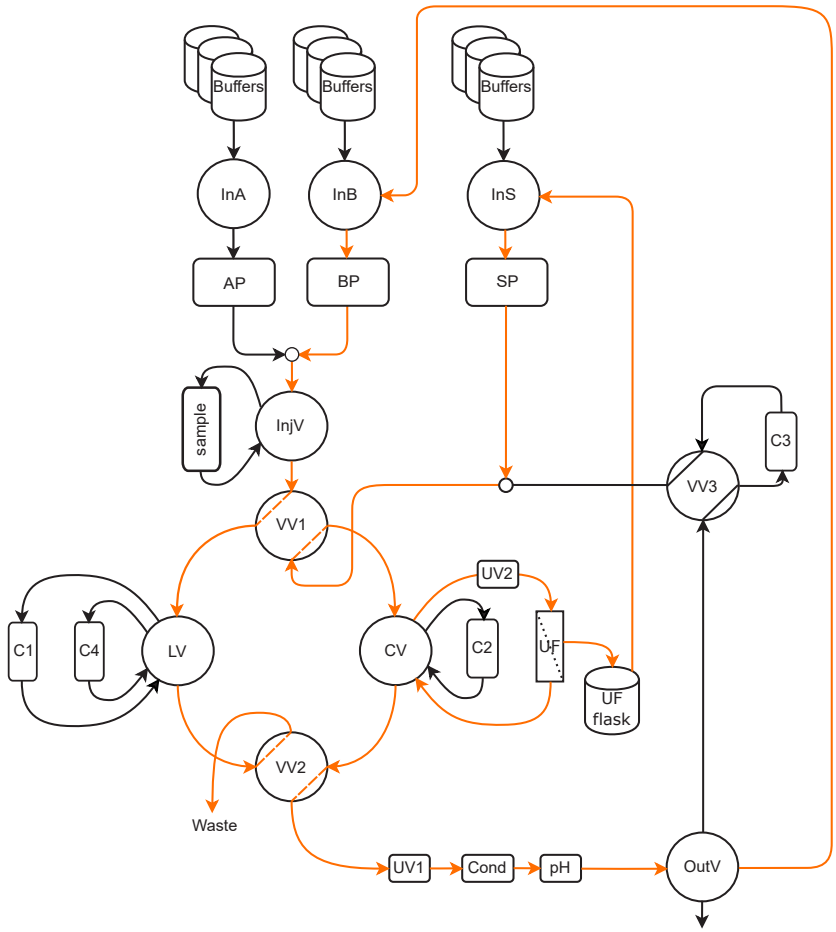


Figure 4.4: The integrated setup used in Paper III. The pathway for the UFDF step is indicated in orange while the rest of the setup is similar to Figure 4.2.

odic countercurrent adsorption, or PCC, (Carta and Perez-Almodovar, 2010) or PCCC, periodic countercurrent chromatography (Jungbauer, 2013). It can be performed in multiple variants with different number of colums (Steinebach et al., 2016). The basic concept can be explained with three columns (Warikoo et al., 2012), see figure 4.6 and 4.7. The feed stream is constant to the three columns. Column 1 is initially loaded while column 3 is eluted, in the next step the product not binding in to column 1 anymore is directed to column 2 while column 3 is regenerated. The next step in the cycle starts again but with column 2 receiving the feed stream instead. This continues until all six steps have been performed and then the cycle restarts.

Cost-savings for PCC have been predicted to around 30% for early stage manufacturing and around 5% for manufacturing (Pollock et al., 2013).

There are several variables to take into account when choosing the best method for

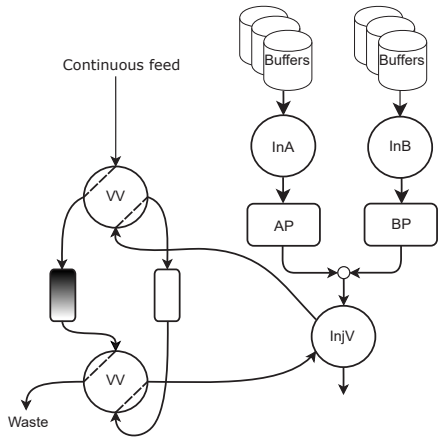


Figure 4.5: A twin-capture concept on the injection valve, where a continuous feed is loaded on alternating columns. While one is loaded, the other can be eluted and recycled.

the continuous capture step. One variable is the productivity. Another variable is the resin utilization, which the captureSMB and the PCC substantially improves. For these more complex methods there is a constraint called the feed continuity constraint which states that all phases other than the loading phase, i.e. recovery and regeneration, must have a duration that is shorter than the loading phase (Godawat et al., 2012). Among the tradeoffs between the more complex methods are the productivity, scheduling constraints and the hardware complexity.

Virus inactivation is still a major challenge when implementing continuous processes (Zydney, 2015; Hammerschmidt et al., 2014). It can be performed through different methods, including solvent/detergent (Martins et al., 2019) and low pH (Martins et al., 2020). In Paper VI, the solvent/detergent option was chosen and put before the PCC, allowing a continuous flow through the reactor. No product was found in the PCC flowthrough.

4.2.1 Multi-machine with PCC

The setup from paper VI, shown in Figure 4.7, contains a PCC and a continuous virus inactivation step in the left system and a polishing step in the right system. The current setup that is shown in the figure corresponds to cycle two in Figure 4.6, where two columns are interconnected and loaded, and the third column is regenerated, i.e. going through the phases elution, CIP, regeneration and equilibration.

UV2 is located so that the UV-level of the breakthrough, after the first column and before the second column, always is read. The other sensors on the first system detects the pool, eluted from C3 in the current setup, that is pumped to the second system. The pool is diluted after UV1, before binding in to column C.

The second system is designed so that the pool can pass the sensors both before it is loaded onto the column as well as after during the elution step of column C by going around one more lap through the three versatile valves. UV2 in that system is located

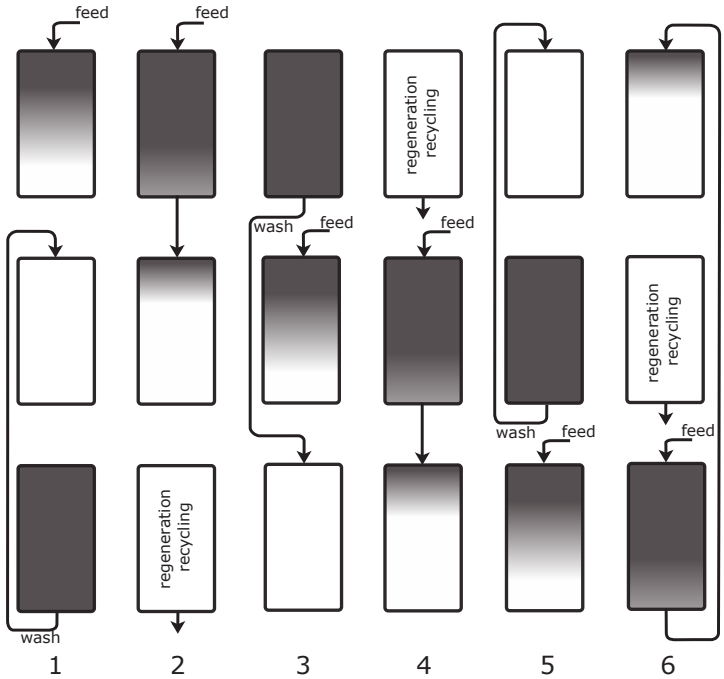


Figure 4.6: A three-column PCC concept with 6 cycled phases. Cycles 2, 4 and 6 are the main cycles where two columns are interconnected, so that the breakthrough from the first column can bind in to the second, and the third column is regenerated and recycled. The pool is also collected in this step. Cycles 1, 3 and 5 are shorter cycles where only a single column is loaded at a time. At the same time one of the other columns is washed, and that wash is pumped through the third column so that any potential product would not be wasted.

so that any breakthrough during the loading phase would be detected.

The dual setup, containing multiple complex phases that happen in parallell, requires that phases are in sync over the systems. When a column in the PCC is fully loaded, it is, by design, eluted and pumped to the next system. If that system is not ready to recieve the pool it would be wasted. The syncing is described in more detail in Chapter 5.

4.3 Integrated results

The result graphs from papers III and VI are shown in Figures 4.8 and 4.9, respectively. It is important to realise that the chromatograms are not only a product of the sequence of the steps and their phases, but just as important is where the sensors are located in the setup relative to valves and columns. The importance of PAT was discussed in Chapter 3.

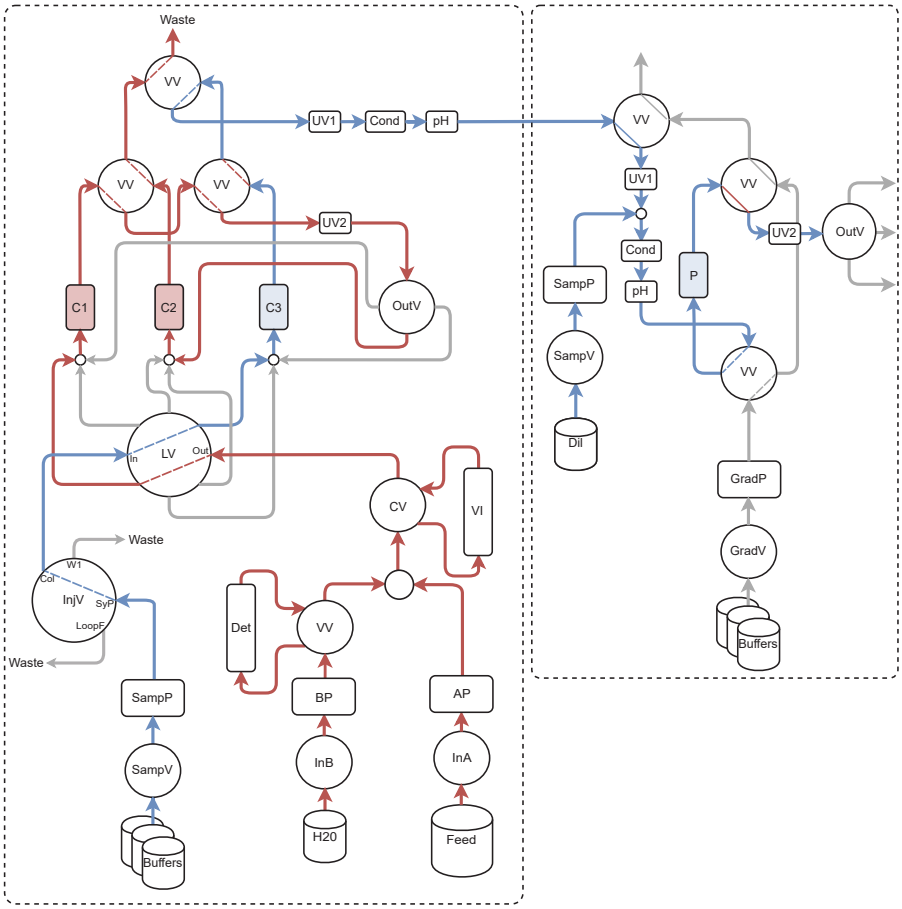


Figure 4.7: A setup with two connected systems. On the left system, the feed is pumped through the virus inactivation reactor at the column valve (CV), mixed with detergents (Det), before reaching the PCC. The loop valve (LV) directs the flow path to either of the 3 columns, C1, C2 or C3. The versatile valves (VV) can be rotated 90 degrees for a different flow path. The grey lines are inactive as the system is set up in this figure. The red line shows the path that the feed is pumped, through both columns C1 and C2 and then to waste. The blue dashed path is active at the same time and it goes through C3 towards the second system where a final column step is located.

An example of where some product loss might occur is in the Figures 4.4 and 4.8. During a coupled elute and load phase, the pool is pumped from one column, through VV2, the sensors, back to the next column and then to waste. A third and extra sensor could be placed on the waste stream to detect if there was any product loss during an experiment.

Some direct quantitative measures for the integrated continuous sequences are easy to obtain. The main results from the case study in Paper II is the reduction of seven

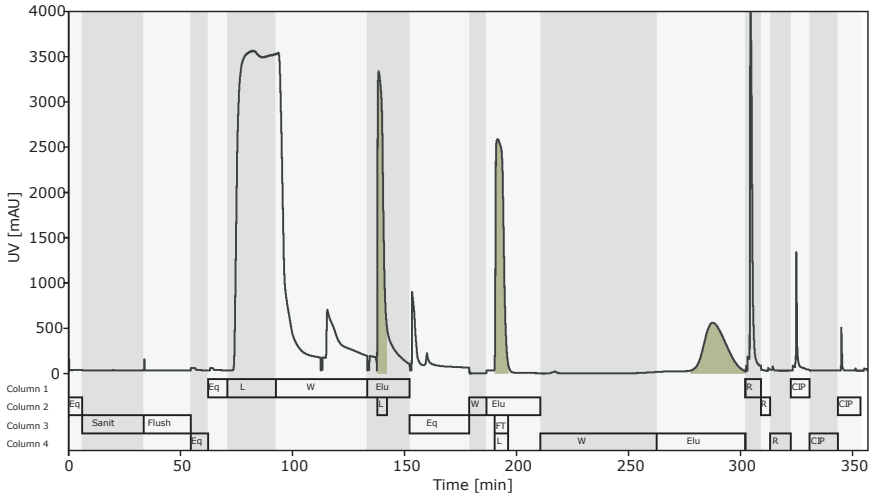


Figure 4.8: Results from paper III. All phases are marked with an alternatingly shaded background and below the graph the steps are marked according to which step they belong. The pool that is sent to the next step is shaded blue.

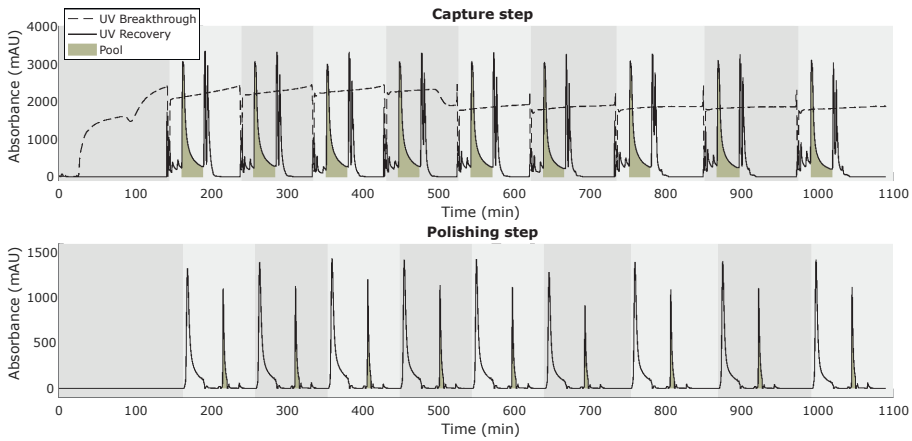


Figure 4.9: Graphs from paper VI. The upper graph is from the UV-sensors of the PCC system. Both the UV-level from the PCC breakthrough and the eluate is seen in the graph. The lower graph is from the following polishing step, where both the received pool and the final pool can be seen.

manually handled steps to two manually handled steps, allotting time for non-manual labor while also allowing overnight runs. The follow up study to that, in Paper VI, with the same process but instead had a continuous capture with PCC, showed clear improvements in both productivity (22.6 to 44.1 mg day^{-1} mL^{-1}) and yield (68.3 to 87.5 %). The quantitative result from Paper III, comparing a manual process to the integrated, is a productivity increase from 0.83 to 1.09 mg mL^{-1} h^{-1}, with a process time reduction

from two working days to a single day.

Another direct, although less tangible, benefit is the increased robustness, due to the closed and automated production processes, that comes with reduced risks of mixups and contamination, and is in better compliance with good manufacturing practice.

4.4 Column to column design

An important factor for the design is simplicity and ease of use. A simple setup reduces downtime in case of problems as well as startup time during new process installations.

The design of the integrated setups described above comes with the aspect that columns must be placed on alternating valves. They can not be placed on the same valve because the recirculation through the versatile valve can only be directed to the other loop/column valve, see Figure 4.2, and the pool would thus “bite its own tail” . The same principle makes flow through columns with low volumes problematic. The entire pool would have to be momentarily parked in the column before the versatile valves are switched and the new phase can begin.

In the setups where streams are recirculated, it is important to be cautious about contaminants and other remains that might get stuck anywhere in the system for future runs. Washes in between every phase can be introduced to counteract this, with sodium hydroxide followed by the new phase buffer. The washes are then pumped through all flow paths, filling the entire system.

Continuous or semi-continuous viral inactivation must be placed in an integrated system so that cleared product never be exposed to non-cleared product. In Paper VI, the setup is built so that the virus inactivation is performed continuously before all chromatography steps.

Pools that are recirculated may need to be diluted with a stream for the pool to be able to bind in to the next step, e.g. pH or ion concentration adjustments. See Figure 4.4 for an example of where the buffer from the sample pump can dilute the recirculating stream. However, the mixing of these streams is not trivial. A mixing chamber offers good mixing, but the cost is band-broadening of the pool and a long tailing peak. A study was made on a simple T-cross intersection which concluded that there is no mixing performed in the intersection and in the tubes, other than diffusion which only is effective at lower flow rates (Åberg and Shareef, 2018).

4.4.1 Alternative designs

Downs and Skogestad (2011) states that current practice in the chemical industry is usually focused on optimization of unit operations. Their recommendation is however to have a holistic view of the design with a mix of top-down and bottom-up hierarchical approaches. This also applies to the biopharmaceutical downstream industry, to the best of my knowledge, where most of the continuous optimizations are often for single unit operations, e.g. PCC and SMB. An interesting example related to this is of a fully continuous end-to-end purification scheme, made up of a capture PCC where the pools are eluted into a surge tank, followed by a second PCC step for polishing (Godawat et al., 2015). This concept does not require any commununication between the systems

since they are decoupled from each other. A process wide SCADA system has recently been developed in Matlab for control of a bioreactor, chromatography steps and atline HPLCs (Feidl et al., 2020). The chromatography steps were performed using two setups of ChromaCon CUBE (Zurich, Switzerland) with a virus inactivation hold in between.

For systems with sequential chromatography steps there is an alternative approach to direct transfer of the pool column to column, which is to have an intermediary loop or vessel to momentarily hold the pool. Bio-Rad (Hercules, California, US) have developed a family of customizable systems, which are similar to the ÄKTA series, with this as a custom option. The benefit of intermediate vessels is that there is no pressure drop from two columns simultaneously. If the double pressure drop is not accounted for in column to column pooling, the first column in the series could potentially break.

A concept that is indifferent to the design will be explored in the next chapter, which is the design of individual columns with regard to their interconnected state during pooling/loading. Their volume and associated flow rates are optimized for total sequence time and resin volumes or ability to handle continuous reactor feeds.

5

Optimization

Optimization is a tool that can be used for many purposes and especially in process systems engineering. A main reason for that is that there often are many possible solutions to a problem with slightly different inputs and it is therefore not easy to find the best one (Biegler, 2010). This chapter deals with some optimization problems related to downstream processing, starting with integrated systems.

5.1 Optimal integration of chromatography steps

In the context of this thesis, optimization is mainly used to find the lowest material costs combined with the fastest processing time. In Paper VI, we demonstrated a mathematical setup that takes specific column properties and use them as constraints in the optimization algorithm. Since the column properties are continuous and because the size of a specific column in a sequence depend on previous column sizes, it is a non-linear problem (NLP). A general NLP problem is shown in 5.1 (Biegler and Grossmann, 2004).

$$\begin{aligned} \text{minimize} \quad & f(x) \\ \text{with respect to} \quad & x \\ \text{subject to} \quad & h(x) = 0 \\ & g(x) \leq 0 \end{aligned} \tag{5.1}$$

where $f(x)$ is the objective function and $h(x)$ are equality constraints and g(x) are inequality constraints. Bounds can also be implemented on the variable x and will be demonstrated below.

In an integrated system, the pool is often directly transferred through one column to another as seen in Chapter 3. In Figure 5.1, this is demonstrated in a general sequence where the sizes and flow rates can vary. The pressure drop, which is a limiting factor, is directly related to the flow rate (Stickel and Fotopoulos, 2001). The task is to find the highest flow rates while keeping the size of the columns as small as possible. Equation 5.2 is the most basic and foundational relationship in this chapter, which occur when the pool from the previous column, $i-1$, is directly loaded onto column i, with V [ml/column] and F [CV/min]. During the eluate/load phase, the volumetric flow rate

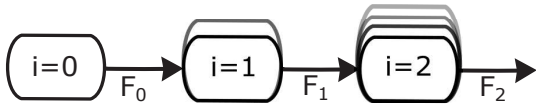


Figure 5.1: A general sequence of columns where both the flow rates and the sizes may vary. Note that only two columns are connected at a time and only when the pool is transferred between them.

of $i-1$ is limited by the maximum flow rate of column i. This correlation is necessary when the flow is coupled, as it is during elute and load phases.

$$V_i F_{i,L,max} \geq V_{i-1} F_{i-1,E} \iff \frac{V_i}{V_{i-1}} \geq \frac{F_{i-1,E}}{F_{i,L,max}} \tag{5.2}$$

Some variables are introduced in equations 5.3 and 5.4 based on equation 5.2. Note that the length of the columns is assumed to remain constant during scale up. This means that the maximum flow rate increases proportionally to the size of each column. Variables x and y, defined as:

$$x_i = \frac{V_i}{V_{i-1}} \tag{5.3}$$

$$y_i = \frac{F_{i-1,E}}{F_{i,max}} \tag{5.4}$$

exist for every connected pair of columns which means that in a general sequence there will be $2(n-1)$ variables to find an optimal solution for where n is the number of columns. For each step in the algorithm, the total volume and the process time are calculated with equations 5.5 and 5.6, respectively. In the paper version of this chapter a robustness and a dilution factor is included in all equations.

$$V_{tot} = V_0 + V_0 \sum_{i=1}^{n} \prod_{j=1}^{i} x_j \tag{5.5}$$

$$t_{tot} = \sum_{i=1}^{n} \left(y_i \frac{\phi_{E,i-1}}{F_{i-1,E}} \right) \tag{5.6}$$

5.1.1 Constraints

For the optimization algorithm to not choose infinitely high flow rates and column volumes there must be some kind of limitations. These limitations are called constraints. The details below are simplified to what is described in the Paper VI and the associated supplemental material.

Equation 5.7 is a representation of the flow rate limit that must not be exceeded. It is called the *current FR-limit*, where *FR* is short for flow rate.

$$x_i \geq y_i \tag{5.7}$$

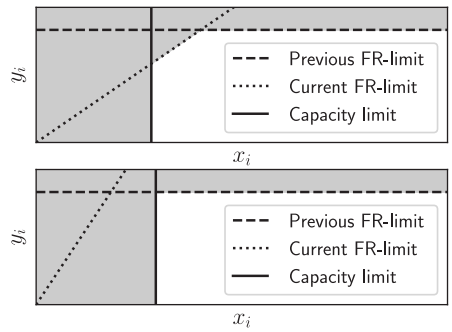


Figure 5.2: The two general graphs of plotted x- and y-values. The white area is inside the constraints.

The flow rate must not exceed the maximum flow rate of the previous column, which is described by constraint equation 5.8 and called the *previous FR-limit*.

$$y_i \leq \frac{1}{R_i} \frac{F_{i-1,max}}{F_{i,max}} \tag{5.8}$$

The absolute capacity, $c_{i,w}$, in mass, of potentially bound in product of each column must be greater than the first column's capacity, ($c_{0,w}$), and the product sum of the intermediate recovery yields, u, which is why constraint equation 5.9 also must be fulfilled for all connections. Note that the product sum of x is equal to the last column volume divided by the first, e.g. $\prod_{j=1}^{3} x_j = x_3 x_2 x_1 = \frac{V_3}{V_2}\frac{V_2}{V_1}\frac{V_1}{V_0} = \frac{V_3}{V_0}$. This constraint is called the capacity limit.

$$\prod_{j=1}^{i} x_j \geq \frac{c_{0,w}}{c_{i,w}} \prod_{j=0}^{i-1} u_j \iff x_i \geq \frac{c_{0,w}}{c_{i,w}} \frac{\prod_{j=0}^{i-1} u_j}{\prod_{j=1}^{i-1} x_j} \tag{5.9}$$

As y is a ratio between non-negative flow rates, it can't be zero or below. This is described by equation 5.10 and is implemented as a bound.

$$y_i > 0 \tag{5.10}$$

These three constraints and the bound can be plotted in a simple graph demonstrated in Figure 5.2. The allowed values for variables x and y is the white area in the figures, confined by the constraints together with the lower bound of zero. The difference between the graphs is where the current FR-limit crosses the previous FR-limit, before or after the capacity limit. Optimal solutions will always be placed on as low x-values and as high y-values as possible since at those values the column volumes will be lowest and the flow rates highest. In the upper graph in the figure, the entire *current FR*-line consists of optimal points for the optimization algorithm and in the lower graph there is only one point which is the upper left corner.

5.1.2 Pareto optimizations

In many situations there are multiple and conflicting objectives. In the integrated sequence there are several variables that we could minimize. In Paper IV, we looked at process time, column volume and buffer consumption. To handle these instances a common tool is to use so-called Pareto optimization (Ngatchou et al., 2005; Chitra et al., 2011; Józefowska and Zimniak, 2008). One method to handle incommensurable objectives is to introduce a weight parameter, w, which balances the competing objectives for the optimization algorithm (Yamanee-Nolin, 2020). The weight shifts on a scale between 0 and 1, giving the objectives different balances, and thus multiple optimization results - a Pareto front.

The following optimization problems come from a setup containing five columns with a twin-capture step so that feed can be continuously loaded from a bioreactor. The setup must be able to handle the continuous feed but other than that there are no benefit in having a faster process time. Instead, the total volume of the columns, V_{tot}, is compared to the total buffer consumption, B_{tot}, in equation 5.12. The variables x and y that are real numbers are solved for in n number of connections as well as the starting volume. The problem is subject to the above constraints and bounds.

$$\begin{aligned} \text{minimize} \quad & w\frac{V_{tot}}{V_{tot,min}} + (1-w)\frac{B_{tot}}{B_{tot,min}} \\ \text{w.r.t.} \quad & V_0 \in \mathbb{R} \\ & x, y \in \mathbb{R}^{\mathrm{n}} \\ \text{s.t.} \quad & \text{Equations}\,(5.7) - (5.10) \end{aligned} \tag{5.11}$$

The result of one of these optimizations can be seen in Figure 5.3. The column properties are seen in the supplemental material to the paper. Note that all individual dots are one optimization each and that they all are optimal solutions depending on the value of the weight. Three chosen weight values at 0.0, 0.2 and 0.8 are written out to show the distribution of the solutions.

Another optimization problem is performed with equation 5.12 where the total volume of the columns will be compared to the total process time. The starting volume is omitted since it is only a matter of scale. There are three different columns in this integrated setup and their specific properties are also found in the supplemental material.

$$\begin{aligned} \text{minimize} \quad & w\frac{V_{tot}}{V_{tot,min}} + (1-w)\frac{t_{tot}}{t_{tot,min}} \\ \text{w.r.t.} \quad & x, y \in \mathbb{R}^{\mathrm{n}} \\ \text{s.t.} \quad & \text{Equations}\,(5.7) - (5.10) \end{aligned} \tag{5.12}$$

Both the connections for the three columns in this example have the *current-FR*-line 5.2 as most optimal, meaning that there are more than one optimal point for both connections. The results of the Pareto optimization are shown in Figure 5.4 in the solid dots. All corners are plotted in the figure but only three of them are Pareto-optimal.

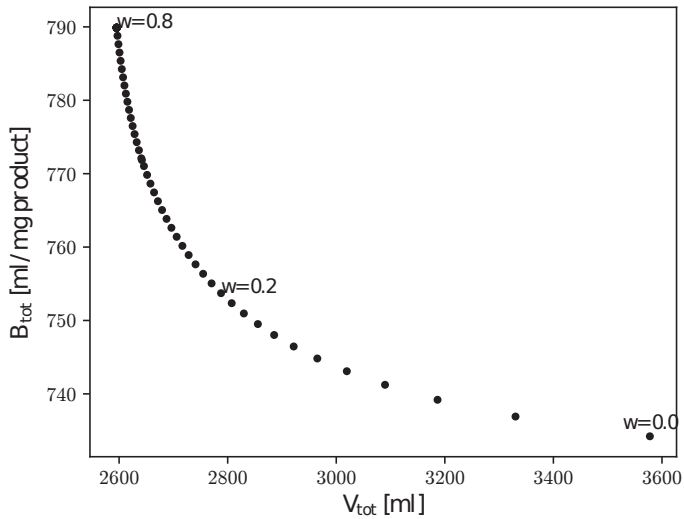


Figure 5.3: A Pareto front comparing total column volume and total buffer consumption in an integrated sequence of five columns. Some of the weights have their corresponding value written out next to them.

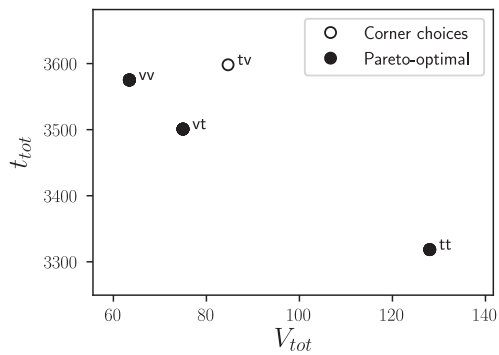


Figure 5.4: All circles were obtained when choosing corners in the $x-y$-plots. The symbols represent the choice in each solution: *vv* is volume-then-volume, *vt* is volume-then-time, *tv* is time-then-volume and *tt* is time-then-time. The full circles are choices selected by the Pareto optimization.

Solution *vv*, volume-then-volume, and *tt*, time-then-time are minimizing the volume and the process time, respectively, while solution *vt* and *tv* are mixes of the two, where *tv* was exempted in the Pareto optimization.

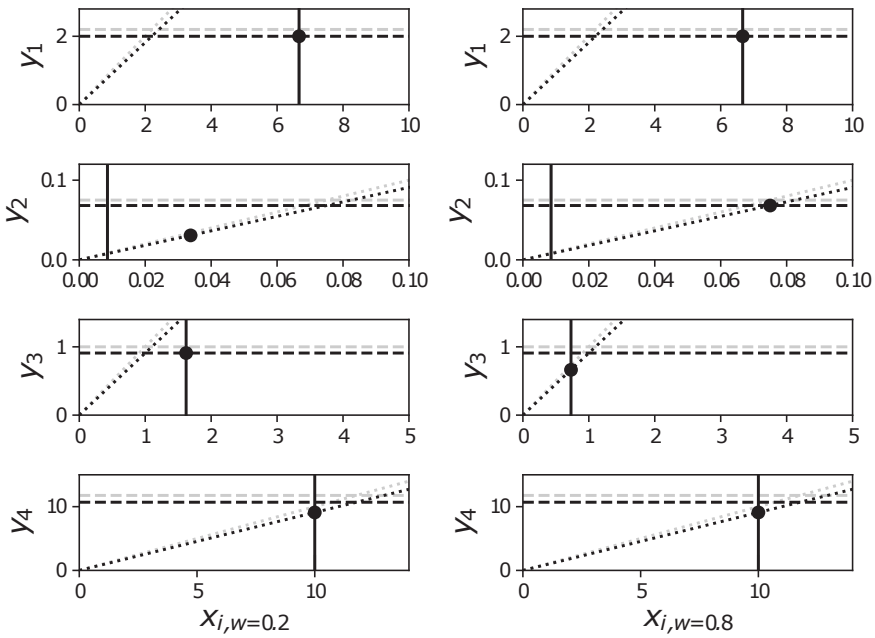


Figure 5.5: $x-y$-plots over the five-column sequence with the arbitrarily chosen $w = 0.2$ in the left sub figures and $w = 0.8$ in the right sub figures. The grey lines are without an included robustness factor which is included in the paper.

5.1.3 Multiple or single optimal solutions

A general trend is that most solutions are in the corners and not on the line. However, in the case with the five-column sequence, there are in some solutions optimal points on the line between the corners. This is seen in Figure 5.5 in the second subplot with $w = 2$. This is explained by the size difference in the second column in the sequence relative to the other columns, see x-values in the figure. It is bigger because it is of type size exclusion which require the volume to be scaled in proportion to the received pool.

The large column affects the total column size which is why the algorithm punishes high x-values. Too small x-values on the other hand will severely limit the flow rate as the difference in capacity is great and the y-value then becomes minimal, following the *current FR limit*. This aspect is primarily a product of having buffer exchange and size exclusion columns in the middle of the sequence. Another equation, 5.13, is used for capacities where ϕ denotes the receiving pool volume in units column volumes and c is now based in volumetric capacity to get a good separation or buffer exchange.

$$x_i \geq \frac{\phi_{E,i-1}}{c_{i,volume}} \tag{5.13}$$

As a side note, it is possible to calculate if there is a single optimal solution or if

all solutions on the *current FR-limit* are solutions. If equation 5.14 is satisfied there are multiple optimal solutions on the line.

$$\frac{F_{i-1,max}}{F_{i,max}} \leq \frac{c_{o,weight}}{c_{i,weight}} \frac{\prod_{j=0}^{i-1} u_j}{\prod_{j=1}^{i-1} x_j} \tag{5.14}$$

A general recommendation is to focus on keeping volumes down because later columns in the sequence would have to keep up with higher volumes to keep the flow rate high. In practice, there will most often be only a single choice when selecting flow rate and column volumes since modern bind and elute-resins usually have a high capacity, and especially capture columns which are first in the sequence.

Potential reactors or UFDF steps between interconnected chromatography steps would split the sequence in two because the elute and load steps would not occur simultaneously.

There is a great difference in costs between resin materials. An improved optimization study would include resin cost instead of simply the total resin volume. The suppliers also sell chromatography columns in discrete sizes with varying lengths and then also different maximum flow rates or pressure drops. A mixed integer non-linear study would be interesting to perform on actual columns from vendors, including prices as well as their pre-packed column selection.

5.2 PCC optimization

This section will give a brief description of the optimization for the PCC step in Paper VI. The purpose is to maximize the resin utilization of the PCC without losing any product in the second column.

Primarily, the PCC had to be designed so that continuity of feed is maintained, implying that the load time must be greater or equal to all recovery steps. This is called the feed continuity constraint (Godawat et al., 2012), which also the following polishing step must adhere to. An empirical model described by Godawat et al. (2012) was used for the optimization of a PCC. It is shown in Equation 5.15 below. A breakthrough curve can also be modelled with thermodynamic kinetics and the mass transfer theory (Gomis-Fons et al., 2020; Perez-Almodovar and Carta, 2009).

$$C = C_F \frac{1}{2}\left(1 + erf\left(\frac{x - k_s}{\sigma}\right)\right) \tag{5.15}$$

where C and C_F are the outlet and feed concentration in mg/mL, respectively, erf is the error function, x is the loaded mass in mg/mL resin, k_s is the binding capacity in mg/mL resin, and σ is an empirical dimensionless constant.

Interconnected PCC cycles, which are even numbers in Figure 4.6, correspond to V_{cycle} in Figure 5.6, between V_1 and V_2. At the start of this cycle, the first column is already loaded with the product corresponding to the area k_3, and the area under the curve, k_2, has been lost. The area k_1 is the breakthrough which is loaded on the second column, while k_4 continues to bind in to the first column. To not lose any product in

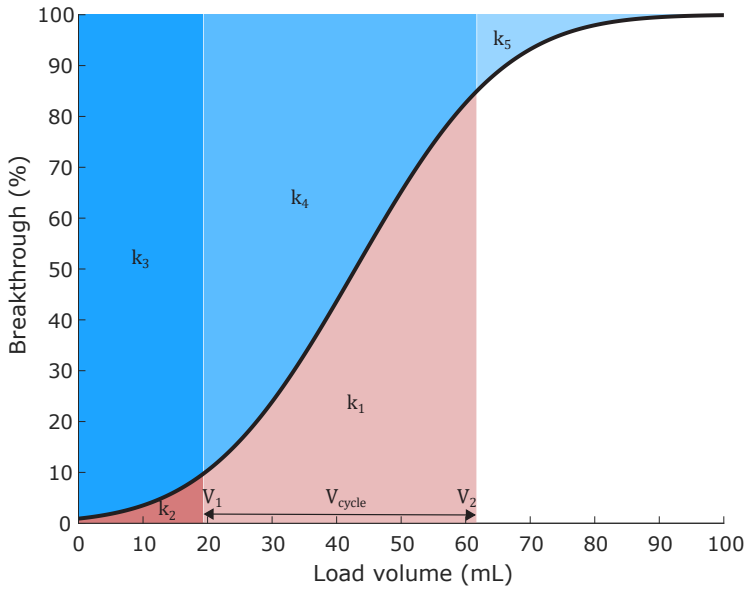


Figure 5.6: Breakthrough curve based on experiments for designing the PCC. V_1 and V_2 are set in this figure based on a 100 % capacity and are for example purposes only.

the breakthrough in the second column in an interconnected state, V_2 and V_1 must be chosen so that k_1 is less than k_3.

After each cycle the capacity of the column decreases slightly, leading to a decrease in the yield. Since the product can find less sites to bind to, it is lost in the breakthrough. This was accounted for and explained in greater detail in Paper VI, where a 10 % capacity loss was assumed. Based on the breakthrough curve in Figure 5.6, 90 % column utilization was found at a loaded volume of 45 ml, which became the new V_2. According to the reasoning above, V_1 was set to 9 ml, which led to a cycle volume of 36 ml. All calculations are dependent on a single flow rate through the column and can thus not be changed, unless the experiment is performed anew.

5.3 Optimal loading

In contrast to the integrated optimization, a local optimization of a capture step was performed and presented in Paper V. The purpose of the study was to optimize the loading step with a number of different loading flow rates. The problem was solved through a model formulated in the paper.

Three key performance indicators (KPIs) together forms the overall objective function. The KPIs are process yield, Y(t), which is the amount of product adsorbed per amount of product loaded, productivity, P(t), is the amount of product adsorbed per time of the complete capture step, and finally the resin utilization, U(t), which is the

amount of product per amount of stationary phase. The final objective function for the optimization is expressed in equation 5.16 and 5.17.

$$-[\omega_Y \widetilde{Y}(t_f) + \omega_P \widetilde{P}(t_f) + \omega_U \widetilde{U}(t_f)] + R_Q \sum_{i=1}^{N_Q-1} \Delta \dot{Q}_i^2 \tag{5.16}$$

$$\sum_{i \in Y,P,U} \omega_i = 1 \tag{5.17}$$

where the term inside the brackets is the sum of the weighted and normalized KPIs. The second term is included to penalize drastic changes in loading flow rates which can cause unwanted pressure spikes, where Q is a flow rate, N_Q the number of different flow rates and R_Q the penalizing factor. The variables to optimize in the complete problem consists of piecewise constant flow rates, the load time, state variables in the model (amount of product in mobile and stationary phases), and an algebraic variable in the model for the adsorption kinetics.

5.3.1 Optimal loading results

The lower figures in Figure 5.8 are solutions with a single flow rate setting while the upper figures have 50 different flow rates, $N_Q = 50$. The left figures have the same weights and so do the right figures. With the option to have multiple flow rates, the solution to the optimization algorithm shows that the flow rate can start high since the product will not compete for as many binding sites in the column, because at the start they are empty. After a while when the sites starts to get used up, the flow rate has to start going down for the product to find empty seats. This continues until the flow rate is too low to keep the productivity at a reasonable level.

While comparing a) to c) and b) to d), the result of the four solutions, shown in Figure 5.8, is that the yields, productivities and utilizations are all significantly improved with a multi-flow rate implementation.

Multiple weight combinations are represented with a Pareto front, see the 3D and 2D plots in Figure 5.8. The multi-flow rate strategy more clearly is superior to the single flow rate strategy in this context.

To be able to make use of optimized loading schemes or intricate and integrated downstream designs from the previous chapter, an advanced and customizable software was devised. It will be introduced in the next chapter.

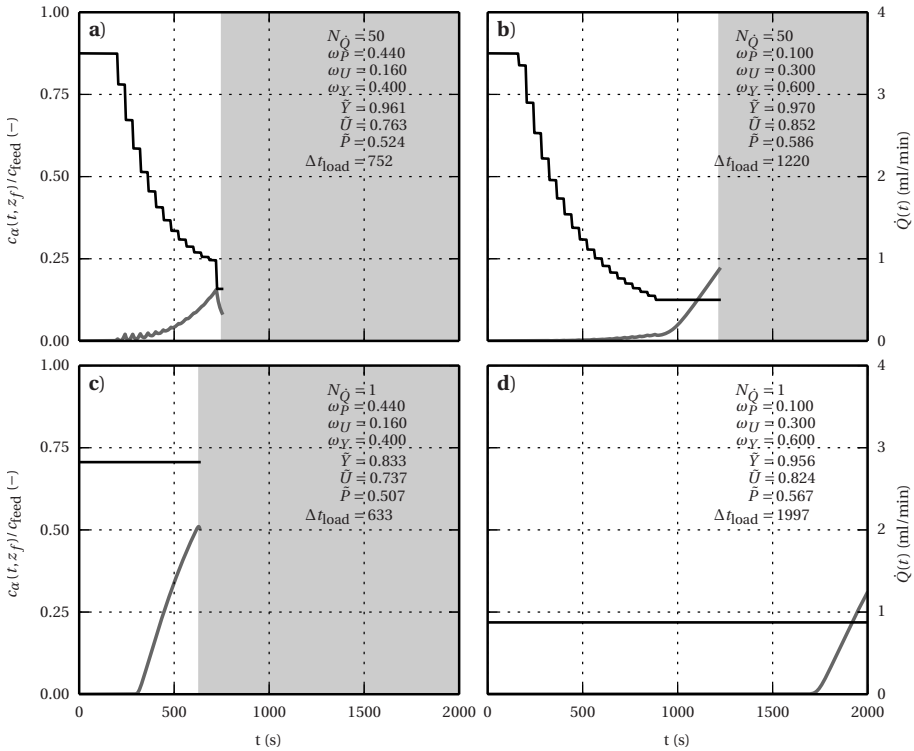
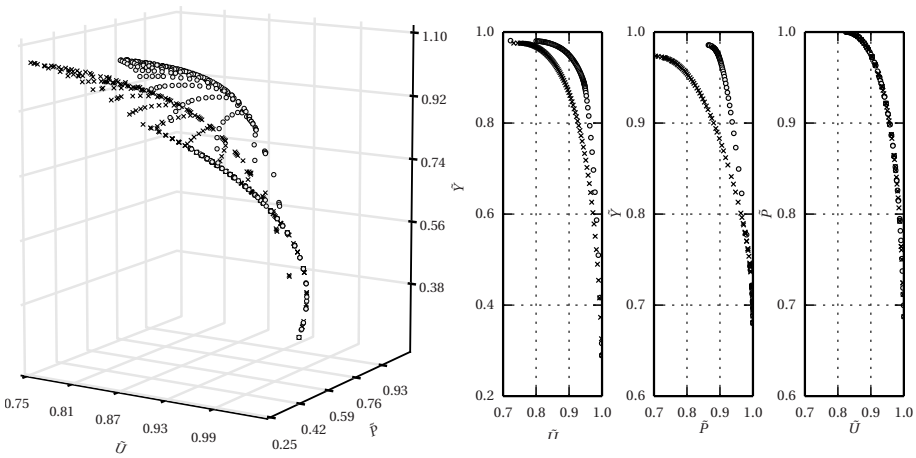


Figure 5.7: The upper figures are solutions for $N_Q = 50$ while lower figures are solutions for $N_Q = 1$. The left figures have the same weights and so do the right figures. The black line is the flow rates while the grey line is product breakthrough at the outlet. The grey zone is time not active.



(a) 3D view

(b) 2D view

Figure 5.8: Multi-flow rate solutions are marked as *o* and single flow rate solutions are marked as *x*. Multi-flow rates outperform single flow rates for most weight combinations.

6

Supervisory control

This chapter will introduce some of the concepts and techniques that in some regards were a prerequisite for the case studies in this thesis. The software that was used for controlling and supervising the systems is called *Orbit*, which was developed at the Department of Chemical Engineering at Lund University (Nilsson et al., 2017). It originally started as a coded implementation of an iterative learning controller (Holmqvist and Sellberg, 2016). It was also used for a discretized multi-level elution trajectory which is similar in the optimization approach to Paper V. The tool made the transition from an optimized trajectory to an experimental implementation seamless. It soon became apparent that there were several benefits to the script-based approach to controlling chromatographic equipment, including its flexibility and customizability as well as the access to the powerful and extensive python libraries.

Orbit has been a tool for several published (including papers I, II, III and VI) and unpublished works since its conception. It was built in Python which is an interpreted, high-level and general-purpose programming language which also supports an object-oriented approach (Rossum, 1995).

6.1 Virtual environment

Gomes et al. (2015) have a vision for integration of PAT with process control where the master controller is an expert system which has a process-wide control strategy. They outline three points that should be fulfilled: a knowledge database that is continuously updated, defined rules of engagement with process sub-units based on PAT and quality attributes, and finally a coupled neural network to predict and detect faults in the running process. The *Orbit* architecture, as will be shown, is a suitable starting point for this vision with its simultaneous control over various unit operations, and due to the fact that it is built in the machine learning-friendly programming language Python.

The purpose of *Orbit* is to be a flexible tool for all kinds of processes related to downstream processing of biopharmaceuticals, including chromatography, UFDF, pH control in reactors. The physical setup is at its core built on the versatile ÄKTA series (Cytiva, formerly GE Healthcare, Uppsala, Sweden), and in particular ÄKTA Pure. The ÄKTA machines are shipped with the software UNICORN^{TM} which has a graphical interface as well as some support for line programming. The user interface of UNICORN

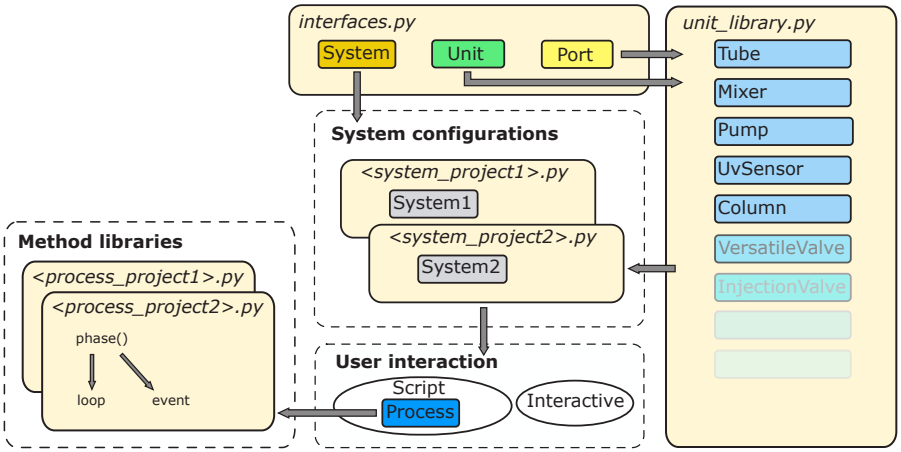


Figure 6.1: The structure of *Orbit*. The files inside the dashed lines are managed by the user. Objects are created based on the system configuration, which than can be controlled through either a predefined script or interactively. The script approach is based on the definition of phases which are interpreted and can perform advanced actions.

communicates with the UNICORN instrument servers which in turn communicate with the hardware. The instrumental server is also accessible to *Orbit* through other softwares, such as OpenOPC *(http://openopc.sourceforge.net/)*, a free open source OPC (OLE for Process Control), or through the REST API extension that is provided in newer versions of UNICORN. This topic will not be further explored for the sake of generality.

Outside the ÄKTA sphere, Bio-Rad have also developed a family of customizable systems for downstream processing. The approach for their software seems to be similar to UNICORN for the ÄKTA, with a slightly more customizable GUI for columns in series.

The virtual structure of *Orbit* can be seen in Figure 6.1. It is built with an object-oriented approach which is appropriate due to the direct physical counterpart in valves, pumps etc. All rectangles inside the files *interfaces.py*, *unit_library.py* and in the system configurations are potential objects that can be instantiated and controlled. Unit objects are the main individual objects that can be controlled and read and ports are the inlets and outlets of the units which, connecting them in a network. System objects is the network, or part of it.

The user interacts in two ways with the system. By either directly controlling it in an interactive way, i.e. reading and writing to the system one line at a time, or through a written script which has predefined phases and processes. The interactive approach also uses the system setup, with its network of units and ports. It is used mostly for simple things that can be performed with a few instructions. The script-based approach utilizes a prepared list of phases, e.g. wash and elution, as well as more complex processes, such as pH-adjustment and UV-pooling. A process object is created in the script file, which contain all of the desired phases in the script, with an example in Listing A.4.

These high-level instructions are decoded in the corresponding method library, to the left in Figure 6.1 and will be further explored below.

6.1.1 Coding

The code in Listing 6.1 is an example of high-level instructions which would perform three phases on a typical bind-and-elute column. Phase A would pump the A1 buffer through column 1 which containing washing buffer, B would load the feed that is on inlet A2 and C would pump eluting buffer from inlet B1 to the column, then pool by volume and direct it to column 2.

```
1          A = Phase(name='wash col1',
2                  SetInlet= {'A':1, '%B':0.0},
3                  Column = col1,
4                  FlowRate = 1.0,
5                  Time = 3.0*60.0)
6
7          B = Phase(name='load col1',
8                  SetInlet= {'A':2, '%B':0.0},
9                  Column = col1,
10                 FlowRate = 2.0,
11                 Time = 10.0*60.0)
12
13         C = Phase(name='elute col1',
14                 SetInlet= {'B':1, '%B':100.0},
15                 Column = col1,
16                 FlowRate = 2.0,
17                 Time = 6.0*60.0,
18                 PoolbyTime = {'startTime':0,
19                               'stopTime':6.0*60.0,
20                               'Next':col2})
21
22         phases = [A,B,C]
```

Listing 6.1: Three example phases with washing, loading and eluting of a single column. In phase C, a more advanced strategy is employed for pooling.

Most instructions, such as *SetInlet* and *FlowRate*, are trivial. They are easy to interpret and simple to perform on most setups. Little knowledge is needed about the system. There are, however, types of instructions that are more advanced and non-trivial, see for example line 18 in phase C, *PoolByTime*. It is an instruction that pools the eluate from *col1* to *col2*, starting at time 0 and ending at minute 6. To perform such an instruction there must be knowledge about how the system is set up and what valves need to turn.

The required knowledge for instructions are stored in two separate files. The first file is the *system* file where units, such as valves, pumps and columns, are instantiated. Instantiation means that the virtual objects are created so that they can be used, and the template for all units are found in the *unit library* file. In the system file (during the instantiation), the units can be given properties to simplify the use of meta paths, e.g. pooling or UFDF.

6.1.2 Phases, Events and Loops

The other file, where knowledge about the system is stored, is the method library where the instructions are interpreted. In this file, a list is created containing new low level instructions for all high level instructions in all phases. A high level instruction, such as *FlowRate* on line 4, is interpreted in a function specifically for *FlowRate* and must

contain specific information on what to do and at what time they should occur, e.g. at time 0, the system pump (which was instantiated in the first file) and the flow rate that is specificed in the value, 1.0. A more advanced high level instruction, such as *PoolByVol*, also has its own interpreting function, but in this case several low level instructions are added to the low-level list which all depend on how the system is set up. Several valves should turn at specified times and the path to the next column opened.

Some instructions are even more complex and require input during the actual process. They are called *Events*. An example of this is UV-based pooling, which constantly checks the UV level for below or above a specified value. When the value is reached, the event is finished and the next instructions can continue. Loops are recurring events that is best performed with another process thread, such as constantly checking the amount loaded in the PCC in paper VI.

6.2 Multiple units

With more complex downstream processes, the need for more equipment increases. Weight scales are useful in some processes, such as in paper III. Other external equipment such as HPLC, splitter valve and fractionaters can also be incorporated if they have a communication port to a PC.

It is also advantageous to communicate with other chromatography systems when there are parallel processes. The design of the PCC in paper VI had to be planned with the polishing step in mind so that each PCC cycle was longer than the polishing step. This is because the PCC has to handle the continuous feed with minimal losses and can not be delayed. After a polishing step was finished it was set to wait for the PCC cycle to sync and send a product pool. The synchronization between the systems was performed with event flags. The extension of the PCC loading time in paper VI, detailed below, also shows the usefulness of synchronization instead of relying on a clock based strategy.

6.3 Advanced strategies

Real-time adaptive algorithms are necessary for robust operation of biopharmaceuticals. Script-based softwares offer in theory unlimited opportunities for implementation of both low-level and high-level controls.

A critical part in producing a consistently high quality product is to have control over the pooling step. Varying concentrations in a continuous feed can affect the cut off points so that unwanted impurities may contaminate the final product if for example the volume is lower so that the set UV level for pooling is detected too late. This was accounted for in Paper VI with a feed forward adaptive pooling control. The product had a tendency to form aggregates which was the cause for the tailing peak. In each cycle, the integral of the pool was calculated to determine the back-end cut off point. A linear relationship between the integral and a desired cutoff level had been established beforehand. The results can be seen in Figure 6.2. This approach is a simple tool to

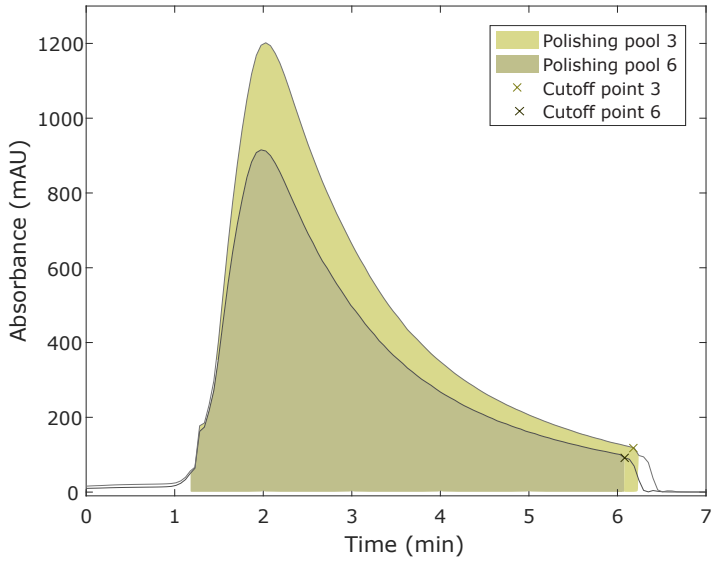


Figure 6.2: Two pools overlaid to demonstrate the adaptive UV-pooling strategy. The cutoff level on the back-end is related to the peak height.

demonstrate the potential in handling biological drifting with PAT tools. Other similar methods for UV-pooling are further explored by Mendhe et al. (2015). In appendix A, a tool for autonomous peak detection is outlined.

6.3.1 Dynamic loading volume of a PCC

As per Chapter 3, there are several methods to handle the feed from upstream directly with direct capture processes. However, the major remaining challenge is how to handle a varying flow rate from the reactor (Fisher et al., 2019). Dynamic steady state control of complex chromatography steps have previously been performed on a model of a Multicolumn Countercurrent Solvent Gradient Purification Process (Papathanasiou et al., 2017). A working real time iterative learning controller, ILC, of a PCC is found in paper VI.

The purpose with an ILC is to keep repetitive commands on a desired trajectory, by adjusting the feedback controller one iteration to the next (Longman, 2000). The controller used in paper VI is a proportional type controller, or previous cycle learning, which in its function uses only signals from the previous iterations (Ouyang and Pipatpaibul, 2010; Xu et al., 2004). The ILC controller is depicted in the diagram in Figure 6.3. The purpose of the controller was to keep the utilization high at a stable level and achieve constant quality product output. The proportional gain of the controller, K, is multiplied with the error, which is the difference between the pool area, y_k, and the target pool area y_{ref}, see Equation 6.1.

$$u_k = u_{k-1} + K(y_{ref} - y_{k-1}) \tag{6.1}$$

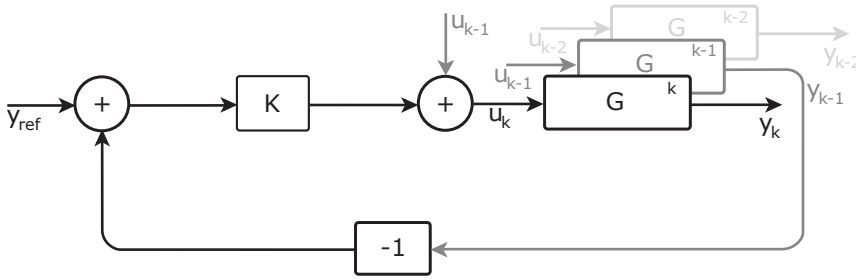


Figure 6.3: The iterative learning controller scheme used for PCC loading time in Paper VI. Each iteration depends on the previous with the purpose of returning the product output, y_k, to the reference value, y_{ref}. K is the controller gain and G is the relationship between load volume and pool integral.

The relationship between u_k and y_k is approximated with G in Equation 6.2.

$$y_k = Gu_k \tag{6.2}$$

The controller gain is set with regards to the convergence rate in Equation 6.3, based on a parameter a (Xu et al., 2004).

$$||1 - GK|| = a < 1 \tag{6.3}$$

Equation 6.3 is rewritten as Equation 6.4. The value of a is between 0 and 1. If the value is set to 0, the controller might become too aggressive since the relationship is not exactly reflected in G. A value of close to 1 would be extremely slow. In the paper, a was set to 0.3.

$$K = (1 - a)G^{-1} \tag{6.4}$$

Equations 6.1, 6.2 and 6.4 together are rewritten to form Equation 6.5. This relationship was used in each iteration to find the value of u_k to determine the loading volume.

$$u_k = u_{k-1}(a + (1 - a)\frac{y_{ref}}{y_{k-1}}) \tag{6.5}$$

A step reduction of 20% in the load concentration was introduced to test the controller and the loading time of the PCC was successfully increased to match the pool after the first cycle, see the c1A output signal and cycle load increase in Figure 6.4. C1B is an extrapolated comparison of the same process with no controller.

6.3.2 Programming considerations

Some practical aspects of operating *Orbit* is detailed in this section, which are critical for getting up and running quickly. Python is a scripting language meaning that is not compiled before the code is executed and any errors that come up during the live process will interrupt and possibly ruin the current experiment.

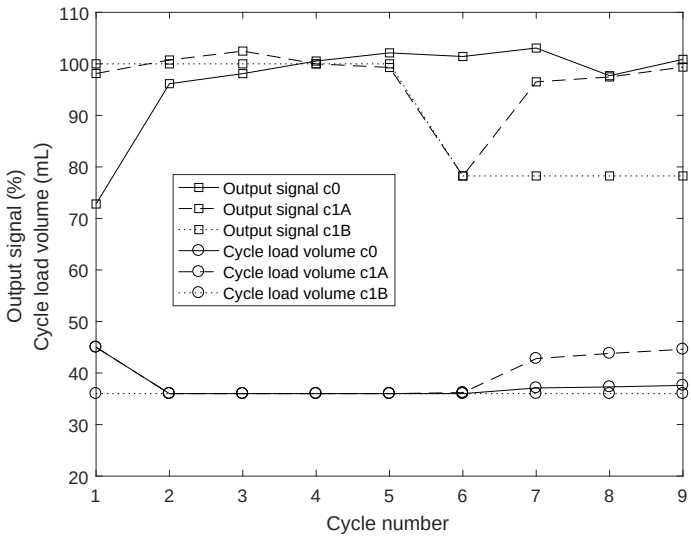


Figure 6.4: The output signal is the resulting area of the product peak in the polishing step in percent of the average, plotted together with its corresponding cycle load volume. c0 has no disturbance, c1A has a disturbance and with the controller that regulates up the load volume so that the output signal returns to the set point, and c1B is an extrapolated version with a disturbance but has no controller.

There are mainly two types of errors to be aware of when writing code. They are classified as syntax errors and logic errors. Syntax errors are the most simple type and arise when a command line can not be interpreted due to incorrect typing or phrasing. It will be highlighted and is easily fixed. Logic errors are harder to detect because often the program will not crash, just behave incorrectly. Examples of logic errors in *Orbit* are badly programmed pathways through the system, lack of timing and sequential ordering during complex phases. Simulated runs can be performed with fake signals to counteract simple errors.

With physical systems there are more than only programming considerations to be aware of. Sometimes it can be hard to distinguish if an error is caused by a programmed logic error or if there is something wrong with the hardware, the buffer solutions, or even the product. An example of this is if the product would not be binding to a column after being diluted inline. Were the correct pathways opened or closed at the correct time? Was the diluting buffer properly prepared? Did the dilution mix sufficiently with the pool so that the target pH and conductivity was reached? Did both pumps work so the proportions were correct? Was everything upstream performed accurately or was the product already spoiled?

With all these questions to a single error it is apparent that a holistic approach to the design and the programming is necessary. Since many operations interact with each other it is an advantage if as many as possible of the potential errors can be prevented proactively by looking for and detecting illogical phases, such as pooling to a column

that is placed on the same valve as the eluting column is placed on.

During building of phases and corresponding low-level instructions and events it is easy to slip up with timings. A safeguard was introduced to quickly check the timing to avoid any simple mistakes. The hardware was monitored with UNICORN. Incorrect opening and closing of valves could be avoided partly when creating meta-pathways so that slip ups are minimized. The important buffers and their CQAs have to be systematically prepared and monitored, and the mixing of the dilution buffer and the pool would have to be tested properly.

6.4 Data handling

In light of Sections 3.4 and 3.5, it is imperative to have a systematic approach to be able to make use of acquired data during currently running processes and from previous processes. All raw data from the sensors is stored in vector format in the virtual sensor objects created at the start of the project. It is accessible throughout the run over all systems. Additional variables can be created to store data which was performed for ILC controller to update the integral value.

To help the user with supervising the process, data from unit operations is constantly written in an html file that is accessible in the working folder of the project. It contains all necessary data for phases, events and loops.

6.5 Plant-wide control

Plant-wide control is concerned with finding which design structure is most optimal for a complete plant. The most important aspect is which variables to measure and control to keep within regulatory requirements. To design such a process, Downs and Skogestad (2011) propose both a top-down analysis, to select controlled variables, combined with a bottom-up synthesis of the control loops. The control loops form a stabilizing layer in the plant and the top-down approach creates control layers with the purpose of maintaining CQAs at a set point (Lakerveld et al., 2013). A reason why this approach has not been fully implemented is due to the multitude of CQAs, system complexity, lack of real-time testing and drifting of biological systems (Fisher et al., 2019; Hong et al., 2018).

The studies in this thesis have explored the potential of connecting multiple purification steps with a small factory footprint and some control loops have been demonstrated with good results. The *Orbit*-platform offers potential for more real-time testing to monitor CQAs, and with access to a multitude of advanced libraries connected to Python, some of the visions of Gomes et al. (2015) could soon become reality.

7

Concluding remarks

The work in this thesis was mainly focused on techniques for improving downstream effectiveness and flexibility. The integrated systems were shown to effectively process industrially relevant material in an efficient manner in regards to productivity and quality, reducing the number of in-between steps significantly.

The first integrated system, explored in Paper I, was a concept study with model proteins. The second integrated system in Paper II is a follow-up to the first study, applying the tools for an industrially relevant case. In Paper III, another industrially relevant and more complex process is exemplified, combined with an integrated UFDF technology. The final integrated system in this thesis is demonstrated in Paper VI which was a multi-system setup with a continuous PCC, adaptive pooling technology and an iterative learning control scheme to handle varying product titers.

An optimization study was performed to explore the relationship between process time and column volumes, and find tools for designing complete integrated downstream process. Another optimization study was performed to showcase the performance when implementing a multi-flowrate approach to the loading step of a capture column.

The software design has a demonstrated flexibility for various purposes and supports radical design modifications. Furthermore, advanced control algorithms and layers have effectively been implemented which showcases its power in dealing with future needs.

With higher productivity, smaller factory footprint and a high degree of flexibility, these new techniques are tools that can be part of a changing industry and increase the availability of coming biopharmaceuticals. One of the most direct challenges for implementation is in making the systems user friendly for production or research personnel.

7.1 Future work

From a business perspective, user friendliness is an important characteristic for actual implementation, if that is the immediate goal. Other important improvements should focus on quality control, due to the added complexity. More sensors can be implemented for a direct access to CQAs, such as at line HPLC systems. There are advanced machine learning algorithms, such as neural networks that can be used for controlling CQAs, which often behave non-linearly. To implement these systems, a large amount of data needs to be collected and analysed, meaning that data systems should be implemented

as early as possible. Knowledge management is a discipline with visualization tools, statistical analyzers, capture tools, and document management systems, that is not yet represented in a scientific context related to pharmaceutical production.

Downstream processing unit operations are generally built for batch-processing purposes even though they can be used semi-continuously, such as in PCC. To have a truly continuous process, individual unit operations need to be re-designed with that in mind. This would preferably be performed by vendors to more quickly reach the industry.

Other potential future work would be to develop expert systems for optimizing complete downstream processes, using knowledge about the target protein and its impurities. The optimization would not only be applied to column sizes and flow rates, but also the type of resin and in which order they should be placed.

A

Peak detection

In this Appendix, a detection tool for peaks is outlined. It is based on the smoothed derivatives of the UV output signal. The example below is demonstration purposes and is a reaction that is sampled and occur over time.

Example reaction

In Figure A.1, a reaction can be seen over 10 analyzed samples. The red line, which is the last peak that is detected, decreases in area, while the areas of the other peaks increases. Samples from iteration 0 and 10 are shown in Figures A.2 and A.3, respectively.

Detection algorithm

Smoothed values and derivates are necessary for handling the data. Otherwise the first and especially the second derivatives would be too noisy to use. A Savitzky-Golay filter was used for this purpose with a window length of 61 and poly order of 2.

First, a base line is established. This is performed by finding all the points where the smoothed first derivative is around 0, plus/minus 1 or any other reasonable value. In order to safely establish that these values are at the baseline and not on a plateau, another condition must apply. It is that the smoothed derivative value must be around 0 over a specified range. In this example this range was set to 40 x-values. When all values that match these conditions are found, they are avaraged and a base line value is established. These conditions are applied in Figure A.4 and sample code is found in Listing A.1.

```
for idx,tVal in enumerate(t[:-derHorizonLen]):
    if all(np.absolute(val) < derivativeLimit for val in \\
    uvPrimSav[idx:idx+derHorizonLen]):
        uvZeroSum.append(uv[idx])
```

Listing A.1: Base line calculation

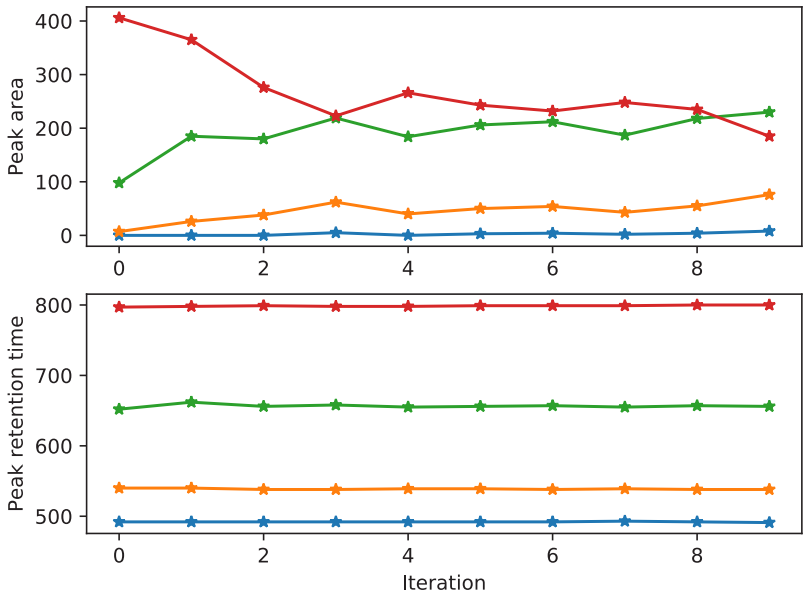


Figure A.1: Areas and retention times detected for each sampled instance. Notice that the red line decreases in area while the other increases. The retention times are not moving.

The mean noise value is the mean absolute difference between each y-value and its corresponding smoothed value.

To find a peak, the entire curve is traversed again, looking for values matching a new set of conditions matching a peak top. The first condition is that the y-value should be positive. The second condition is that the first derivative should cross zero, i.e. be positive or zero and the value in the next position negative. The third and final condition is that the second derivative must be negative since the first derivative goes from positive to negative. See Figure A.5 and Listing A.2.

```
for i, uvPrimval in enumerate(uvPrimSav):
    if uvBisSav[i] < 0 and uvSav[i] - uvZeroBaseLine > peakStartLevel \\
    and uvPrimSav[i] <= 0 and uvPrimSav[i-1] > 0:
        topIndex.append(int(i))
```

Listing A.2: Peak detection

When the top values are found, the peak starts and ends must be identified. They can be either connected to the base line or directly to another peak. Both of these occurrences are seen in Figure A.5. Two conditions can find the start of a peak as in Listing A.3. The first condition is if the UV value, when traversed backwards from the top index, is below a specified value for peak detection, minus the baseline value. This specified value can be set to any reasonable value, and in this case it was set to 5. The

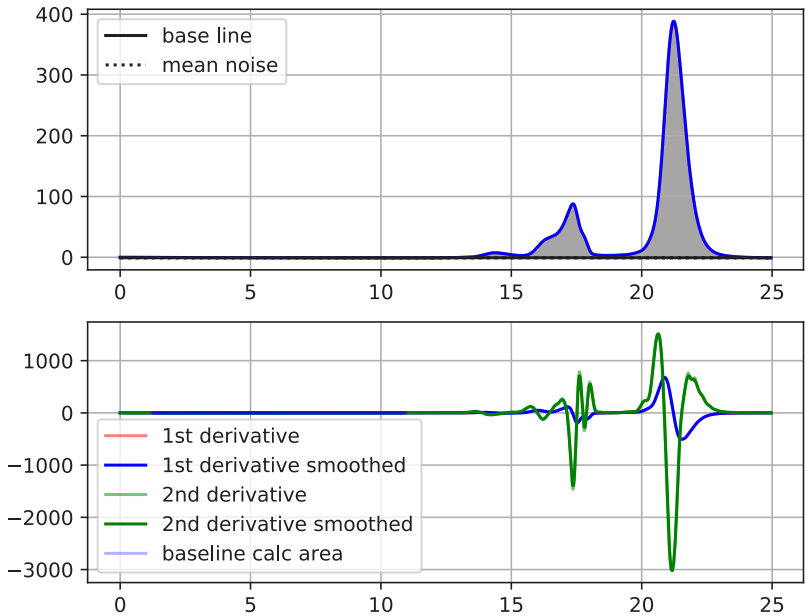


Figure A.2: Sample 1. The grey areas in the upper figure are the detected peaks. The lines in the lower figure are the derivatives of the UV, and a line for showing where the baseline is calculated.

second condition that can signal a peak start is if the first derivative crosses zero in negative direction (traversed backwards) and the second derivative is positive. A peak end is detected and identified in a similar fashion.

```
for i, tVal in reversed(list(enumerate(t[:topIndexValue]))):
    if uvSav[i] - uvZeroBaseLine < peakStartLevel:
        return int(round(i))
    elif uvBisSav[i] > 0 and uvPrimSav[i] >= 0 and uvPrimSav[i-1] < 0:
        return int(round(i))
```

Listing A.3: Finds peak start or valley

The areas of the peaks are calculated easily when the indexes for peak starts, tops and ends, as well as the baseline, is found.

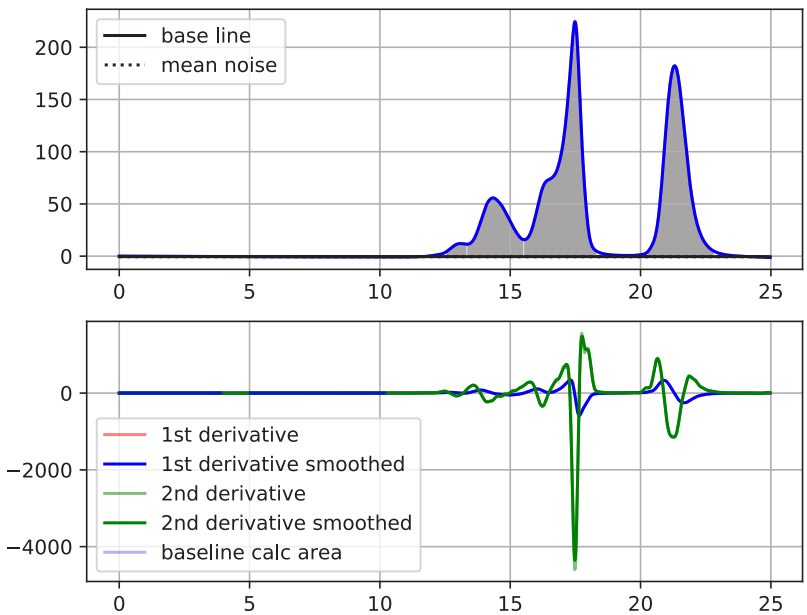


Figure A.3: Sample 10. Same principles as in Figure A.2.

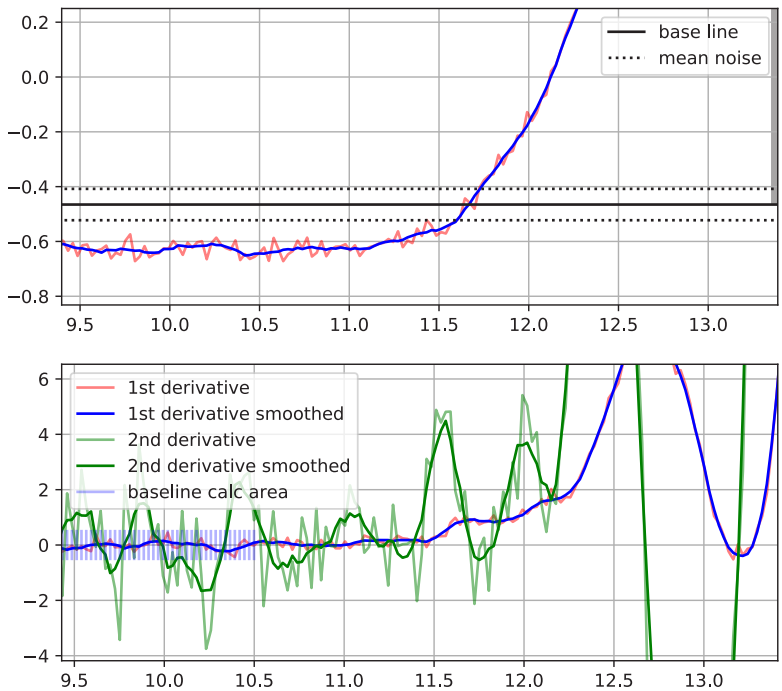


Figure A.4: Zoom in on the base line with the zone (to the left in the lower figure) for which the base line is calculated. The zone in which the base line is calculated in is only valid where the derivative is around zero.

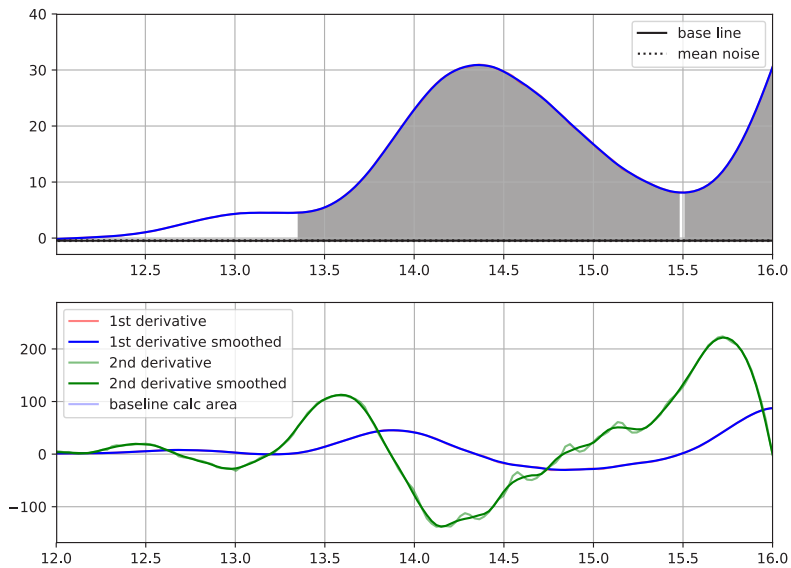


Figure A.5: Zoom in on the first peak with the derivative shifts. It is mainly the first derivative which determines the peak starts, tops and ends.

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Manufacturing of biopharmaceuticals is a costly and time consuming process due to its inherent complexity and variability. The downstream process is the most expensive part and it involves multiple non-trivial steps. Continuous integrated manufacturing is a new concept that is encouraged by authorities. The advantages include uninterrupted operation, automation, consistent product quality, lower residence times and decreased capital and operational costs.

Novel techniques and concepts require a holistic process understanding as well as a technical competence to implement them, and to do that, there are multiple practical challenges that must be overcome. There is a growing need to rapidly adjust to market demands and also to decrease costs as the industry gets increasingly competitive. The ability to adjust a facility or production line, to manufacture a variety of biopharmaceuticals, must be taken into consideration when developing integrated systems. It is also important to be aware of how to monitor critical quality attributes to properly ensure product quality. Another important attribute of integrated systems is intuitive and how easy to use they are.

The aim of the thesis is to demonstrate, based on a selection of case studies, how some of the presented challenges can be overcome. The case studies have integrated systems that are flexible and have advanced process analytical technology. One of the papers in this thesis also focuses on the optimization of coupled columns sequences with the purpose of reducing costs. Overall, the results of this thesis shows the potential of continuous integrated designs for purification of biopharmaceuticals while simultaneously increasing both performance and product quality.

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