Design and Control of Integrated Continuous Processes for the Purification of Biopharmaceuticals

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DOCTORAL DISSERTATION

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Title and subtitle

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Abstract

The production of biopharmaceuticals so far has been based on batch processes that are robust and well-known, but very inefficient and inflexible, causing the products to be very expensive and process development to be slow and costly. The production of biopharmaceuticals is thus changing towards integrated continuous biomanufacturing (ICB) in order to reduce costs and increase flexibility in a constantly changing market. Continuous processing has been successfully implemented in upstream operation through the use of perfusion bioreactors, but the maturity required for the widespread use of ICB on commercial scale has not yet been achieved in integrated continuous downstream processes (ICDPs). The research presented in this thesis provides several tools for the design, optimization, control and scale-up of ICDPs for the purification of biopharmaceuticals with the aim of reducing the technological gap in downstream processing. The feasibility of implementing these processes in general platforms on laboratory and pilot scale has also been demonstrated.

Process control and automation form a central part of the work presented in this thesis, including the development of several control strategies such as controlling the loading phase in the chromatography column, optimal product pooling in the elution phase, and adjusting and monitoring the pressure in an ultrafiltration process. In addition, existing research software has been further developed to enable automation in a number of different applications.

The implementation of an ICDP requires a specific design approach to enable process integration and continuity of the feed from the upstream process. Several design equations were used for process integration. Feed continuity was achieved by employing periodic multi-column chromatography in the capture step. Process scheduling is therefore very important in this case, as the cycle time must be matched to the product recovery time. The effects of different integration approaches on process scheduling, and thus the overall productivity, was studied. Periodic multi-column chromatography not only allows for a continuous feed, but can also lead to increased productivity and resin utilization, as in the case of the periodic counter-current chromatography (PCC) process described in Paper III, where model-based optimization was performed. Another tool used to increase process efficiency in a downstream process was model-aided flow programming (Paper V), where a variable flow rate was used in the loading phase to achieve higher productivity and resin utilization.

The feasibility of ICDPs was demonstrated by implementing them in different applications. Chromatography and ultrafiltration technologies were integrated in a single system (Paper I), and a complete ICB process was developed for the production of monoclonal antibodies (mAbs) (Paper II). Paper III describes the integration of a 3-column PCC capture step in a downstream process for the purification of mAbs. Continuous solvent/detergent-based virus inactivation and continuous capture were combined in an ICDP (Paper IV). The downstream processes described in Papers II and III were further developed to allow for the purification of pH-sensitive mAbs (Paper VI), and this process was coupled with the upstream system and run on pilot scale, demonstrating its feasibility on a larger scale (Paper VII).

The results of this research show that ICDPs outperform traditional manual batch downstream processes. Automation, integration and continuous biomanufacturing lead to higher productivity, shorter process time, more rapid development of biopharmaceuticals, and lower investment costs.

Key words

Integrated Continuous Biomanufacturing, Downstream Processing, Process Design and Optimization, Process Control, Biopharmaceuticals

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Tot està per fer i tot és possible
(Everything is yet to be done,
yet everything is possible)

— Miquel Martí i Pol

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Popular Scientific Summary

Getting one step closer to global access to life-saving pharmaceuticals

Biopharmaceuticals are drugs that are produced using living organisms, and are used for the prevention of infectious diseases, as in the case of vaccines, and for the treatment of a broad range of diseases, such as cancer and hereditary disorders, having become an essential part of modern medicine. The drawback is that they are very expensive. For example, the cost of cancer treatment with a biopharmaceutical such as monoclonal antibodies is about $100,000 per patient per year. One way of reducing the cost is to increase the efficiency of the production process.

Speeding up the development of biopharmaceuticals

One of the things we have learnt from the COVID-19 pandemic is that reducing the development time of a biopharmaceutical can save many lives in the case of a global health emergency. In addition, it also reduces the price of the drug as the development of a biopharmaceutical is very costly, and accounts for a significant part of the total production cost. Therefore, developing new processes that allow rapid and straightforward scale-up should be a priority.

Integrated continuous processes for the purification of biopharmaceuticals

Integrated continuous biomanufacturing can be used to speed up the development of biopharmaceuticals and reduce costs, thus improving global access. In an integrated continuous process, all the operations are physically connected to achieve a single continuous flow, unlike the traditional batch processes, where there is no connection between the operations. This approach provides higher productivity, requires less equipment, and offers greater flexibility, and thus a lower production cost and a shorter development time. The purification of biopharmaceuticals is a very important part of the production process, but is very costly. For this reason, several methods of designing, controlling and optimizing integrated continuous processes for the purification of biopharmaceuticals are described in this thesis.

Process modelling, automation, design and optimization

Process modelling was used to predict the process performance based on factors affecting the process, and the information obtained was used to improve process control and design. A continuous process must be automated so that it can work without human intervention. Various methods of controlling and automating the process were thus investigated. The design of the unit operations in an integrated continuous purification process is complex and requires specific approaches that are presented in this thesis. Several examples of ways in which the optimization of

a process can be aided by modelling are also presented, as a means of increasing process efficiency.

These new techniques were successfully tested experimentally on laboratory and pilot scale for the integrated continuous purification of biopharmaceuticals. In all the cases studied, the unit operations in the purification processes were integrated with minimal use of equipment, and the processes were automated and controlled using Orbit, software created in my research group and further developed in this work to allow the application of the complex process sequences that are necessary in an integrated continuous process. The potential of integrated continuous biomanufacturing to reduce production costs and minimize the development time of a biopharmaceutical was demonstrated by the high productivity, high utilization of the equipment and increased automation, allowing the process time to be reduced from days to hours.

Populärvetenskaplig Sammanfattning

Ett steg närmare global tillgång på livräddande läkemedel

Biologiska läkemedel är läkemedel som produceras med hjälp av levande organismer och som används för att förebygga smittsamma sjukdomar, så som är fallet med vaccin, och för att behandla ett brett spektrum av sjukdomar, såsom cancer, samt ärftliga sjukdomar. De har blivit en viktig del av modern medicin. Nackdelen är att de är väldigt dyra. En cancerbehandling med ett biologiskt läkemedel såsom monoklonala antikroppar kostar cirka 100 000 USD per patient och år. Ett sätt att sänka kostnaderna är att öka effektiviteten i produktionsprocessen.

Att påskynda utvecklingen av biologiska läkemedel

En av sakerna som COVID-19 pandemin har lärt oss är att minskad utvecklingstid av ett biologiskt läkemedel kan rädda många liv i en global nödsituation. Detta leder också till ett lägre läkemedelspris, eftersom utvecklingen av biologiska läkemedel är väldigt dyr och står för en betydande del av den slutliga kostnaden. Utvecklingen av nya processer som möjliggör snabb och enkel uppskalning av produktionen borde därför vara en prioritet.

Integrerade kontinuerliga processer för upprening av biologiska läkemedel

Integrerad kontinuerlig bioteknisk produktion kan användas för att påskynda utvecklingen av biologiska läkemedel och samtidigt minimera kostnaderna, och på så sätt förbättra den globala tillgången. I en integrerad kontinuerlig process är alla processteg fysiskt sammankopplade, för att möjliggöra ett kontinuerligt flöde i tillverkningsprocessen, i motsats till de traditionella satsvisa processerna, där det inte finns någon koppling mellan processtegen. Det här tillvägagångssättet ger högre produktivitet, kräver mindre utrustning och erbjuder högre flexibilitet, vilket resulterar i både lägre produktionskostnader och kortare utvecklingstid. Upprening av bioläkemedel är en väldigt viktig del av produktionsprocessen, men är väldigt kostsam. I den här avhandlingen beskrivs flera olika metoder för att designa, styra, reglera och optimera integrerade kontinuerliga processer för upprening av biologiska läkemedel.

Processmodellering, automatisering, design och optimering

Processmodellering med utgångspunkt från kända faktorer som påverkar processen användes för att förutsäga processprestandan, och den information som erhölls användes i sin tur för att förbättra processregleringen och designen. En kontinuerlig process ska vara automatiserad så att den kan fungera obemannad. Därför undersöktes olika strategier för att styra och automatisera denna process. Designen av en integrerad kontinuerlig process är komplicerad och kräver särskilda metoder som presenteras i den här avhandlingen. Några exempel på hur

modellering kan vara ett hjälpmedel för att optimera processen och således förbättra processeffektivitet presenteras också.

Dessa nya metoder testades med framgång på både laboratorie- och pilotskala för integrerad kontinuerlig produktion och upprening av biologiska läkemedel. De integrerade processerna implementerades med minimal användning av utrustning i alla studerade fall, och processerna automatiserades och styrdes med hjälp av Orbit, en mjukvara som har utvecklats i min forskargrupp och som i det här arbetet anpassades för att möjliggöra styrning av de komplexa processekvenser som krävs i en integrerad kontinuerlig process. Den potential som integrerad kontinuerlig bioteknisk produktion har för att minimera produktionskostnaderna och utvecklingstiden demonstrerades genom den höga produktiviteten, det höga nyttjandet av utrustningen och den ökade graden av automatisering, vilket gjorde att processtiden kunde reduceras från dagar till timmar.

Resumen de Divulgación Científica

Un paso más cerca del acceso global a medicamentos imprescindibles

Los biofármacos son medicamentos que se producen mediante el uso de organismos vivos y se usan para la prevención de enfermedades contagiosas, como en el caso de las vacunas, y para el tratamiento de un gran número de enfermedades, como por ejemplo el cáncer o ciertos trastornos hereditarios, habiéndose convertido en un componente esencial de la medicina moderna. El inconveniente de los biofármacos es que tienen un precio muy elevado. Por ejemplo, el coste de tratamiento de un cáncer con un biofármaco como los anticuerpos monoclonales es de alrededor de 100.000 dólares estadounidenses por paciente y año. Una manera de reducir este coste es aumentando la eficiencia del proceso de producción.

La importancia de acelerar el desarrollo de biofármacos

Una de las cosas que hemos aprendido de la pandemia de COVID-19 es que reducir el tiempo de desarrollo de un biofármaco puede salvar muchas vidas en caso de emergencia sanitaria global. Además, también permite reducir el precio final del medicamento, ya que el desarrollo de un biofármaco tiene un alto coste y supone una parte importante del coste final de producción. Por lo tanto, la creación de nuevos procesos que permitan un desarrollo y escalado rápidos y sencillos debe ser una prioridad.

Procesos integrados y continuos para la purificación de biofármacos

Los bioprocesos integrados y continuos permiten la aceleración del desarrollo de biofármacos y la reducción de costes, facilitando así el acceso global a estas medicinas. En un proceso integrado y continuo, todas las operaciones están físicamente conectadas para obtener una corriente continua, a diferencia de los tradicionales procesos por lotes, donde no hay una conexión entre las operaciones. Este tipo de procesos tienen una mayor productividad, requieren de menor equipamiento y ofrecen una mayor flexibilidad, por lo que tanto el coste de producción como el tiempo de desarrollo del fármaco son menores. La purificación de biofármacos es una parte del proceso de producción muy importante, pero supone un gran coste. Por esta razón, en esta tesis, se presentan varios métodos de diseño, control y optimización de procesos integrados y continuos para la purificación de biofármacos.

Modelización, automatización, diseño y optimización del proceso

En este trabajo, se hizo uso de modelos para predecir el rendimiento del proceso, y así mejorar el diseño y control del proceso con la información obtenida. Un proceso continuo debe estar automatizado para que pueda funcionar sin intervención humana. Por esta razón, se investigaron diversos métodos para el

control y automatización del proceso. El diseño de las operaciones unitarias en un proceso de purificación integrado y continuo es complejo y requiere de unos procedimientos específicos que se presentan en esta tesis. Además, también se enseñan algunos casos de optimización con la ayuda de modelos para incrementar la eficiencia del proceso.

Los métodos desarrollados fueron probados experimentalmente con éxito en escala de laboratorio y piloto, para la purificación integrada y continua de biofármacos. En todos los casos estudiados, las operaciones unitarias en los procesos de purificación fueron integradas minimizando el uso de equipamiento, y la automatización y control de los procesos se consiguió gracias a Orbit, un programa creado en mi grupo de investigación y que se ha seguido desarrollando como parte de este trabajo para permitir la aplicación de secuencias de proceso complejas que son necesarias en un proceso integrado y continuo. En esta tesis, se ha demostrado el potencial de los bioprocesos integrados y continuos para reducir costes de producción y minimizar el tiempo de desarrollo de biofármacos, debido a su alta productividad, su alto aprovechamiento de los equipos y su mayor automatización, lo cual permite reducir el tiempo de proceso de días a horas.

List of Publications

This thesis is based on the publications listed below, which will be referred to by their Roman numerals throughout the thesis.

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

II. **Gomis-Fons J**[*], Schwarz H[*], Zhang L, Andersson N, Nilsson B, Castan A, Solbrand A, Stevenson J, Chotteau V. Model-based design and control of a small-scale integrated continuous end-to-end mAb platform. *Biotechnology Progress.* 2020; 36(4): e2995.

III. **Gomis-Fons J**, Andersson N, Nilsson B. Optimization study on periodic counter-current chromatography integrated in a monoclonal antibody downstream process. *Journal of Chromatography A*. 2020; 1621: 461055.

IV. Löfgren A[*], **Gomis-Fons J**[*], Andersson N, Nilsson B, Berghard L, Lagerquist Hägglund C. An integrated continuous downstream process with real-time control: A case study with periodic countercurrent chromatography and continuous virus inactivation. *Biotechnology and Bioengineering*. 2021; 118(4): 1645-1657.

V. **Gomis-Fons J**[*], Yamanee-Nolin M[*], Andersson N, Nilsson B. Optimal loading flow rate trajectory in monoclonal antibody capture chromatography. *Journal of Chromatography A*. 2021; 1635: 461760

VI. Scheffel J[*], Isaksson M[*], **Gomis-Fons J**[*], Schwarz H, Andersson N, Norén B, Solbrand A, Chotteau V, Hober S, Nilsson B. Design of an integrated continuous downstream process for acid-sensitive monoclonal antibodies based on a calcium-dependent Protein A ligand. *Journal of Chromatography A*. 2022; 1664: 462806

VII. Schwarz H[*], **Gomis-Fons J**[*], Isaksson M[*], Scheffel J[*], Andersson N, Andersson A, Castan A, Solbrand A, Hober S, Nilsson B, Chotteau V. Integrated continuous biomanufacturing at pilot scale for pH-sensitive monoclonal antibodies. 2022; Submitted for publication.

[*]Shared first authorship

Other related publications

Löfgren A, Yamanee-Nolin M, Tallvod S, Gomis-Fons J, Andersson N, Nilsson B. Optimization of integrated chromatography sequences for purification of biopharmaceuticals. *Biotechnology Progress*. 2019; 35:e2871.

Moreno-González M, Keulen D, Gomis-Fons J, Lopez-Gomez G, Nilsson B, Ottens M. Continuous adsorption in food industry: The recovery of sinapic acid from rapeseed meal extract. *Separation and Purification Technology*. 2021; 254: 117403

Tallvod S, Yamanee-Nolin M, Gomis-Fons J, Andersson N, Nilsson B. A novel process design for automated quality analysis in an integrated biopharmaceutical platform. 2022; Submitted for publication.

Related oral presentations (Lead Author/Presenter)

Gomis-Fons J, Löfgren A, Andersson N, Nilsson B, Berghard L. Design and implementation of an integrated and automated downstream bioprocess including a final UF/DF step. Oral presentation at the 14th International PhD Seminar on Chromatographic Separation Science; March, 2018; Burghausen, Germany.

Gomis-Fons J, Andersson N, Nilsson B. Optimization study on periodic counter-current chromatography integrated in a monoclonal antibody downstream process. Oral presentation at Integrated Continuous Biomanufacturing IV; October, 2019; Brewster, Massachusetts, USA.

My contributions to the studies

I. I planned and performed all the experiments. I designed the integrated downstream process and wrote the code for process automation and control. I analysed the data and wrote the article.

II. I planned and performed the experiments with HS. I designed the downstream process and wrote the code for the implementation of the process. I analysed the data from the downstream part of the process, while HS analysed the data from the upstream part. I co-wrote the article together with HS.

III. I planned and performed all the experiments. I wrote and ran the optimization code and implemented and calibrated the model. I wrote the code for process control. I analysed the data and wrote the article.

IV. I planned and performed the experiments together with AL. We designed the process and wrote the code for the process control. I was involved in the analysis of the results, and I co-wrote the article together with AL.

V. I was involved in the development of the optimization code with MYN. We planned and performed all the experiments. I wrote the software code for the experimental implementation of the process. I was involved in the analysis of the results, and I co-wrote the article together with MYN.

VI. I designed the integrated process and optimized the process conditions in the virus inactivation and polishing steps together with MI, while JS was responsible for the optimization of the capture step and the development of the protein A resin. The three of us planned and performed the experiments with the integrated process and co-wrote the article. I wrote the software code for the implementation of the process together with MI.

VII. I designed the downstream process and was responsible for process control in the downstream process together with MI. JS was responsible for the development and production of the protein A resin, and HS was responsible for the upstream part. The four of us planned and performed the experiments and co-wrote the article. I was involved in the analysis of the results regarding the purification process.

Acknowledgements

The first person I would like to thank is my main supervisor, Prof. Bernt Nilsson. He has had so much faith in me since I came to the Department of Chemical Engineering at Lund University, and has supported me in every way. He gave me the freedom to work independently, which I really appreciate. I am also grateful to my co-supervisor, Niklas Andersson, who developed the software Orbit, without which none of this work would have been possible. He was always willing to help me adapt Orbit to the needs of each project.

I believe that the best way to carry out research is through collaboration, and I am grateful that most of my work was performed with other researchers, both inside and outside our group. One of them is Dr Anton Löfgren, whom I want to thank for instilling calmness in me when things were not going well, and for making me not worry too much. I am also grateful to Mikael Yamanee-Nolin, for his enthusiasm, positivism and willingness to always do the right thing. I would also like to thank Simon Tallvod for developing new laboratory gadgets to help me with the implementation of the processes, and Daniel Espinoza for his wonderful attitude to learning. I want to express my special thanks to Madelène Isaksson, for being the best research companion, for her commitment, and for having taught me so much. I am so happy that we started as colleagues and now I can call you my friend. I would also like to thank other people outside the group: Hubert Schwarz, for his hard work with the bioreactor and professionality; and Monica Moreno González, for her welcoming attitude and initiative that made my wonderful stay at TU Delft possible.

None of the research carried out during these years would have been possible without the involvement of external partners. In particular, I am grateful to the companies SOBI, Novo Nordisk and Cytiva for their contributions in terms of resources and expertise. I would also like to thank the competence centre AdBIOPRO for providing me with opportunities for valuable collaboration and projects. It has been an honour to be part of one of the leading competence centres in the world in continuous biomanufacturing.

There are others at the Department who have made my PhD studies a pleasurable journey: Miguel Sanchis Sebastiá, you have been my role model from the moment we came to Sweden together. Thank you for everything we have experienced together, and for guiding me along the right path, although I'll never admit that you are right; Mariona Battestini Vives, thank you for being the smile of the Department and for introducing me to the Catalan community in Copenhagen; Ruben Juárez Cámara, thank you for always bringing joy and laughs to the coffee room; Marta Lemos Ferreira, Catarina Oliveira and Gonçalo Rodrigues, thank you for an amazing time during your time at the Department.

Last but not least, I would like to thank my friends and family in Spain and in Sweden, even if they still do not understand what I am doing. In particular, I want to thank my parents, my parents-in-law and my siblings for their unconditional support. It would be unfair not to mention my dog Goku, because see him jump up with so much excitement and love when I get home makes my day. My wife Maria deserves my special thanks, for believing in me more than I do, for always having the right words to make me feel better, and for bringing so such happiness into my life. Finally, I would also like to thank my son Arnau because, even if he is not aware of it yet, his birth just a few months before the defence of this thesis has made me the happiest person in the world.

Contents

Abbreviations and symbols

Abbreviations

AEMC	Anion-exchange membrane chromatography
AEX	Anion-exchange chromatography
BT	Breakthrough
CEX	Cation-exchange chromatography
CIP	Cleaning-in-place
DBC	Dynamic binding capacity
DOE	Design of Experiments
FDA	U.S. Food and Drug Administration
HIC	Hydrophobic interaction chromatography
HPLC	High performance liquid chromatography
ICB	Integrated continuous biomanufacturing
ICDP	Integrated continuous downstream process
ICS	Integrated column sequence
mAb	Monoclonal antibody
MMC	Mixed mode chromatography
PAT	Process analytical technology
PCC	Periodic counter-current chromatography
UF	Ultrafiltration
DF	Diafiltration
VI	Virus inactivation

Symbols

A	Constant in the Van Deemter equation related to eddy diffusion (dm)
B	Constant in the Van Deemter equation related to molecular diffusion (dm^2/min)
c	Extra-particle mobile phase concentration (g/L)
C	Constant in the Van Deemter equation related to mass transfer resistance (min)
c_F	Feed concentration (g/L)
c_p	Concentration in the mobile phase inside the particle (g/L)
c_{pool}	Concentration of the pool (g/L)
c_S	Concentration of modifier in the column (M)
c_{SF}	Feed concentration of modifier (M)
c_{tank}	Concentration in the tank and at the outlet (g/L)
$c_{tank,in}$	Concentration at the inlet of the tank (g/L)
D	Dilution factor in the inline conditioning step (-)
D_{ax}	Axial dispersion coefficient (dm^2/min)
d_c	Diameter of the column (dm)
D_{eff}	Effective pore diffusivity (dm^2/min)
d_p	Particle diameter (dm)
e	Error signal in the proportional integral controller (-)
$F_{tank,in}$	Flow rate at the inlet (L/min)
F_F	Feed flow rate (L/min)
H	Height equivalent of a theoretical plate (dm)
H_0	Henry's equilibrium constant (M^{β})
k	Adsorption rate constant (min^{-1})
K	Langmuir equilibrium constant (g/L)
K_{ILC}	Controller gain in the iterative learning control law (-)

K_0	Empirical constant in the Blake-Kozeny equation (-)
k_{eff}	Effective mass transfer coefficient (m/s)
k_f	Mass transfer coefficient for the external particle film (dm/min)
k_{kin}	Kinetic constant (min^{-1})
K_P	Controller proportional gain in the proportional integral controller (-)
L	Column length (dm)
L_{max}	Maximum column length (dm)
Pe_c	Column-based Peclet number (-)
Pe_p	Particle-based Peclet number (-)
q	Concentration of adsorbed molecules in the stationary phase (g/L)
Q	Protein load (g/L)
q_{eq}	Concentration in the stationary phase in equilibrium (g/L)
q_{max}	Maximum adsorption capacity (g/L)
r	Radial distance from the centre of the particle (dm)
r_p	Particle radius (dm)
Re_p	Particle-based Reynolds number (-)
t_{cycle}	Cycle time in the purification process (min)
T_i	Integration time in the proportional integral controller (min)
t_{load}	Loading time (min)
u	Control signal (-)
v	Superficial fluid velocity (dm/min)
$V_{UF/DF,min}$	Minimum initial working volume in the UF/DF process (L)
V_c	Volume of the column (L)
V_{load}	Loaded volume (L)
V_{pool}	Volume of the pool (L)
V_{tank}	Tank volume (L)

y	Output signal (-)
y_{ref}	Reference value for the output signal (-)
z	Longitudinal distance from the inlet of the column (dm)
α	Lumped empirical constant for the estimation of the pressure drop (bar min dm^{-2})
β	Equilibrium modifier-dependence parameter (-)
ΔP	Pressure drop along the column (bar)
ΔP_{max}	Maximum permissible pressure drop along the column (bar)
ε	Total column void (-)
ε_c	Extra-particle column void (-)
ε_p	Particle porosity (-)
μ	Dynamic viscosity of the fluid (bar min)
ρ	Density of the fluid (g/L)
τ	Residence time (min)
τ_{load}	Residence time during the loading phase (min)
τ_{pool}	Residence time during the pooling phase (min)

1 Introduction

1.1 Biopharmaceuticals and their production

Biopharmaceuticals (also called biologics) are drugs derived from biological systems. They are typically proteins or peptides, and are essential for healthcare throughout the world, being used in numerous treatments such as hormone and enzyme replacement therapies and cancer.[1] The market for biopharmaceuticals is growing rapidly, which has led to increased focus on the development of production processes.[1-4]

A biomanufacturing process usually consists of an upstream system and a downstream system. A typical platform for the production of monoclonal antibodies (mAbs), which represent a large proportion of the total revenue in the biopharmaceutical industry,[4-7] is presented in Figure 1. The most important unit in the upstream system is the production bioreactor, where the biological reaction with living cells takes place. An adequate number of cells in the production bioreactor is ensured by the seed train, represented by the N-2 and N-1 bioreactors in Figure 1. The cells produce the active pharmaceutical ingredient together with other components, such as host cell proteins, DNA fragments and endotoxins.[8] Purification is thus necessary to remove these impurities and obtain a pure product. The cells are removed from the fermentation broth using a process called clarification. The clarified harvest is then purified using a number of chromatography-based unit operations, which make up the downstream system. Chromatography is a very selective separation method in which the components present in the mobile phase are adsorbed onto a stationary phase (also called resin), which usually consists of porous particles packed in a column. The separation is achieved due to the different interaction of the components with the stationary phase.[9] The first chromatography step in the purification process of a biopharmaceutical is usually called the capture step; the objectives of which are to remove most of the impurities that are not product-related and reduce the process volume.[9] Affinity chromatography is common in this step as it is very product-specific. An example of this is protein A chromatography, which is used for antibody purification.[8,10] After the capture step, virus inactivation (VI) takes place, usually by incubation at low pH or with a solvent/detergent mixture.[11-14] One or two chromatography steps, called polishing, usually follow this to further increase the purity. Product-related impurities are often removed using cation-exchange

chromatography (CEX), anion-exchange chromatography (AEX) or hydrophobic interaction chromatography (HIC).[8,10-12,15] Finally, the product is concentrated, and the buffer composition is changed for suitable long-term storage in an ultrafiltration/diafiltration (UF/DF) step.[10,16-18]

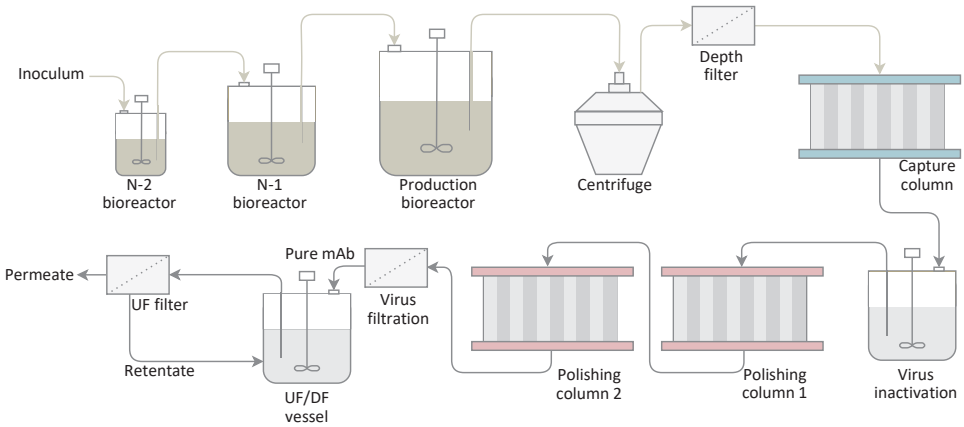

Figure 1. Block diagram of a typical mAb biomanufacturing process.[8,19]

The chromatography steps can be run in bind-and-elute mode or flow-through mode.[20] In bind-and-elute mode, the different process phases are: loading, where the protein is adsorbed on the available binding sites, while some of the impurities flow through the column; washing, where the impurities present in the mobile phase are removed; elution, where a non-binding buffer is used to desorb the protein, and the product is pooled to be loaded onto the next column or to be collected; stripping, where the strongly adsorbed molecules are desorbed; cleaning-in-place (CIP), where the column is cleaned, usually with a caustic solution; and equilibration, where the column is equilibrated with a binding buffer. In flow-through mode, the product is not adsorbed and flows directly through the column, while the impurities are bound. The process phases in this mode are: loading and pooling, where the product is loaded and at the same time pooled at the outlet of the column; washing, where the desired product, present in the mobile phase, is flushed out of the column; stripping, where the bound impurities are desorbed; followed by CIP and equilibration, where the column is cleaned with a caustic solution and equilibrated with a non-binding buffer for the next purification cycle. UF/DF includes the following process phases: concentration to reduce the volume; diafiltration (DF), where the volume is kept constant and the new buffer is added batchwise or continuously; a second concentration phase, to adjust the final product concentration; recovery, where the product in the system is extracted and collected in the vessel; CIP of the system and the filter; and finally equilibration of the filter.[18]

1.2 Integrated continuous biomanufacturing

Although the biologics segment is growing very rapidly, the production cost still limits the application of these drugs worldwide. For example, the cost of treatment for rheumatoid arthritis with a monoclonal antibody (adalimumab) is approximately $50,000 per patient per year.[1] One of the reasons for this is that biopharmaceuticals are currently mainly produced using batch processes, which are inefficient and inflexible to changes in product demand.[21] The portfolio of products is becoming increasingly more complex, markets are evolving rapidly, and there is increasing pressure for cost reduction to facilitate global access to biopharmaceuticals.[2,21,22] Therefore, more efficient and flexible processes are required in the biomanufacturing industry.[1,2,23]

This can be achieved through integrated continuous biomanufacturing (ICB).[2,22,24] ICB has been implemented in other sectors, such as the petrochemical, steel casting and food sector, where significant reductions in operational cost and increased flexibility have been achieved.[2,22] The advantages of continuous processing include steady-state operation, short cycle times, lower capital cost, smaller/less equipment, and higher productivity.[2,22] Furthermore, the U.S. Food and Drug Administration (FDA) supports ICB as a means of ensuring product quality.[25]An increase in productivity has been achieved in the biopharmaceutical industry in upstream processing, by shifting from batch to continuous mode, but downstream processing is still mostly discontinuous, and over 60% of the total production cost is attributable to the purification of the product.[24,26] In this thesis, "ICB process" denotes an integrated continuous process for the production and purification of a biopharmaceutical, thus including both upstream and downstream unit operations, while "integrated continuous downstream process" (ICDP) only refers to the downstream process.

Although most of the downstream processes in the biopharmaceutical industry are carried out in batch mode and in separate steps,[23,24] continuous periodic chromatography processes have attracted interest in recent years. Examples of this are periodic counter-current chromatography (PCC)[27,28] (as illustrated in Figure 2), a two-column simulated moving-bed process (called CaptureSMB),[28,29] and multi-column counter-current solvent gradient purification[30,31]. These processes allow a continuous feed and provide higher productivity and resin utilization, while maintaining a similar yield to that in batch processes.[28,31]

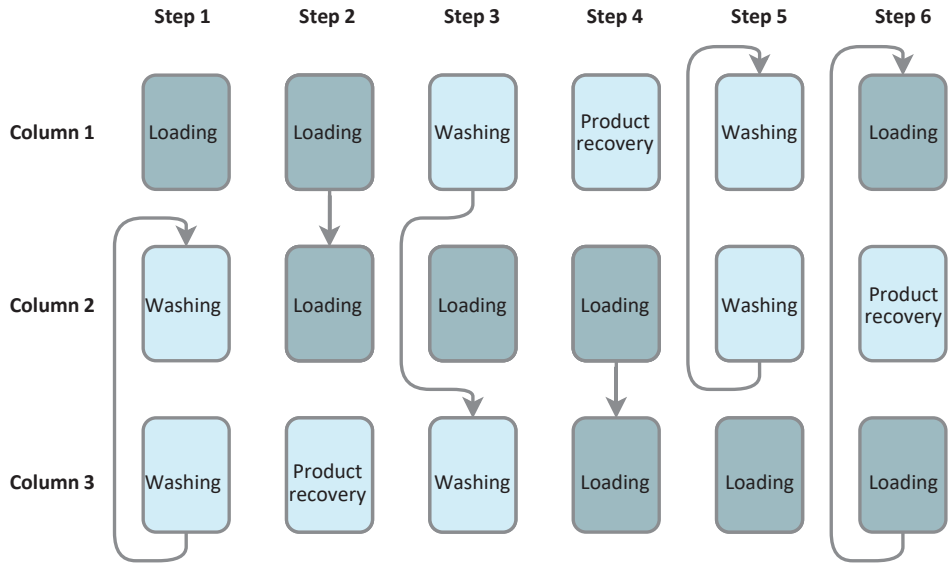

Figure 2. The principle of a 3-column PCC process.

Continuous processing cannot achieve its true potential without process integration. In the context of biomanufacturing, an integrated bioprocessing platform consists of physically connected unit operations where the use of storage tanks between the steps is minimized, thus reducing the hold-up volumes and the size of the equipment.[32] Integrated biomanufacturing offers higher productivity than a process performed in separate steps or with storage tanks between the steps, as the process time is shorter due to the reduction in holding times between the steps.[32-34] Furthermore, process integration is associated with a higher degree of automation, which reduces the need for manual labour, while improving the reproducibility and robustness of the process.[22,32,35] Process integration can be achieved by directly loading the pool from one column onto the next one (Figure 3). This concept is called the integrated column sequence (ICS),[11,34] and reduces the equipment required as several separation steps can be connected in a chromatography system.

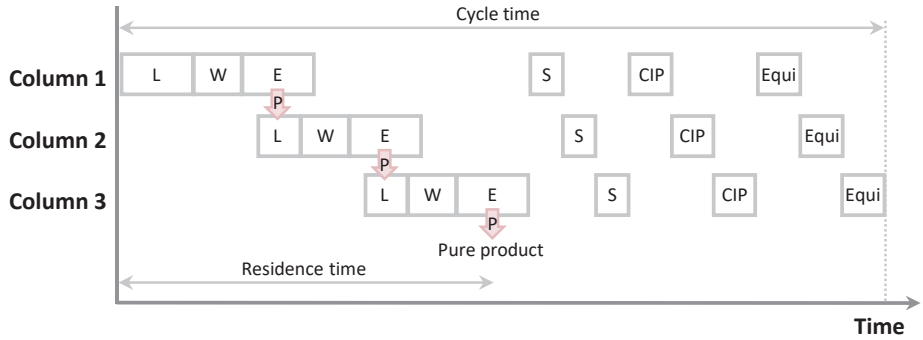

Figure 3. Gantt chart illustrating the principle of ICS with a 3-column process in a single system. After loading (L) and washing (W), the pool (P) obtained from elution (E) is directly loaded onto the next column. Stripping (S), cleaning-in-place (CIP) and equilibration (Equi) then take place.

1.3 Aim and outline

The aim of the work presented in this thesis was to develop and test tools for the efficient design, control and implementation of ICDPs for the purification of biopharmaceuticals. Mechanistic modelling was used for process design and optimization and to implement control strategies to achieve automation. The ICS process concept[11,34] was applied to integrate the downstream steps with minimal equipment requirements. Multi-column periodic chromatography was used to allow a continuous inlet stream into the downstream process, and the research software Orbit[34] was used and further developed to control and automate the implemented processes. The ICDPs were tested on both laboratory and pilot scale, demonstrating that the tools can safely be applied for process scale-up.

Chapter 2 presents the mechanistic models used for the design, optimization and control of ICDPs. The control and automation strategies developed in this work and implemented in the studies are addressed in Chapter 3. Several design criteria for ICDPs are introduced in Chapter 4. In this chapter, model-based optimization is presented as a design tool, and is applied to optimize a PCC capture step integrated in a downstream process, and to obtain an optimal flow rate trajectory in the loading of a mAb capture step. Chapter 5 describes five case studies carried out in this work. Finally, Chapters 6 and 7 present the conclusions drawn from the results of these studies and suggested future research in this field to continue the path established in this work.

2 Process Modelling

Process development in the biopharmaceutical industry has typically been achieved using the statistical method of Design of Experiments (DOE).[36-42] This approach involves the design of numerous experiments to find the relationship between several factors and outputs affecting the process.[42,43] It allows relevant and accurate information to be obtained about the process through experimental work. However, it requires the performance of many experiments, and is thus resource intensive.[42] For example, in a two-level factorial DOE of a chromatography process involving four factors (e.g., elution time, washing time, loading time and elution gradient slope), the number of experiments that would have to be performed is $2^4 = 16$.[38]

Mechanistic modelling is an alternative method where the physical phenomena are described in detail using mass balances. These mass balances, together with the use of models to describe adsorption, provide valuable information on the performance of the process, significantly reducing the need for experiments.[42,44] However, a mechanistic model will include some unknown parameters, and a small number of experiments must be carried out to calibrate it.[45] The best values of these parameters are obtained when the difference between the simulated and the experimental data is a minimum. Once the model parameters have been determined, the mechanistic model can be used to obtain relationships between the process parameters and outputs, allowing simulations to find the optimal running conditions and to predict the behaviour of the process.[15,46,47]

Mechanistic modelling can also be used as a tool in the quality by design approach.[48-51] Quality by design is a manufacturing concept in which the process and control strategies are designed to ensure product quality based on the understanding of the risks that can compromise product quality.[52-55] The FDA considers that quality by design is key in achieving a process that consistently ensures product quality.[25,56] This kind of modelling has already been used extensively in biologics purification processes. For example, Baur et al. used mechanistic modelling in the protein A-based capture step in the purification of mAbs to optimize the process and compare different process configurations.[29,57] Klatt et al. also used mechanistic modelling to optimize and control chromatographic processes,[58] while Shi et al. used it to optimize a PCC process .[59]

Different models can be used to model a protein purification process, depending on the process step and the aim of applying the model in the particular case.[15,45,60,61] The model should not be more complex than necessary for the specific purpose, but it should be able to provide an accurate estimate of the desired outputs. The models used in the present work can be divided into three categories: those used in affinity chromatography, those used in ion-exchange chromatography and those used for other components in the process, such as non-chromatographic packed-bed columns, tubes, loops and mixers.

2.1 Models in affinity chromatography

Affinity chromatography is used to isolate a molecule based on highly specific binding interactions.[62,63] The selectivity of this process is very high, and only a small number of product-related impurities are eluted with the purified product.[62] It is normally used as the first step in the purification sequence, also known as the capture step, in order to increase the purity and the concentration.[8,10] The product binds to the resin, while most of the impurities flow through the chromatography column. The concentrated product is then obtained in the elution phase, where there is usually only one peak in the chromatogram corresponding to a relatively pure product. The high selectivity means that separation usually occurs during the loading phase, and not during the elution phase.[62,63] The modelling approach used thus focuses on the loading phase, while it is not usually necessary to predict the shape of the chromatograms in the elution phase. In the loading phase, the information that is most important for process design is provided by the break-through (BT) curve,[47,60,61] which gives the concentration of the product in the effluent stream as a function of time. The BT curve determines how much product can be loaded onto a column. Process parameters such as loading flow rate, feed concentration and particle size will affect the shape of the BT curve.[57,59,64] Therefore, the objective of modelling this chromatography step is to predict the BT curve for a number of given process parameters.

Different models can be used to model affinity chromatography.[45,60,61,65,66] One is the external film model, also known as the transport-dispersive model (Eqs. 2.1-2.4).[45,66] In this model, the column is modelled as a plug flow reactor with dispersion. It is assumed that there is a film on the external surface of the particles, which means that the concentration in the mobile phase outside and inside the particle are not equal, leading to a driving force that causes mass transfer between the mobile phases outside and inside the particle. The molecules adsorbed on the binding sites on the internal surfaces of the particle are in equilibrium with the molecules in the mobile phase inside the particle. The Danckwerts boundary conditions are applied at the inlet and the outlet of the column (Eqs. 2.1a and 2.1b).

$$\frac{\partial c}{\partial t} = D_{ax}\frac{\partial^2 c}{\partial z^2} - \frac{v}{\varepsilon_c}\frac{\partial c}{\partial z} - \frac{1-\varepsilon_c}{\varepsilon_c} j_A \tag{2.1}$$

$$\frac{\partial c}{\partial z} = \frac{v}{\varepsilon_c D_{ax}}(c - c_F) \qquad at\ z = 0 \tag{2.1a}$$

$$\frac{\partial c}{\partial z} = 0 \qquad at\ z = L \tag{2.1b}$$

$$j_A = k_f \frac{3}{r_p}\left(c - c_p\right) \tag{2.2}$$

$$\frac{\partial c_p}{\partial t} = j_A - \frac{\partial q}{\partial t} \tag{2.3}$$

$$\frac{\partial q}{\partial t} = k\left[(q_{max} - q)c_p - q/K\right] \tag{2.4}$$

Here, c is the extra-particle mobile phase concentration, c_F is the feed concentration, c_p is the concentration in the mobile phase inside the particle, q is the concentration of adsorbed molecules in the stationary phase, D_{ax} is the axial dispersion coefficient, v is the superficial fluid velocity, k_f is the mass transfer coefficient for the external particle film, ε_c is the extra-particle column void, ε_p is the particle porosity, r_p is the particle radius, z is the longitudinal distance from the inlet, L is the column length, q_{max} is the maximum adsorption capacity, K is the Langmuir equilibrium constant, and k is the adsorption rate constant.

A variant of this model is the internal film model (Eqs. 2.5-2.7),[29,47] in which it is assumed that the film is on the internal surfaces of the particle, and not on the external surface of the particle, as in the transport-dispersive model. Therefore, the concentration in the mobile phase inside the particle is assumed to be the same as the concentration in the mobile phase of the column, which simplifies the model.

$$\frac{\partial c}{\partial t} = D_{ax}\frac{\partial^2 c}{\partial z^2} - \frac{v}{\varepsilon}\frac{\partial c}{\partial z} - \frac{1-\varepsilon}{\varepsilon} j_A \tag{2.5}$$

$$\frac{\partial c}{\partial z} = \frac{v}{\varepsilon\, D_{ax}}(c - c_F) \qquad at\ z = 0 \tag{2.5a}$$

$$\frac{\partial c}{\partial z} = 0 \qquad at\ z = L \tag{2.5b}$$

$$j_A = k_{eff} \frac{3}{r_p}\left(q_{eq}(c) - q\right) \tag{2.6}$$

$$\frac{\partial q}{\partial t} = j_A \tag{2.7}$$

Here, k_{eff} is the effective mass transfer coefficient, ε is the total column void (including extra-particle and intra-particle void), and $q_{eq}(c)$ is the concentration in the stationary phase in equilibrium with the mobile phase concentration c.

Another model commonly used to predict BT curves in affinity chromatography is the general rate model (Eqs. 2.8-2.10),[60] which is the chromatography model with the highest degree of complexity as both extra-particle and intra-particle mass transfer phenomena are taken into account. Unlike the film model, there is a concentration gradient inside the particle along the radius. The same boundary conditions as in the previous models are applied to the column (Eqs. 2.8a and 2.8b), while for the particle, the boundary conditions are obtained by considering symmetry in the concentration profile in the centre of the particle (Eq. 2.9a) and continuity of flux through the particle surface (Eq. 2.9b).

$$\frac{\partial c}{\partial t} = D_{ax}\frac{\partial^2 c}{\partial z^2} - \frac{v}{\varepsilon_c}\frac{\partial c}{\partial z} - \frac{1-\varepsilon_c}{\varepsilon_c}\frac{3}{r_p}k_f\left(c - c_p\big|_{r=r_p}\right) \tag{2.8}$$

$$\frac{\partial c}{\partial z} = \frac{v}{\varepsilon_c D_{ax}}(c - c_F) \qquad at\ z = 0 \tag{2.8a}$$

$$\frac{\partial c}{\partial z} = 0 \qquad at\ z = L \tag{2.8b}$$

$$\frac{\partial c_p}{\partial t} = D_{eff}\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_p}{\partial r}\right) - \frac{1}{\varepsilon_p}\frac{\partial q}{\partial t} \tag{2.9}$$

$$\frac{\partial c_p}{\partial r} = 0 \qquad at\ r = 0 \tag{2.9a}$$

$$\frac{\partial c_p}{\partial r} = \frac{k_f}{D_{eff}}(c - c_p) \qquad at\ r = r_p \tag{2.9b}$$

$$\frac{\partial q}{\partial t} = k\left[(q_{max} - q)c_p - q/K\right] \tag{2.10}$$

The adsorption phenomena in affinity chromatography are usually defined by a Langmuir isotherm (Eq. 2.11).[47,60,65,67] Since the selectivity is so high, the adsorption of the impurities is negligible, and it can be assumed that only the desired product adsorbs on the available sites. Therefore, the adsorption is not competitive, and this is the reason why more complex equilibrium models are not needed in this case.

$$q_{eq}(c) = q_{max}\frac{Kc}{1 + Kc} \tag{2.11}$$

The partial differential equation systems obtained from the above models can be converted into ordinary differential equation (ODE) systems by discretizing the special derivatives using the finite volume method.[68] The resulting ODE system can then be solved with one of the many commercially available ODE solvers (e.g., *ode15s* in MATLAB™ or the *scipy.integrate.solve_ivp* function in the Python library *scipy*).

In the studies presented in Papers II, III and V, a general rate model was used to predict the breakthrough in a protein A capture step in the purification of mAbs. Figure 4 shows the curves obtained with the validated model together with the experimental data for mAbSelect PrismA™ resin (Fig. 4a and 4c) and mAbSelect SuRe™ (Fig. 4b and 4d). Some deviation can be observed, especially in the uprising part of the BT curve. This is probably due to wall effects. These affect the void volume along the wall due to the different geometry of the particle and the column wall, and friction between the wall and the bed.[69] The lower void fraction in this region leads to product breaking through the column earlier than expected. These effects become more important as the ratio between the column diameter and the particle diameter decreases.[69,70] The recommended minimum value for this ratio is 200.[70] For the calibration experiments of the general rate model, a 1 mL HiTrap™ column (0.7 x 2.5 cm) was used, and the particle sizes were 60 µm (mAbSelect PrismA) and 85 µm (mAbSelect SuRe). This corresponds to column-to-particle diameter ratios of 117 and 82, both of which are considerably below the recommended minimum value, and indicate that wall effects can in fact be significant in these experiments. In spite of this, small errors in the BT curve can be acceptable as long as the total area under the curve (which represents the amount of material leaving the column) is similar in the simulated and the experimental cases, as this value is used to calculate process performance attributes such as yield, productivity and resin utilization.[59,64] In addition, wall effects will be less important on larger scales as the column diameter increases,[69] and thus the results obtained on small scale using this model should be applicable at larger scales.

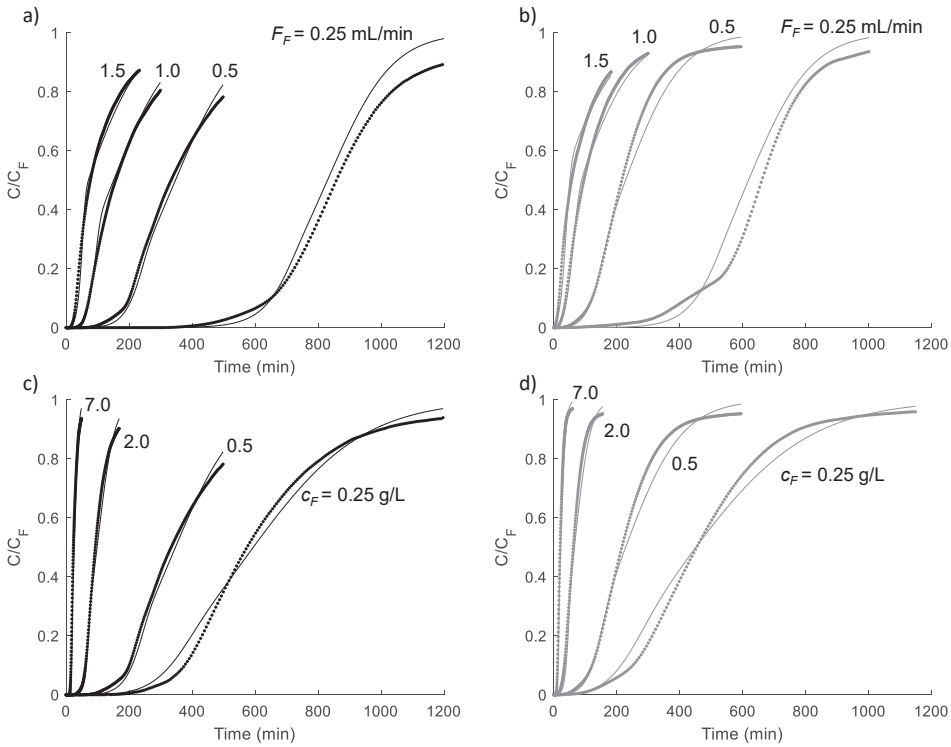

Figure 4. Calibration of the general rate model: Experimental (dots) and fitted breakthrough curves (solid lines) at four different flow rates, at a constant concentration of 0.5 g/L for a) mAbSelect PrismA, and b) mAbSelect SuRe. Experimental and fitted breakthrough curves for four different mAb concentrations, at a constant flow rate of 0.5 mL/min for c) mAbSelect PrismA, and d) mAbSelect SuRe.

2.2 Models in ion-exchange chromatography

In ion-exchange chromatography, two or more molecules are separated based on their charge, which determines their affinity to the resin.[71] The molecules compete with each other to bind to available sites. A modifier (commonly a salt) is used to elute the components, such that the protein molecule is replaced by a molecule of the modifier. Therefore, the higher the modifier concentration, the weaker the interaction between the proteins and the resin. The desired product usually elutes together with product-related impurities, and therefore a compromise must be made between selectivity, purity and yield.[15,72] In this case, the modelling approach focuses on predicting the chromatogram of each component in the elution phase, while the BT curve can be used to predict certain parameters in the adsorption model.[15] Although the models presented in the previous section can also be used in this case, the simpler reaction-dispersive model[66,73] (Eqs. 2.12-

2.14) was used for the prediction of the elution profiles in the present work, using the Danckwerts boundary conditions for both the protein (Eqs. 2.12a and 2.12b) and the modifier (Eqs. 2.14a and 2.14b).

$$\frac{\partial c}{\partial t} = D_{ax}\frac{\partial^2 c}{\partial z^2} - \frac{v}{\varepsilon}\frac{\partial c}{\partial z} + \frac{1-\varepsilon_c}{\varepsilon}\frac{\partial q}{\partial t} \tag{2.12}$$

$$\frac{\partial c}{\partial z} = \frac{v}{\varepsilon_c D_{ax}}(c - c_F) \qquad at\ z = 0 \tag{2.12a}$$

$$\frac{\partial c}{\partial z} = 0 \qquad at\ z = L \tag{2.12b}$$

$$\frac{\partial q}{\partial t} = k_{kin}\left[H_0 c_S^{-\beta}\left(1 - \frac{q}{q_{max}}\right)c - q\right] \tag{2.13}$$

$$\frac{\partial c_S}{\partial t} = D_{ax}\frac{\partial^2 c_S}{\partial z^2} - \frac{v}{\varepsilon}\frac{\partial c_S}{\partial z} \tag{2.14}$$

$$\frac{\partial c_S}{\partial z} = \frac{v}{\varepsilon_c D_{ax}}(c_S - c_{SF}) \quad at\ z = 0 \tag{2.14a}$$

$$\frac{\partial c_S}{\partial z} = 0 \qquad at\ z = L \tag{2.14b}$$

Here, c_S is the concentration of modifier in the column, c_{SF} is the feed concentration of modifier, k_{kin} is the kinetic constant, H_0 is Henry's equilibrium constant, and β is the equilibrium modifier-dependence parameter.

The adsorption model is usually more complex than in affinity chromatography, as the dependency on salt concentration (and, in some cases, pH) must be included in the model for each component. Two examples of adsorption models for ion-exchange chromatography are the steric mass action model,[46] and the Langmuir-based model with dependency on salt concentration,[15,73] which is the one given in Eq. 2.13 and used for the prediction of the CEX and AEX steps in the purification of mAbs (Paper II).

2.3 Models in packed-bed columns, tubes and mixers

The non-chromatographic packed-bed columns, tubes and mixers used in the process must also be modelled in order to predict the product concentration in different parts of the process, as well as to estimate the dispersion of the product throughout the whole system.

For tubes, a plug flow model with dispersion was used (Eq. 2.15). For packed columns without adsorption, the column void was included in the model (Eq. 2.16).

$$\frac{\partial c}{\partial t} = D_{ax}\frac{\partial^2 c}{\partial z^2} - v\frac{\partial c}{\partial z} \tag{2.15}$$

$$\frac{\partial c}{\partial t} = D_{ax}\frac{\partial^2 c}{\partial z^2} - \frac{v}{\varepsilon_c}\frac{\partial c}{\partial z} \tag{2.16}$$

In this case, the correct estimation of the dispersion coefficient is most important in predicting the concentration profile of the effluent. The particle-based Peclet number can be used to estimate the dispersion coefficient in packed beds.[66,74] The Peclet number relates the convective and the diffusive transport phenomena, and is defined as follows:

$$Pe_p = \frac{v \cdot d_p}{D_{ax}} \tag{2.17}$$

where Pe_p is the particle-based Peclet number and d_p is the particle diameter.

The dispersion coefficient can thus be obtained from the Peclet number using Eq. 2.17. The Peclet number is not constant, but varies with the turbulence of the fluid against the particles, which is quantified by the particle-based Reynolds number:

$$Re_p = \frac{d_p \cdot v \cdot \varepsilon_b \cdot \rho}{\mu} \tag{2.18}$$

where Re_p is the particle-based Reynolds number, and ρ and μ are the fluid density and dynamic viscosity.

An empirical expression that correlates the Reynolds number and the Peclet number is then needed. In this work, the correlation proposed by Rastegar et al.[74] was used.

$$Pe_p = \frac{0.18 + 0.008 \cdot Re_p^{0.59}}{\varepsilon_b} \tag{2.19}$$

The dispersion coefficient in non-packed tubes can be estimated using the column-based Peclet number (Pe_c):

$$Pe_c = \frac{v \cdot d_c}{D_{ax}} \tag{2.20}$$

where Pe_c is the column-based Peclet number and d_c is the diameter of the column.

In the present work, the Peclet number in non-packed tubes was obtained experimentally through tests at different velocities with a tracer. It can also be estimated without performing experiments, using computational fluid dynamics[75] or empirical equations.[76]

An ideal tank model was used to predict the concentration at the outlet of the mixers and vessels (Eq. 2.21):

$$\frac{\partial c_{tank}}{\partial t} = \frac{F_{tank,in}}{V_{tank}}\left(c_{tank,in} - c_{tank}\right) \tag{2.21}$$

where $c_{tank,in}$ and $F_{tank,in}$ are the concentration and flow rate of the incoming stream, c_{tank} is the concentration in the tank and at the outlet, and V_{tank} is the tank volume.

According to this model, the equivalent of 3 mixer volumes must pass through the mixer before the concentration at the outlet reaches 95% of that at the inlet. This indicates that such a mixer will have a significant impact on the residence time distribution of the product through the process, and should therefore be taken into account in the model. For example, in the study presented in Paper VII, it was estimated that the residence time of a protein molecule in the harvest tank between the upstream and the downstream processes could be up to 3.6 days. This means that if a product that does not meet the quality requirements enters the harvest tank, the product collected in the harvest tank for almost 4 days must be discarded, which would have considerable economic impact.

3 Process Control and Automation

Process control and automation are crucial to achieve a fully ICB platform. The FDA considers that a control strategy is key in establishing a process that can deliver a product with the specified quality attributes in a robust way.[56] In addition, a high degree of automation is needed to implement an ICDP in a time- and resource-efficient way, as it implies a reduction in manual work and better utilization of the equipment and raw material.[22,35] Process analytical technology (PAT), which provides real-time information on the process parameters, and most importantly, on the product quality attributes, is required to implement an adequate control system.[77,78]

Supervisory control and data acquisition software is an important element in a control strategy in order to integrate the software and hardware of different units.[79-81] In the present work, the research program Orbit, developed at the Department of Chemical Engineering at Lund University, was used as supervisory control and data acquisition software and modified to allow for the control of an ICDP.[34] Orbit was originally created to control ÄKTA™ chromatography systems in order to overcome the limitations of the ÄKTA control system UNICORN™. Unlike UNICORN, Orbit allows the simultaneous control of several systems and pieces of analytical equipment, the use of real-time control, and the implementation of complex automation strategies. Orbit is written in the language Python, and communicates with UNICORN to send instructions at predetermined times and receive data from the system, based on information on the process provided by the user[34] (see Figure 5).

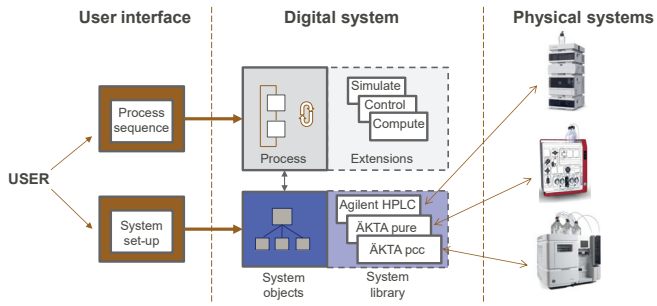

Figure 5. Architecture of the control software Orbit. The user defines the process sequence and the system set-up, and Orbit creates a process with an instruction list and as many system objects as there are physical systems. The created process then generates the instructions according to the process sequence provided by the user. These are sent to the system objects, which are connected to the physical systems using the corresponding system libraries.

Control strategies implemented in Orbit were developed in this work to adapt the software to the needs of an ICDP, and are presented in the sections below.

3.1 Control and automation of a multi-system process

The automation of a whole downstream process often requires the simultaneous control of several systems, which is possible with Orbit. This can be done in two ways: with hierarchy and without hierarchy.

In the first case, an Orbit program is created for each system, called the slave Orbit programs, and another Orbit program, called the master Orbit program, controls the slave Orbit programs, thus creating a hierarchy between the master and the slave programs. The master program synchronizes the actions of the slave programs, and the slave programs only send process instructions to their respective system when instructed to do so by the master program. All the process data are saved in the master program.

The second method involves a non-hierarchical relationship between the Orbit programs. An Orbit program is created for each system, as in the previous case, but there is no master program controlling them. Instead, communication between the programs ensures the process synchronization required in a multi-system process (see Figure 6). In general, each Orbit program saves only the process data related to the system it controls. However, it is sometimes necessary for several Orbit programs to share process data, and such data are saved in all the Orbit programs that require them. Synchronization between the programs is achieved using flags, which are implemented as Boolean variables. For example, when two systems need to perform a task simultaneously, System 1 sends a flag to System 2 when System 1 is ready to perform the task, and waits for the corresponding flag from System 2, which is sent when System 2 is also ready. When both systems have sent their respective flags and received the flag from the other system, they proceed with the simultaneous task. This approach was used in this work, in particular, in the studies presented in Papers III, IV, VI and VII.

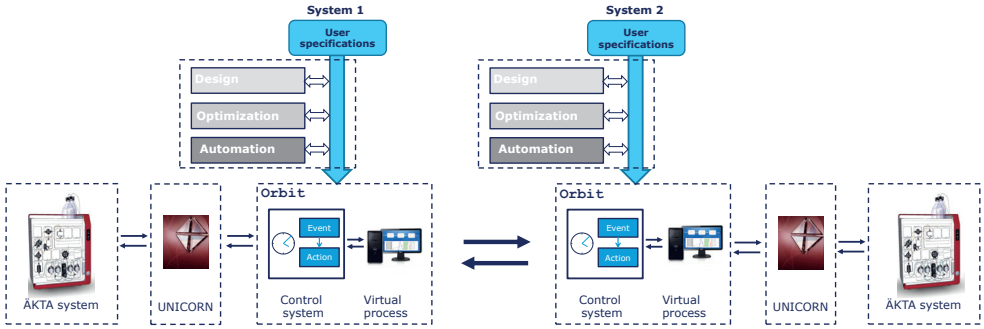

Figure 6. Schematic representation of the multi-machine system in non-hierarchical mode. Each system has an Orbit program with user-entered specifications of the process. The Orbit programs communicate with each other using flags allowing for process synchronization.

In addition, Orbit allows the connection of small pieces of equipment without the need to create a separate Orbit program. For example, in the studies described in Papers II and VII weight balances were connected to the computer using a serial interface or Ethernet connection. External pressure sensors, pH and temperature sensors, and laboratory mixers are other examples of small equipment that can be included in Orbit, thus allowing the extensive use of PAT.

Orbit allows the implementation of a digital twin of the process, which is a model-based simulation of the real process. A digital twin of a mAb downstream process was developed using the models presented in the previous section to simulate the entire process and support the implementation of several control strategies, as well as to monitor the downstream process and evaluate the process performance indicators in real time (Paper II).

3.2 Loading control

In the capture step, the productivity and the resin utilization are maximized by loading as much product as possible, but excessive loads can lead to product losses in the BT stream.[59,64] In addition, variations in the process may influence the product loading; the most important ones being the feed concentration, the loading flow rate, and the reduction in the column capacity over time.[28,57] It is therefore important to have a loading control strategy. The loading of the column can be controlled in different ways, based on time, the volume loaded, online detection of the UV absorbance of the BT stream, iterative learning control, modelling or online analysis.

3.2.1 Time-based loading control

This is the simplest approach to determine the duration of the loading of a column, and was the method used in the studies described in Papers I and III. A constant loading time is pre-defined in the user specifications of the process. The loading time is usually obtained based on the protein load, the feed concentration and the flow rate, according to Eq. 3.1. The protein load (Q) in a batch process depends on the dynamic binding capacity (DBC), which is usually defined at 10% of BT.[82,83] To ensure robustness, and to account for capacity loss, 80% of the DBC at 10% BT was used for the protein load.[83]

$$t_{load} = \frac{Q\ V_c\ (1 - \varepsilon_c)}{c_F\ F_F} \tag{3.1}$$

Here, t_{load} is the loading time, c_F and F_F are the feed concentration and flow rate, and V_c is the volume of the column.

The DBC decreases as the loading flow rate increases, due to mass transfer limitations. For this reason, the DBC must be obtained for the highest flow rate expected during operation. Similarly, the concentration used in this calculation must be the highest expected.

3.2.2 Volume-based loading control

Volume-based loading control is similar to the time-based approach, but the flow rate is removed from the equation. Instead of loading for a specific period of time, the loaded volume is calculated continuously by measuring the flow rate, and loading is stopped when a certain volume has been reached. This approach is especially convenient when the flow rate is not constant (see Papers VI and VII). The loaded volume (V_{load}) is calculated as follows:

$$V_{load} = \frac{Q\ V_c\ (1 - \varepsilon_c)}{c_F} \tag{3.2}$$

3.2.3 Absorbance-based loading control

This method involves measuring the UV absorbance of the BT stream from the column, i.e., inline UV measurements. Loading is stopped when the absorbance reaches a set value, indicating the presence of product in the BT stream. This method is robust to changes in the concentration and flow rate, and even to reduction in the column capacity over time, but it has the disadvantage that some product is lost in the breakthrough. In addition, when the concentration of

impurities is very high and that of the product is low (as is usually the case in the capture step), the UV signal from the BT stream is also very high, mainly due to the impurities, often above the linear range of the UV monitor, and it is difficult to detect the product. This method is therefore limited to applications where the feed concentration is high. This is usually the case in bioreactors run in fed-batch mode, where titres higher than 5 g/L can be obtained in the production of monoclonal antibodies.[1,6] However, the titres are lower in perfusion bioreactors, typical values ranging between 0.5 and 1 g/L for mAb production,[12,84] making this loading control strategy less suitable in these cases. This method is the built-in loading control strategy in the ÄKTA pcc system, and has been used in previous studies.[59,64]

3.2.4 Iterative learning control

One way of overcoming the disadvantage of absorbance-based loading control is to make use of iterative learning control, also called batch-to-batch control. The idea behind this approach is to use information from previous purification cycles to optimize the loading of the next cycle in a iterative way,[85] as illustrated in Figure 7. The control signal from cycle k (u_k) is modified so as to maintain the output signal (y_k) at the reference value (y_{ref}), using a learning control law such as that in Eq. 3.3, where K_{ILC} is the controller gain.

$$u_k = u_{k-1} + K_{ILC}(y_{ref} - y_{k-1}) \tag{3.3}$$

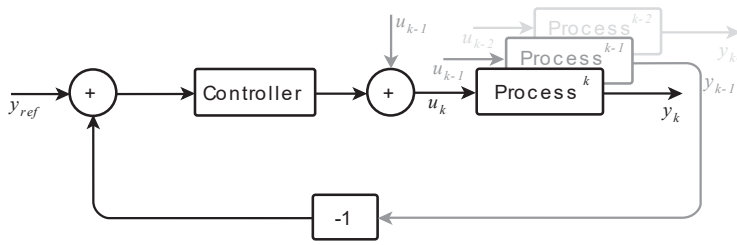

Figure 7. Illustration of the iterative learning control strategy using a previous cycle learning scheme. The variables u_k and y_k are the control and output signals, respectively, for cycle k; and y_{ref} is the reference value of the output signal.

This control strategy was implemented in the study presented in Paper IV. The control signal was the loaded volume, and the output signal was the product concentration in the pool, which was calculated by integrating the area under the elution peak. As the control signal is the loaded volume (and not the loading time), this strategy is robust to variations in the flow rate, as in the case of volume-based loading control. It is also robust to changes in the feed concentration, as a change in the output signal is detected, and the control signal is corrected such that the

output signal is returned to the steady-state value. However, it is not possible to determine whether the reduction in the output signal is due to a decrease in feed concentration or a decrease in the column capacity using this approach. Therefore, the loss in capacity must be considered in the design of the column. In addition, online or inline analysis of the BT stream would be needed to determine whether any product is lost in that stream, thus indicating that the column capacity has decreased.

3.2.5 Model-based loading control

This approach uses modelling to predict the DBC of the column based on the flow rate and the estimated harvest concentration. The flow rate is known, and the harvest concentration is estimated by calculating the area under the elution peak, assuming that there is a linear relationship between these two parameters. The general rate model was used to predict the DBC, to simulate the BT curve, and finally to determine the loaded volume using Eq. 3.2 (Paper II). When the loading control strategy was used, the cycles became longer when the harvest concentration was lower than the design value to maximize the resin utilization. As a result of the longer cycles, the number of cycles was reduced by up to 2.4 times, which led to lower buffer consumption and reduced effect on the column capacity, as the number of CIP cycles to which the column was subjected is also reduced. Another advantage is that the product pool is more concentrated, which reduces the processing time in the following downstream steps. One limitation of the method described in Paper II is that the capacity loss of the column is not included in the model. Therefore, this control strategy is not robust to severe capacity loss. This could be solved by including the capacity loss in the model, in such a way that it could be predicted beforehand. This would allow a more accurate estimate of the DBC. Another limitation is that the relationship between the area under the elution peak and the harvest concentration is not always linear, especially if product is lost in the BT stream, as this leads to a smaller elution peak. A potential solution could be to measure the harvest concentration directly, either online or offline.

3.2.6 Loading control based on online analysis

Another way to determine when to stop loading is by online analysis of certain process streams. However, the time taken to perform the analysis should be shorter than the time taken for the measured properties to change.[86] For example, the feed can be sampled periodically to determine the harvest concentration. In perfusion runs, this concentration does not usually change very quickly, and the concentration can be obtained by size-exclusion HPLC analysis, which can take between 30 and 60 min, so a moderate sampling frequency (1-2 times a day) should be

sufficient to accurately predict the harvest concentration. The breakthrough stream can also be sampled, to determine whether product is breaking through the column (thus not binding to the resin). Monitoring the feed and the breakthrough would be sufficient for a loading control strategy, but the eluate could also be sampled to measure the concentration of the purified product. This would allow the yield to be calculated, providing more information on where product loss takes place in the process.

The information obtained would be the same as that with the aforementioned inline UV absorbance measurements, but with some time delay, thus making loading control slower. For example, if the online analysis took 1 hour to perform, it would be too late to control the current purification cycle, which may take between 1 and 2 hours, and the control action would then be applied during the following cycle. Therefore, this analytical method should be combined with a batch-to-batch loading control strategy. The advantages of this include the robustness of the analysis method, the accuracy of the results, and the possibility of performing additional analysis in an automated way (aggregate content, charge variants, etc.). As a requirement, it must be possible to integrate the analytical system with the chromatography systems, and with the control system.

3.3 Pooling strategy

The size and shape of the peaks during the elution can vary over time, depending on several factors: load concentration, buffer composition, temperature in the room, etc. In order to make the purification process as robust as possible, a pooling strategy was developed and implemented in Orbit. Paper I describes a simple pooling strategy in which pooling of the product was started and ended based on the UV absorbance of the elution peak (the so-called cut-off limits). These cut-off limits were always the same, and did not adapt to changes in the peak size. Paper II describes an adaptive pooling strategy in which new cut-off limits were obtained based on the feed concentration (Figure 8). The feed concentration was estimated from the area under the peak of the feed stream onto the column (left peaks in Figure 8). A modification of this pooling strategy was included in the study presented in Paper IV. The height of the elution peak was used to obtain the cut-off limits instead of the area under the previous peak, which made it simpler to implement. In addition, online measurement of the feed concentration was no longer necessary. However, a drawback of this method is that at very high values of the UV absorbance, the relationship between concentration and absorbance is no longer linear, leading to inaccurate values of the cut-off limits.

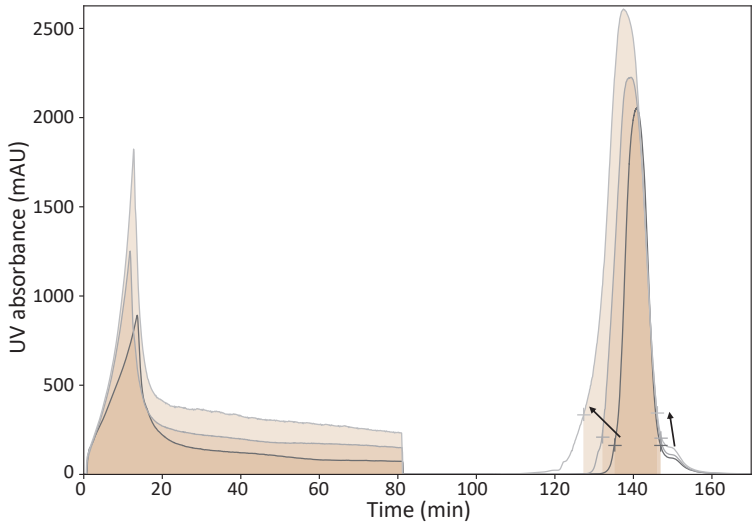

Figure 8. Adaptive pooling based on the absorbance of the elution stream from a CEX column for the removal of mAb aggregates. The absorbance cut-off limits (crosses) adapt to the amount of product loaded.

3.4 Pressure control in a UF/DF process

UF/DF is a common step in the purification of biopharmaceuticals, used to concentrate the purified product and replace the buffer to obtain the desired salt composition and pH.[18] Concentration is performed using an ultrafiltration (UF) filter (also called membrane) that retains the desired product (retentate stream) while allowing the passage of water and salt components (permeate stream). The retentate is returned to the UF/DF vessel, and the permeate is discarded (Figure 9). Buffer exchange is performed by DF, which differs from the concentration step in that the new buffer is added to the vessel, thus maintaining the volume in the vessel. DF can be either continuous, when new buffer is added simultaneously at the same rate as the permeate leaves the system, or sequential, when a number of concentration and subsequent dilution steps are carried out.[18,87]

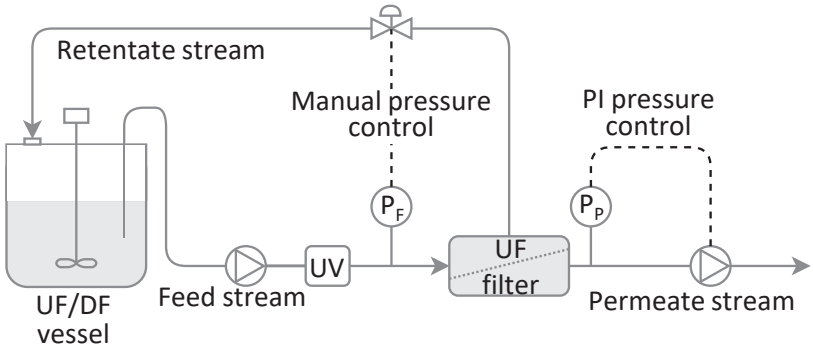

Figure 9. Block diagram of the UF/DF process. There is one pressure sensor on the feed side (P_F), used for manual control of the feed pressure, and another on the permeate side (P_P), used for the proportional integral (PI) control of the permeate pressure.

The pressure difference between the two sides of the membrane (the so-called transmembrane pressure) is important in UF/DF as this determines the flux through the membrane, and therefore the productivity.[16,88] In the study described in Paper I, a control strategy was implemented to maintain a constant permeate pressure and to be able to monitor the permeate flow rate. A proportional integral feedback controller was implemented for this purpose (Eqs. 3.4-3.5). The permeate pressure, measured with an in-built pressure sensor in the ÄKTA pure chromatography system functioned as the output signal (y), and the permeate flow rate was the control signal (u).

$$u = K_P\, e + \frac{K_P}{T_i}\int_0^t e\, dt \tag{3.4}$$

$$e = y_{ref} - y \tag{3.5}$$

Here, K_P is the controller proportional gain, T_i is the integration time, and e is the error, the value of which changes over time. The feedback controller not only allowed the permeate pressure to be maintained, but also provided a measure of the permeate flow rate, which was used to estimate the vessel volume, evaluate the membrane performance, and identify problems related to fouling of the membrane.

4 Process Design and Optimization

The design of an ICDP is more complex than that of a traditional batch mode purification process, as several conditions must be fulfilled. These conditions are presented in this chapter, and can be divided into those that are necessary to enable process integration, and those that are necessary to achieve a continuous process with multi-column periodic capture chromatography. The optimization of flow rate trajectories in the loading phase is also presented in this chapter as a tool to improve process efficiency. Finally, process scale-up, a very important aspect in process design, is addressed at the end of this chapter.

4.1 Integrated downstream processes

An integrated downstream process involves the removal of unnecessary steps and resources, to allow optimal use of the equipment, resulting in a smaller footprint, the minimization of manual intervention, and the reduction of hold-up volumes.[89] In the integration concept adopted in this work (ICS), the hold-up volumes between the chromatography steps are minimized, and the product pool from a column is loaded directly onto the next column, thus reducing process time and space.[34] In order to do so, several conditions must be fulfilled (Eqs. 4.1-4.4).

$$V_{c,i} \cdot Q_i \geq V_{pool,i-1} \cdot c_{pool,i-1} \tag{4.1}$$

$$\frac{V_{c,i-1}}{\tau_{pool,i-1}} \cdot D_{i-1} = \frac{V_{c,i}}{\tau_{load,i}} \tag{4.2}$$

$$\tau_i \geq \tau_{i,min} \tag{4.3}$$

$$V_{pool,N} \geq V_{UF/DF,min} \tag{4.4}$$

V_{pool} and c_{pool} are the volume and concentration of the pool, τ_{pool} and τ_{load} are the residence times during the pooling and loading phases, $V_{UF/DF,min}$ is the minimum initial working volume in the UF/DF process, i refers to the column

number, and D is the dilution factor in the inline dilution process that is necessary to adjust the salt concentration and pH of the eluate from column $i-1$ to load it onto column i (hereinafter, the "conditioning step").

The first condition is related to column capacity: this should be sufficient to accommodate the product from the previous column. The protein load is usually obtained as 80% of the DBC at 10% BT, as in batch mode. The second condition is related to the fact that the loading flow rate of a column must be the same as the elution flow rate of the previous column (plus any possible dilution, if applicable). In addition, the residence time must be the same as or greater than that used in the non-integrated batch process (τ_{min}), if the same process performance and degree of separation are to be achieved (third condition). Finally, if UF/DF is included as the last step in the downstream process, the scale of the process must be adapted to ensure that a sufficient amount of product is supplied (forth condition). The pool volume from the last chromatography step before the UF/DF process must thus be greater than the minimum working volume of the UF/DF process. In the study described in Paper I, for example, the columns were approximately 10 times larger than they would have been without the UF/DF step, with volumes ranging from 8 to 25 mL. Including a UF/DF step would thus increase the cost of early development phases, whereas it is often desirable to minimize the consumption of raw material and buffers in order to reduce costs. For this reason, the UF/DF step was not considered in the other studies presented in the thesis. The column volumes used in the studies described in Papers II, III, IV and VI, where ICDPs were implemented on laboratory scale without a final UF/DF step, were as little as 1 or 2 mL.

4.2 Multi-column periodic capture

Continuous purification of biopharmaceuticals usually involves the implementation of processes with multi-column periodic operation since chromatography in bind-and-elute mode is inherently a discontinuous process.[20] The use of multiple columns run in periodic mode in the capture step is thus an effective way of achieving the continuous flow required to integrate the downstream process with the perfusion bioreactor. Furthermore, the use of multiple columns can increase the productivity, resin utilization and yield.[9,64,89,90] However, the design of a multi-column periodic capture process is slightly more complex than that of a batch capture process, and additional conditions to those mentioned above must be fulfilled.

In a multi-column periodic capture process, several tasks are performed simultaneously in different columns. For example, one column can be loaded while another is being washed, eluted or regenerated, thus saving processing time.

This means that the loading time must be equal to or longer than the product recovery time (i.e., the time taken to wash, elute and regenerate the column) in order to achieve synchronized periodic operation. This translates into a design constraint known as the feed continuity constraint,[64] and is expressed in Eq. 4.5, where t_{cycle} is the cycle time and is equal to the loading time:

$$V_{c,1}\, Q_1 \geq t_{cycle}\, F_F\, c_F \tag{4.5}$$

This means that the volume of the capture columns depends on the cycle time, which is determined by the product recovery time. The cycle time varies depending on the process set-up. Three different set-ups were compared (Paper III), and it was found that integrating the capture step with the rest of the downstream process affected the cycle time, as shown in Figure 10. The first alternative involved the implementation of a complete downstream process in a single chromatography system. In this case, the cycle time was longer because most of the tasks are performed in series, as the number of pumps and valves is limited. The second alternative involved the use of two systems, such that the polishing steps and the capture step could be carried out simultaneously, leading to a 36% reduction in the cycle time. In this alternative, the two chromatography systems must be synchronized, which means that, if one system completes its task before another, this will lead to a delay in the system. Therefore, the cycle time is the highest between the recovery time in the capture step and in the polishing steps. This is undesirable as a system with shorter tasks will remain unutilized, as can be seen in the capture step in Figure 10. The aim of Alternative 3 was thus to solve this problem by introducing a surge vessel between the two systems so that they could be run asynchronously, thus minimizing the waiting time. In this third alternative, the cycle time in the capture step was reduced by 67% compared to the first alternative, however, it is more complex and involves the introduction of a hold-up volume.

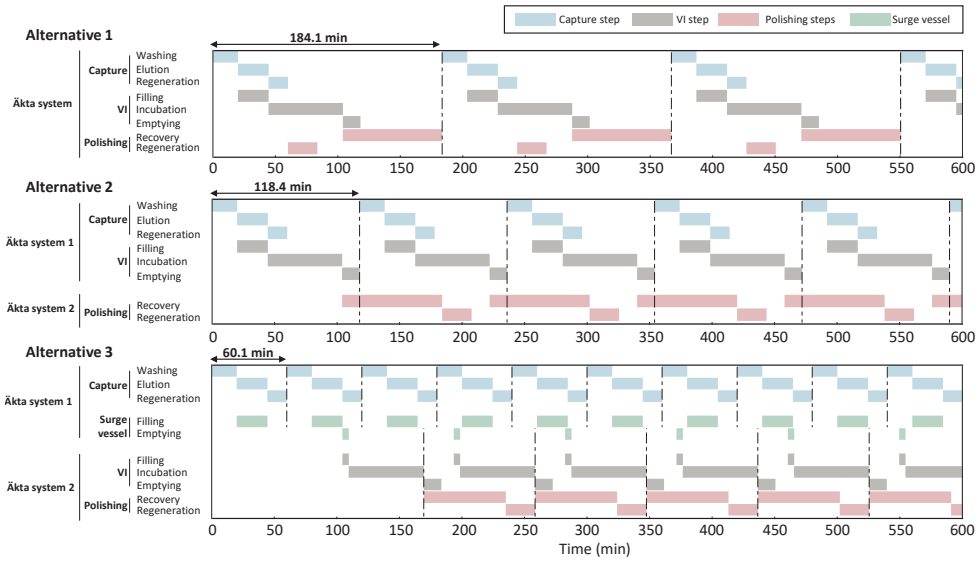

Figure 10. Gantt charts for the three integration alternatives studied. Alternative 1: integrated process in one system, Alternative 2: integrated process with two systems, and Alternative 3: integrated process with a surge vessel and two systems.

Another important aspect of the design is the protein load. This can differ considerably depending on the periodic process in question. Paper II describes a simple 2-column periodic capture process; one column is loaded while the other performs the recovery steps. When the recovery steps have been completed and the other column is completely loaded, the columns' functions change. In this case, the protein load in the column is the same as in batch mode, that is, 80% of the DBC at 10% BT.

PCC is a more advanced process solution, and is used in the studies described in Papers III, IV, VI and VII. In PCC, three or more columns are used, and the main difference compared to the 2-column periodic capture process is that two columns are connected during the loading phase such that the second column captures the product that breaks through the first column.[64] This leads to an increase in resin utilization and productivity, as both the protein load on the column and the loading flow rate can be higher than in the 2-column process, without losing more product.[28,59,64] For this reason, the protein load is calculated differently. In this case, the protein load can obtained with the DBC at high percentages of BT (and not 10%, as in the batch case), typically between 50 and 70%.[12,59,64] The higher the protein load, the higher the resin utilization, but the higher the risk of product loss in the BT stream of the second interconnected column.[59] In addition, a higher loading flow rate leads to higher productivity, but also to lower resin utilization and/or yield, as the BT curve becomes flatter.[59] Therefore, a trade-off must be

made between productivity, resin utilization and yield, and an optimal protein load must be found.

The optimal protein load can be obtained by analysing the BT curve, assuming the BT experiment is run at the same residence time and feed concentration as the ones expected in the PCC process (see Papers IV and VII). Several areas can be calculated from the BT curve to obtain the amount of protein adsorbed in the two interconnected columns and the product loss. During the loading phase in PCC, both the interconnected columns (referred to as Column 1 and Column 2) become loaded with product: Column 1 is loaded with the harvest, and Column 2 is loaded with the breakthrough from Column 1. In the illustrative example in Figure 11, Column 1 is loaded with a total of 60 g/L resin, and the amount of product corresponding to area k_1 (product BT from Column 1) is loaded onto Column 2. The amount of product k_1 in this example is 20 g/L, and constitutes the protein load in Column 2. At 20 g/L, the area under the BT curve (k_2) corresponds to the product loss in the BT stream from Column 2. In the following cycle, Column 2 acts as Column 1, and continues to be loaded with harvest until the total protein load on the column reaches 60 g/L. The protein load during a cycle (Q_{cycle}) is therefore 40 g/L, as Column 2 is already loaded with 20 g/L at the start of the following cycle. The total amount of product adsorbed in Column 1 is given by area k_3, while area k_4 corresponds to the unutilized portion of the resin.

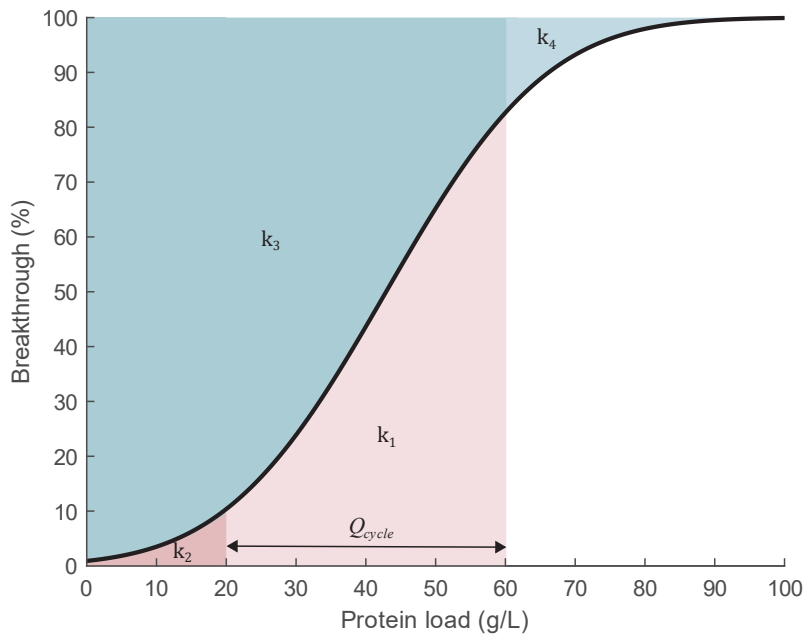

Figure 11. Illustration of a BT curve and the areas defined for the design of a PCC capture process. The X axis is the protein load in g/L resin and the Y axis is the BT percentage defined as the concentration in the BT divided by the feed concentration.

The resin utilization is therefore obtained by dividing the amount adsorbed (k_3) by the total resin capacity ($k_3 + k_4$). The product loss in percentage is obtained by dividing the product loss (k_2) by the total amount of product loaded (Q_{cycle}), while the yield is 100 minus the percentage product loss. In the study presented in Paper IV, different values were obtained for the yield and resin utilization depending on the protein load on the columns (Figure 12). For very high protein loads, the area k_4 becomes very small, resulting in very high resin utilization, however, the area k_2 increases, which reduces the yield.

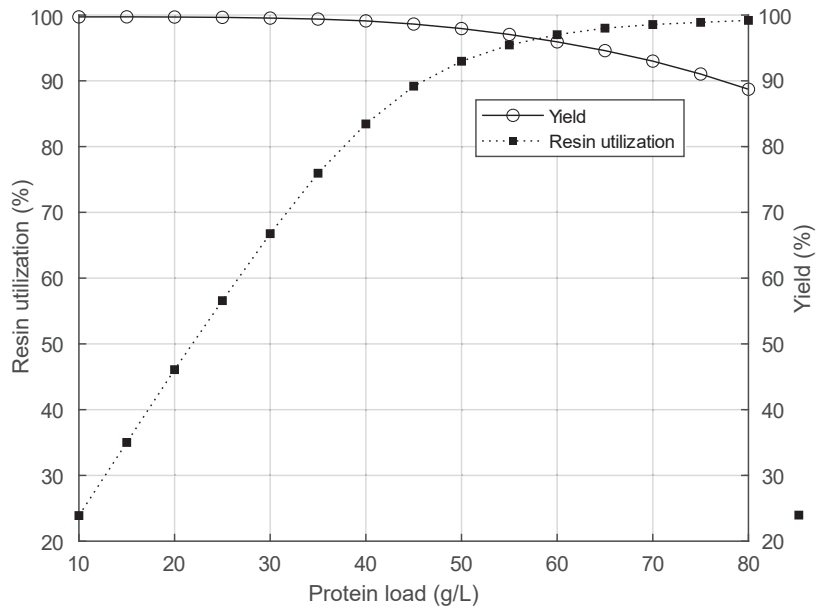

Figure 12. Effect of the protein load on the resin utilization and yield in PCC.

The appropriate protein load can then be determined based on the process parameter that is most important: resin utilization or yield. Once the protein load has been determined, the column volume can be calculated using Eq. 4.5. The residence time of the process can then be obtained from the column volume and the loading flow rate. This method is convenient due to the very little experimental effort required, as, in principle, only a BT curve experiment is needed. However, the residence time at which the BT curve experiment is run may differ from the residence time of the actual PCC process, causing the shape of the BT curve to change (the shorter the residence time, the flatter the BT curve). If the residence time of the actual PCC process is found to be shorter than that used in the BT curve experiment, BT curve experiments should be repeated iteratively until the residence time in the BT curve experiment and the PCC process coincide. Alternatively, a shorter residence time than the one expected in the actual PCC

process could be chosen. The process would thus be designed for the worst-case scenario regarding the residence time, and iterative repetition of the BT experiments would be avoided, although the residence time would not be optimal.

Model-based optimization can be used to determine the optimal residence time and protein load. In this approach, the process is simulated at different process conditions to identify those that give the best process performance.[29,59] In other words, optimal decision variables must be found to maximize (or minimize) one or several objective functions. In cases where there are several objective functions, the method is called multi-objective optimization, and a set of optimal solutions, called the Pareto front, is obtained.[91]

Paper III describes a multi-objective optimization study on PCC, in which different Pareto fronts were obtained. The decision variables were the residence time in the loading phase and the cycle time (which is indirectly related to the protein load), and the objective functions were the productivity and the resin utilization. The yield was set as a constraint, and was therefore constant. The general rate model was used to simulate the process, and optimization was performed using an elitist non-dominated sorting genetic algorithm for constrained multi-objective problems called NSGA-II,[91] built into the MATLAB *gamultiobj* function. Figure 13 shows two Pareto fronts: one for a 3-column PCC process, and the other for a 2-column periodic capture process. The feasible points depend on the process set-up (using the three alternatives shown in Figure 10). In the solutions with higher loading flow rate (shorter residence time), the cycle must be shorter to maintain a high yield, as the BT curve appears earlier, leading to a lower protein load. These solutions thus give higher productivity as a result of the higher loading flow rate, but the resin utilization is decreased since the protein load is lower. As the cycle time is short at these points, they are only feasible for Alternative 3. The points in the Pareto front with higher resin utilization and lower productivity have lower loading flow rate, higher protein load, and longer cycle time. These points are feasible for all three alternatives as the cycle is longer than the minimum required in the three cases. When comparing the 3-column and 2-column processes, it can be seen that the 3-column PCC process is much better in terms of both productivity and resin utilization, due to the interconnection of the columns during the loading phase, which allows increasing resin utilization without negatively affecting the productivity or yield.

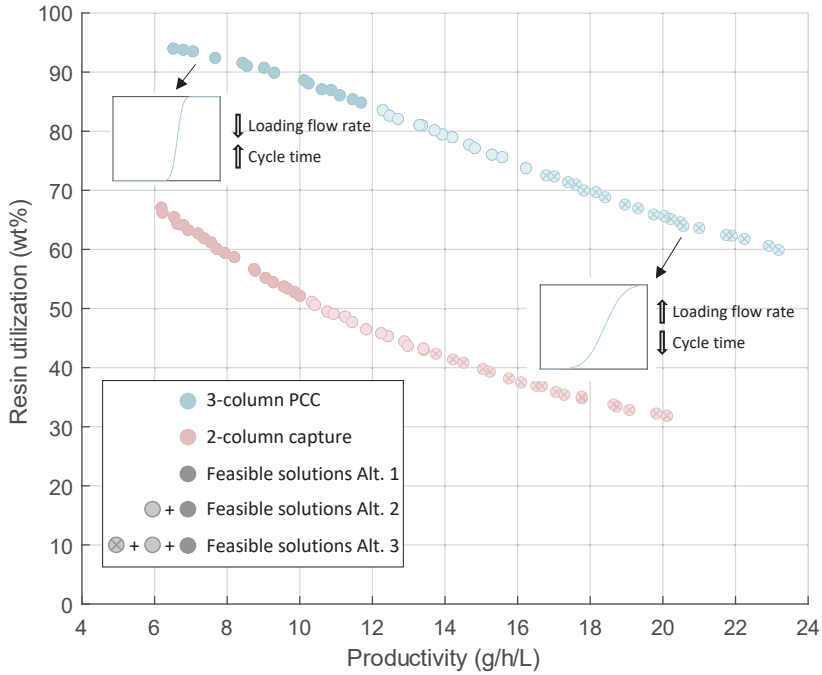

Figure 13. Pareto fronts with optimal solutions for the PCC operation. Three different integration alternatives according to Figure 10 (filled, shaded and crossed points) were considered. The feed concentration was 0.5 g/L and the protein A resin was mAbSelect PrismA. A 3-column PCC process is compared to a 2-column periodic capture process.

4.3 Flow programming

Productivity and resin utilization can also be improved by flow programming.[20,67,83,92] This method consists of applying a stepwise or continuous flow rate trajectory in the loading phase.[20] The flow rate affects the process performance significantly. As mentioned, a higher loading flow rate leads to higher productivity but lower resin utilization and/or yield due to a flatter BT curve.[93] Therefore, applying a higher flow rate at the beginning, when all the binding sites are available, and reducing it towards the end of the loading phase, thus giving the protein molecules more time to diffuse, can give a suitable trade-off between the productivity, resin utilization and yield. The potential of varying loading flow rate trajectories has been demonstrated previously, both with the DOE approach[20] and with a modelling approach.[67]

A comprehensive model-based study of flow programming of a mAb capture step was performed to compare different types of flow trajectories, using the general rate model to predict the BT curves (Paper V). Five-step flow trajectories and

continuous flow trajectories were compared with the same process using a constant flow rate, and multi-objective optimization was performed with the NSGA-II optimization method to obtain flow trajectories that maximized the productivity and resin utilization, with a yield constraint of 99%. Several Pareto fronts were obtained, as shown in Figure 14. Five different points were chosen for comparison:

- Case I – constant flow rate,
- Case IIa – a 5-step flow rate trajectory with higher resin utilization than in Case I, but the same productivity,
- Case IIb – a 5-step flow rate trajectory with higher productivity than in Case I, but the same resin utilization,
- Case IIIa – a linear flow rate trajectory with higher resin utilization than in Case I, but the same productivity,

  and

- Case IIIb – a linear flow rate trajectory with higher productivity than in Case I, but the same resin utilization.

When comparing Case IIa with Case I, an increase of 9% in resin utilization was achieved, while Case IIb gave 12% higher productivity than Case I. The results for Cases IIIa and IIIb (linear flow rate trajectory) were very similar to those obtained in Cases IIa and IIb (5-step flow rate trajectory). Optimization of quadratic trajectories and stepwise trajectories with up to 50 steps was performed, giving similar results to those shown in Figure 14. It was therefore concluded that complex flow trajectories are not beneficial, and that simple, optimized trajectories are sufficient.

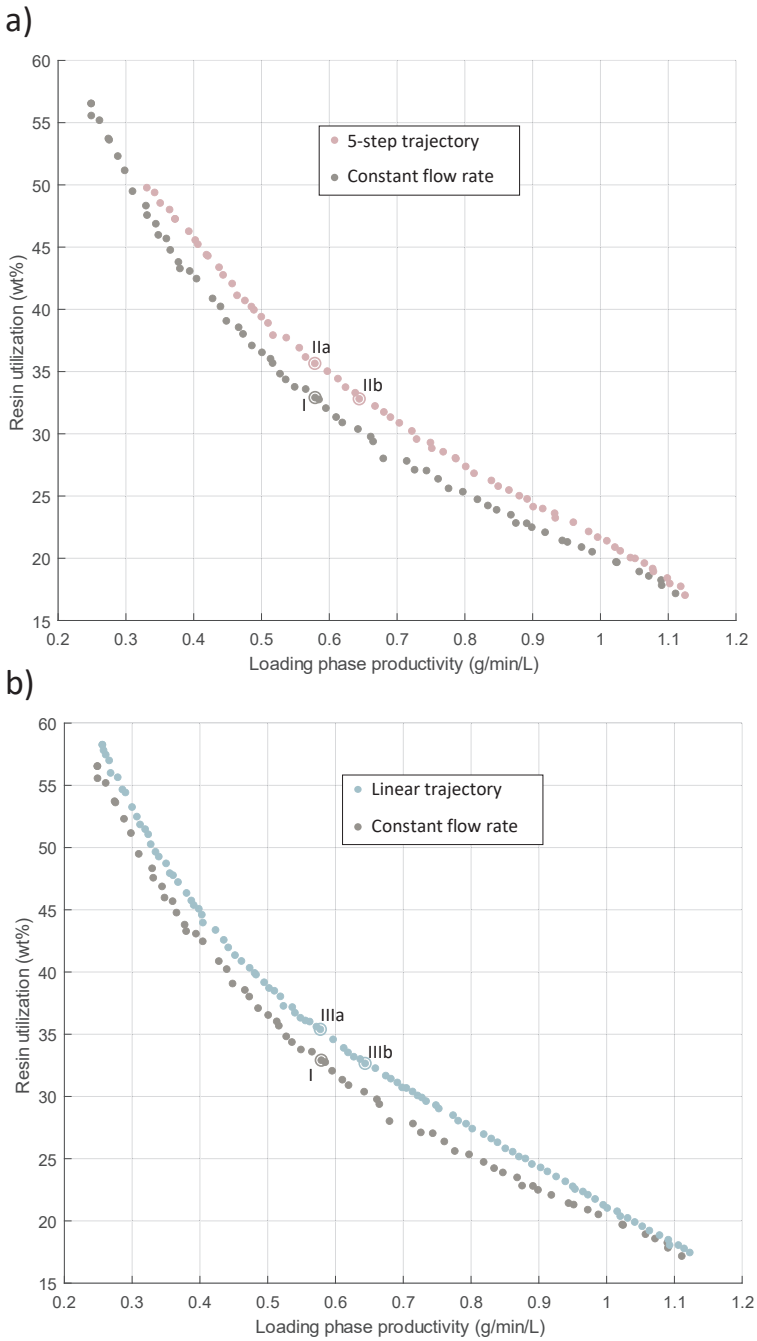

Figure 14. Pareto fronts for different loading alternatives obtained with flow programming. a) Pareto front for 5-step flow rate trajectories, b) Pareto front for linear trajectories. The Pareto front for constant flow rate (Case I) is plotted in both panels for comparison. Note that the loading phase productivity is defined as the amount of product loaded divided by the loading time and the resin volume.

4.4 Process scale-up

Packed-bed chromatography columns are relatively easy to scale up as the process parameters scale linearly.[94] Traditionally, the column length and the fluid velocity (and thus the residence time) are kept constant, while the column diameter is increased.[94,95] However, this method is not practical for scaling up laboratory-scale columns, which typically have lengths of only a few centimetres. Stickel and Fotopoulos have shown that for columns with larger diameters, the pressure drop along the column is higher at constant velocity and length, which can limit the maximum velocity applicable on large scales.[96] To avoid dramatic changes in the aspect ratio, a flexible approach was studied in which the column length was increased (Papers V and VII).[70,94] The main idea behind this method is that the number of theoretical plates (N) must be kept constant (or increased) to obtain the same (or better) separation performance. The definition of the number of theoretical plates, combined with the Van Deemter equation for the height equivalent of a theoretical plate (H),[20] results in the following equation:

$$N = \frac{L}{H} = \frac{L}{A + \frac{B}{v} + Cv} \tag{4.6}$$

where A, B and C are constants in the Van Deemter equation related to eddy diffusion, molecular diffusion and mass transfer resistance, respectively.[20] The molecular diffusion in proteins is very slow as the molecules are very big.[20] Therefore, the B term can be neglected. If the velocity is expressed as the column length divided by the residence time (τ), Eq. 4.6 can be written as below.

$$N = \frac{1}{\frac{A}{L} + \frac{C}{\tau}} \tag{4.7}$$

According to Eq. 4.7, the greater the column length and the residence time, the better the separation, as has been shown previously.[94] This means that the column length does not have to be kept constant. This method of scaling-up is thus based on maintaining the residence time, and increasing the column length and the column diameter, which means that the flow velocity is also changed. The length of the column is limited by the pressure drop along the column. The pressure drop (ΔP) can be calculated with the Blake-Kozeny equation:[96]

$$\Delta P = \mu \frac{K_0}{{d_p}^2} \frac{(1-\varepsilon_c)^2}{\varepsilon_c^3} vL \tag{4.8}$$

where K_0 is an empirical constant. Assuming that the column void, the particle diameter and the fluid viscosity are the same on all process scales, the left term

can be lumped into a single empirical constant, α. Expressing the velocity as the column length divided by the residence time, as above, results in Eq. 4.9.

$$\Delta P = \frac{\alpha L^2}{\tau} \tag{4.9}$$

The maximum column length (L_{max}) can then be obtained from Eq. 4.10.

$$L_{max} = \sqrt{\frac{\Delta P_{max} \tau}{\alpha}} \tag{4.10}$$

The empirical constant α depends on the aspect ratio, being higher for columns with a higher diameter-to-length ratio.[96] The maximum permissible pressured drop (ΔP_{max}) is also scale-dependent, being lower for wider columns.[94] Therefore, these two values must be obtained for the aspect ratio intended for the final scale, which requires pressure data from the resin manufacturer at different aspect ratios.[94] Once the column length is known, the column diameter can be adjusted to obtain the column volume required to maintain the residence time and the protein load.

5 Integrated Continuous Downstream Processes: Case Studies

Once an ICDP has been designed and optimized, and suitable control and automation strategies have been obtained, it must be implemented experimentally. This chapter presents several case studies together with the results obtained from the implementation of the process.

5.1 Case Study I: Integration of chromatography and ultrafiltration

Chromatographic unit operations are usually implemented in systems that are intended for preparative chromatography on different process scales, such as ÄKTA pure or ÄKTA pcc, while UF/DF is usually performed in specially adapted systems for filtration processes, such as ÄKTA crossflow and ÄKTA flux, or the KrosFlo® systems. The integration of chromatography and UF is challenging as there are no commercially available systems in which both processes can be run. In the first case study (Paper I), an ÄKTA pure chromatography system was adapted so as to be able to run both processes in a single system. Several chromatography steps to purify a recombinant protein, were combined with a final UF/DF step to concentrate the final product and exchange the buffer, as illustrated in Figure 15. The first step was the capture of the main impurities in a mixed mode chromatography (MMC) column. A CEX column, an anion-exchange membrane chromatography (AEMC) column, and an AEX column were used in the subsequent polishing steps. Finally, UF/DF provided the desired product in terms of concentration and buffer composition.

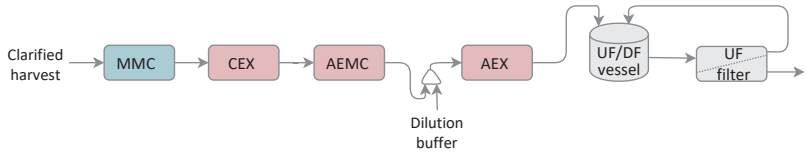

Figure 15. Block diagram of the integrated process described in Paper I, where chromatography and ultrafiltration were combined for the purification of a recombinant protein. The colours represent the function of each unit: blue, capture; red, polishing steps; and grey, product concentration and buffer exchange.

The chromatograms corresponding to the different columns are presented in Figure 16a. The product is first loaded onto the MMC column. It is then eluted from that column and loaded onto the CEX column (first shaded peak). During elution of the CEX column, the pool (second shaded peak) flows through the AEMC column and it is loaded onto the AEX column. Finally, the product is eluted from the AEX column into the UF/DF vessel (third shaded peak). The product was concentrated 10 times in the concentration step of the UF/DF process, and 97% of the buffer was replaced by the storage buffer with 5-step sequential DF (Figure 16b). The UV absorbance in the feed was measured to monitor the protein concentration in the vessel and the conductivity in the permeate was monitored to ensure that the buffer was replaced at the desired rate.

Comparison with the same processes run manually in separate steps revealed that the total process time was reduced from 12.5 h to 7.5 h when using the integrated process. This led to an increase in productivity, from 0.9 to 1.1 g/h/L of the capture column, demonstrating the benefit of process integration and automation.

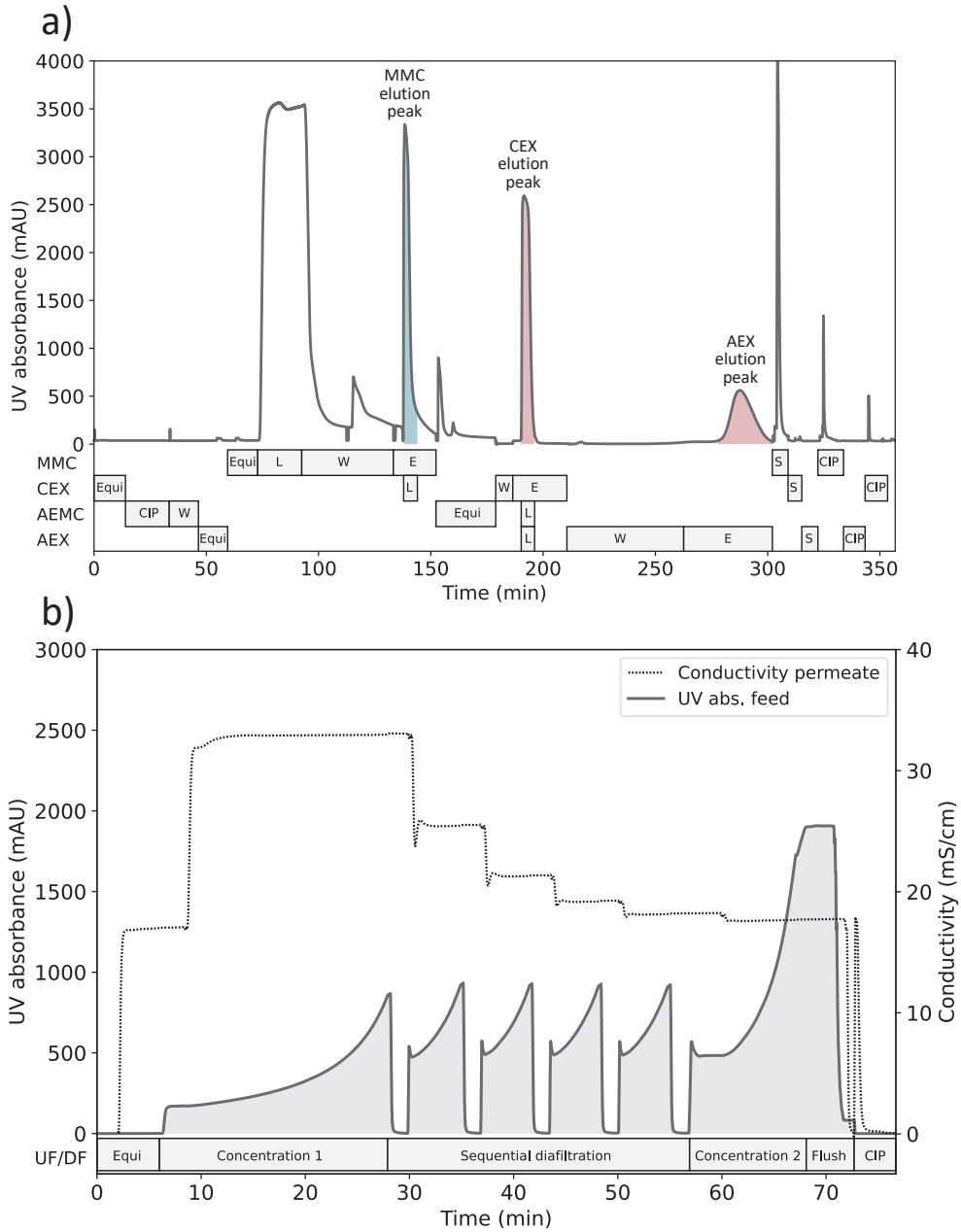

Figure 16. a) Chromatogram showing the MMC, CEX, AEMC and AEX steps. The shaded areas are the peaks eluting from the MMC, CEX and AEX columns. The nomenclature for the process phases is as in Figure 3. b) UV absorbance in the feed and conductivity in the permeate in the UF/DF process.

5.2 Case Study II: ICB process with 2-column continuous capture for the production of mAbs

The integrated downstream process presented in Paper I was developed further to implement an ICB process for the production of mAbs (Paper II). A laboratory-scale bioreactor was run in perfusion mode together with an ICDP consisting of three chromatography steps and a VI step (Figure 17). The capture step was run with affinity protein A chromatography in a 2-column periodic capture process with continuous feed from the bioreactor. The second step was VI, where the product was held at low pH in a vessel. Two polishing steps were then used to increase the purity: a CEX column in bind-and-elute mode, and an AEX column in flow-through mode with a conditioning step by inline dilution at the inlet. The ICDP was implemented in a single ÄKTA pure chromatography system, using a set-up similar to that described in Paper I. The 2-column periodic capture process was made possible by the introduction of two additional valves that allowed loading of one column and simultaneous elution of the other.

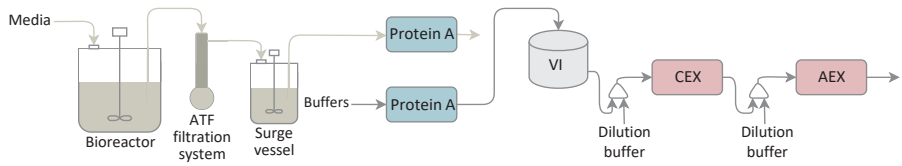

Figure 17. Block diagram of the ICB process for the production and purification of mAb described in Paper II. An alternating tangential flow (ATF) perfusion bioreactor was connected to an ICDP consisting of a 2-column periodic capture step (using protein A resin) with continuous loading, a VI step, and two polishing steps using CEX and AEX columns.

The perfusion culture was run for 13 days with continuous purification of the product. The bioreactor-based productivity reached a maximum of 0.79 g/day/L bioreactor volume, and averaged about 0.6 g/day/L, which is close to the values obtained by others using similar processes (0.67 g/day/L by Godawat et al.[12], and 0.33 g/day/L by Steinebach et al.[84]). In addition, the process scale was the smallest ever used for an ICB process, with a bioreactor volume of only 0.2 L. Previously reported bioreactor volumes in other laboratory-scale ICB implementations are 1.2 and 1.5 L.[84,97] This allowed a very compact design, with a footprint of only a few square metres, enabling the implementation of this ICB process in small labs with limited resources.

5.3 Case Study III: PCC integrated in a downstream process

A 3-column PCC operation in an ICDP for the purification of mAbs was used in the process illustrated in Figure 18 (Paper III). The process was implemented on laboratory scale using two ÄKTA pure systems: the PCC and VI in one ÄKTA system, and the polishing steps in another ÄKTA system.

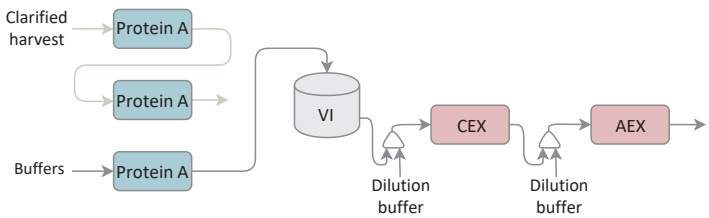

Figure 18. Block diagram of the ICDP with 3-column PCC for the purification of mAbs described in Paper III.

The process was run for 18 hours with clarified harvest at a concentration of 0.5 g/L. Periodic peaks of product at cyclic steady state were observed in each step, as shown in Figure 19. The three peaks seen in the chromatogram of the protein A capture column (Figure 19a) correspond to the washed-out impurities, the eluted product and the strongly bound impurities desorbed in the stripping phase. The chromatographic peaks from the polishing steps (CEX and AEX) are shown in Figure 19b. The first CEX peak is the loading phase of the CEX column when the VI vessel is emptied. The second CEX peak is the elution peak, and the AEX peak is detected at the same time since this column is run in flow-through mode. Its height is approximately half that of the CEX peak as a result of the conditioning step, which is carried out by inline dilution at a 1:1 ratio.

The experimental resin utilization was estimated to be 74%, the downstream productivity was 12 g/h/L protein A resin, and the overall yield was 78%. These results are comparable to state-of-the-art PCC processes with the same feed concentration. Shi et al. studied the effect of residence time in a PCC process, and reported productivities between 2 and 8 g/h/L for a resin utilization of 70% and the same feed concentration,[59] while Baur et al. reported a maximum productivity of approximately 8 g/h/L for a feed concentration of 0.5 g/L using model-based optimization.[28]

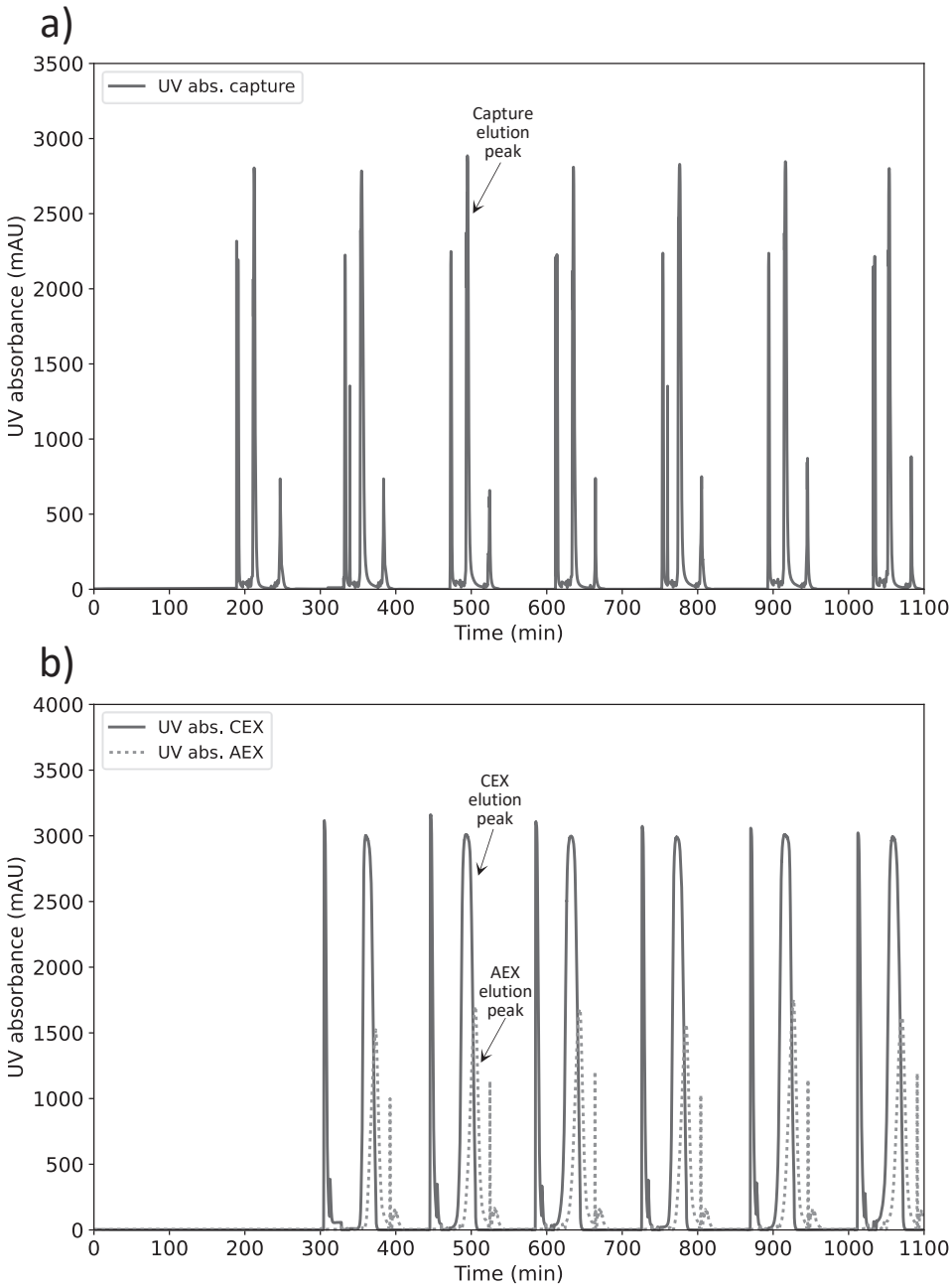

Figure 19. Chromatograms of the capture step (a) and the polishing steps (b) of the ICDP presented in Paper III at cyclic steady state. The labelled peaks represent the eluted product from each chromatographic step.

5.4 Case Study IV: PCC integrated with pre-capture continuous VI

In traditional batch VI, the product is maintained at low pH in a vessel, and the total time during which the product is held under acidic conditions is longer than necessary due to the time taken for filling and emptying the vessel. Long residence times under harsh acidic conditions can lead to the formation of protein aggregates. Continuous VI allows the residence time distribution to be narrowed, while keeping the hold-up volume low.[13] In this case study (Paper IV), a packed-bed column was used to carry out continuous VI with a solvent/detergent method to avoid acidic conditions. A stock solution of 3% tri-n-butyl-phosphate (solvent) and 10% polysorbate 20 (detergent) was mixed by inline dilution with clarified harvest at a ratio of 1:9. The packed-bed column was placed before the capture step, which consisted of a 3-column PCC operation for the purification of a recombinant protein, thus obtaining a continuous stream from the harvest to the capture columns (Figure 20). A polishing step based on HIC was used to further increase product purity. The solvent and the detergent added for VI were removed in both chromatography steps.

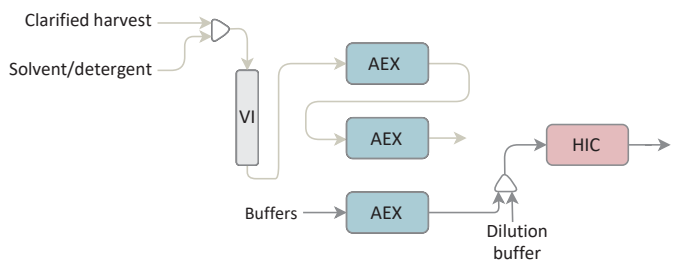

Figure 20. Block diagram of the ICDP with PCC and continuous solvent/detergent VI for the purification of a recombinant protein described in Paper IV. The capture step is based on AEX and the polishing step is based on HIC.

This process was also run in batch mode to evaluate the benefit of adopting a continuous approach. The batch process consisted of VI in batch mode followed by a single-column capture step and the polishing step. The protein load was the same in the continuous and batch runs, and several process performance indicators were compared. It can be seen from Table 1 that the continuous process out-performed the batch process; showing a higher yield, due to the low product loss in the breakthrough of the capture column, and higher productivity, due to a much shorter process time.

Table 1. Process performance indicators in a batch downstream process and a continuous downstream process

Parameter	Units	Batch process	Continuous process
Product output per cycle	mg	22.6	23.5
Resin utilization	wt%	89.2	89.2
Productivity	g/day/L resin	22.6	44.1
Yield	wt%	68.3	87.5
Specific buffer consumption	L/g	16.7	16.0

5.5 Case Study V: ICB process with PCC for the production of pH-sensitive mAbs

Aggregation is a common problem in the production of mAbs. It is caused by the association of two or more protein molecules due to their instability in the environment; the aggregate form being more thermodynamically favourable.[98] These aggregates pose a risk to the patient as they can over-stimulate the patient's immune response, leading to the risk of adverse effects.[99] For this reason, they must be removed in the purification process. Most aggregates are removed by CEX in the polishing step, where the monomer and the aggregates co-elute. Their elution peaks usually overlap, which means that in order to remove the aggregates, a portion of the monomer must also be discarded. Therefore, demands on product quality severely compromise the yield. One way to overcome this problem is to minimize the formation of aggregates. Continuous manufacturing helps reduce aggregate formation due to the shorter residence time in the bioreactor.[19] However, the acidic conditions usually applied in the protein A capture and the VI steps often lead to aggregation, as the pH falls to between 3.2 and 3.5.[29,97] This is especially problematic for aggregation-prone mAb variants such as next-generation bispecific mAbs, IgG2 and IgG4.[100-102] A downstream process with milder conditions is thus needed for the purification of pH-sensitive mAbs.

In this case study, a newly developed protein A ligand called Z_{Ca} was used. The binding of antibodies to this ligand is regulated by calcium, and elution can be carried out at pH values up to 6.0.[103,104] Solvent/detergent-based VI was also used to avoid acidic conditions. The protein A capture step, VI and the two polishing steps were integrated by optimal selection of the process conditions (Paper VI). In particular, the pH and salt concentration were chosen to be the same in the elution phase of the capture step, the VI step and the loading phase of the CEX step to avoid the need for conditioning steps. This reduced the process complexity, the loading volumes and the buffer consumption. This ICDP, shown in Figure 21, was run on laboratory scale with clarified harvest as feed and with column volumes ranging from 1 to 2 mL.

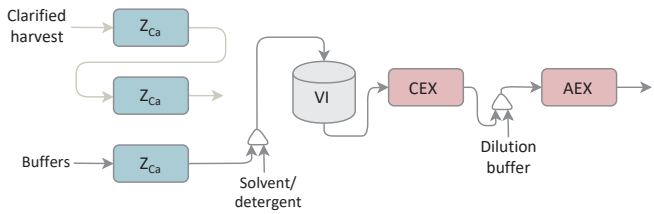

Figure 21. Block diagram of the laboratory-scale ICDP described in Paper VI. A 3-column PCC capture step, with the Z_{Ca} protein A ligand, a solvent/detergent-based VI step and two polishing steps are used for the purification of pH-sensitive mAbs.

Figure 22 shows the chromatograms corresponding to the different process steps. The larger peak in Figure 22a (capture step) corresponds to the impurities that are removed in the washing phase, while the second peak, shaded blue, is the eluting product. The capture eluate was mixed inline with the solvent/detergent stock solution, which was the same as the one used in Case Study IV, and loaded into the VI vessel. After 60 minutes' incubation, the virus-inactivated product was loaded onto the CEX column. The plateau seen in the first 17 minutes in Figure 22b (polishing steps), corresponds to the product being loaded onto the CEX column. The first large peak, shaded darker red, is the CEX eluate. The product is loaded directly onto the AEX column with 1:1 inline dilution to adjust the pH and salt concentration. As the AEX column is run in flow-through mode, the product is obtained directly from the AEX column after a certain delay volume, which corresponds to the second, smaller, peak, shaded lighter red. The minimum pH to which the product was exposed was 5.5. As a result of the mild conditions used in the process, no aggregates were detected, and product loss in the CEX step was significantly reduced.

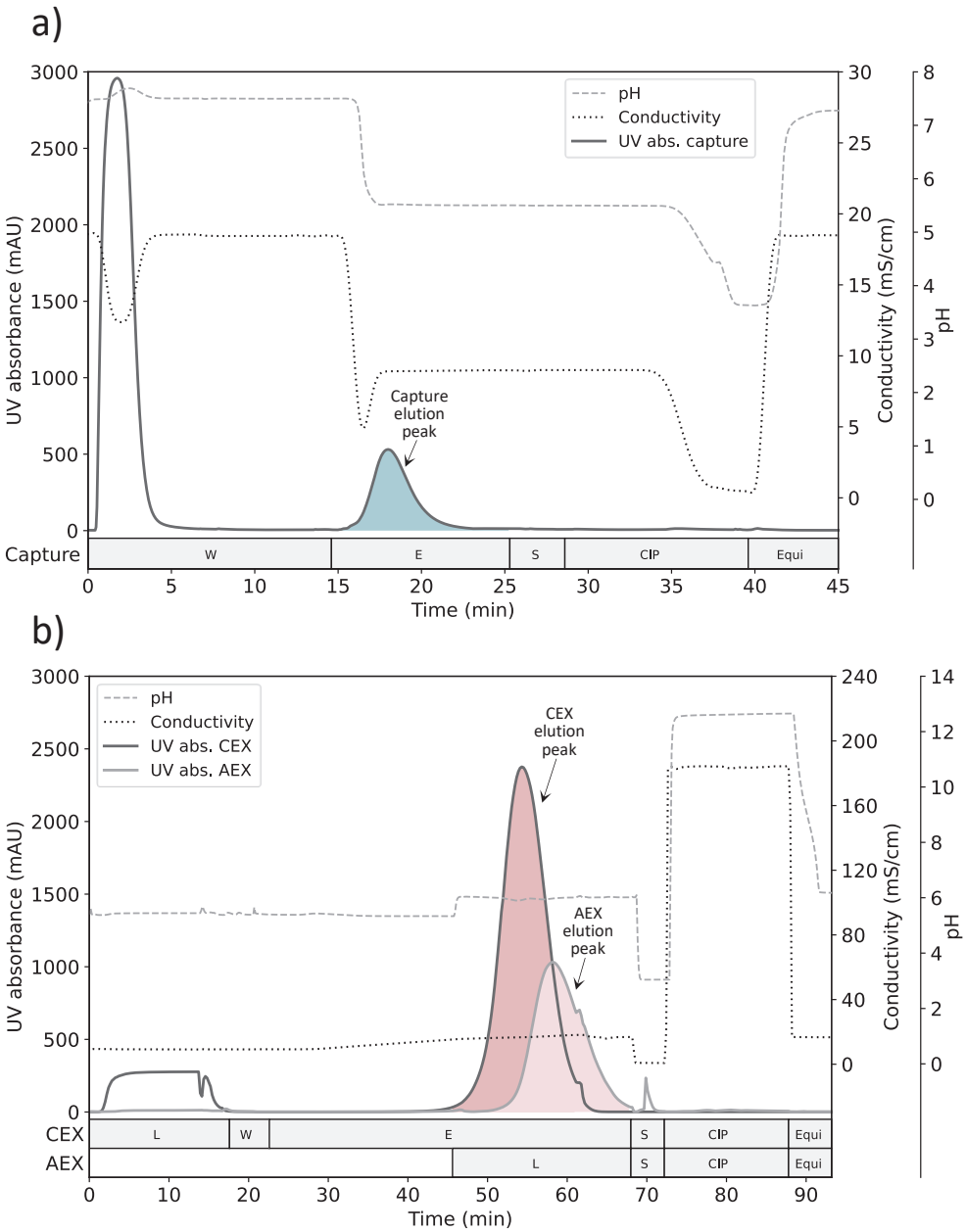

Figure 22. Chromatographic profiles for the capture step (a) and the polishing steps (b) of the ICDP presented in Paper VI for the purification of pH-sensitive mAbs. The shaded areas correspond to the product pools in each step. The nomenclature for the process phases is as in Figure 3.

The downstream process for pH-sensitive mAbs was then scaled up and coupled to a 30 L tangential flow filtration perfusion bioreactor to demonstrate the feasibility of the process on pilot scale in a 17-day run (Paper VII). The process is illustrated in Figure 23. The capture step consisted of a 3-column PCC process with the aforementioned Z_{Ca} protein A ligand implemented in an ÄKTA pcc system, and the polishing steps were implemented in an ÄKTA pure system. The integration approach used was Alternative 3 in Figure 10, i.e., a two-system set-up with a surge vessel in between, as this minimizes the waiting times, leading to more efficient synchronization between the capture step and the polishing steps. As explained in the previous section, one drawback of this integration approach is the higher process complexity and the inclusion of a hold-up volume in the process due to the use of the surge vessel. However, in this case the VI vessel acted also as the surge vessel, meaning that the process complexity was not increased, and there were no additional hold-up volumes. In a low-pH VI step, traditionally used in mAb purification processes,[10] the VI vessel could not act as a surge vessel as a long residence time would promote the formation of aggregates. This is an advantage of the solvent/detergent method, which results in higher productivity due to better synchronization between the systems. A 2-vessel periodic process was used in the VI step so as to be able to fill one of the vessels and empty the other one simultaneously.

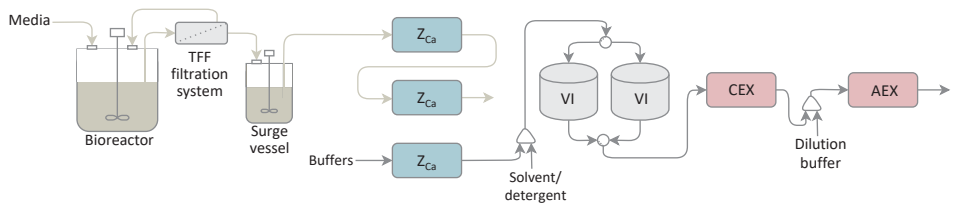

Figure 23. Block diagram of the pilot-scale ICB process for the production and purification of pH-sensitive mAbs described in Paper VII. A tangential flow filtration (TFF) perfusion bioreactor is connected to an ICDP consisting of a 3-column PCC capture step, with the Z_{Ca} protein A ligand, a solvent/detergent-based VI step and two polishing steps.

The results of the long-term cyclic steady-state operation of the pilot-scale ICDP are shown in Figure 24. In PCC, it is important to monitor the BT curves from the capture steps, and these are shown in Figure 24a. The first of the two interconnected columns was loaded up to BT percentages of 30% (defined by the concentration in the BT divided by the feed concentration), while the mAb concentration in the BT of the second column was low, which means that some product was lost in the BT of this column, but the amount was small. The process time for the capture step was about 40 min, while the process time for the polishing steps was approximately 120 min. Therefore, the polishing steps were run with product collected from three capture cycles, thus avoiding unproductive waiting times in the capture step.

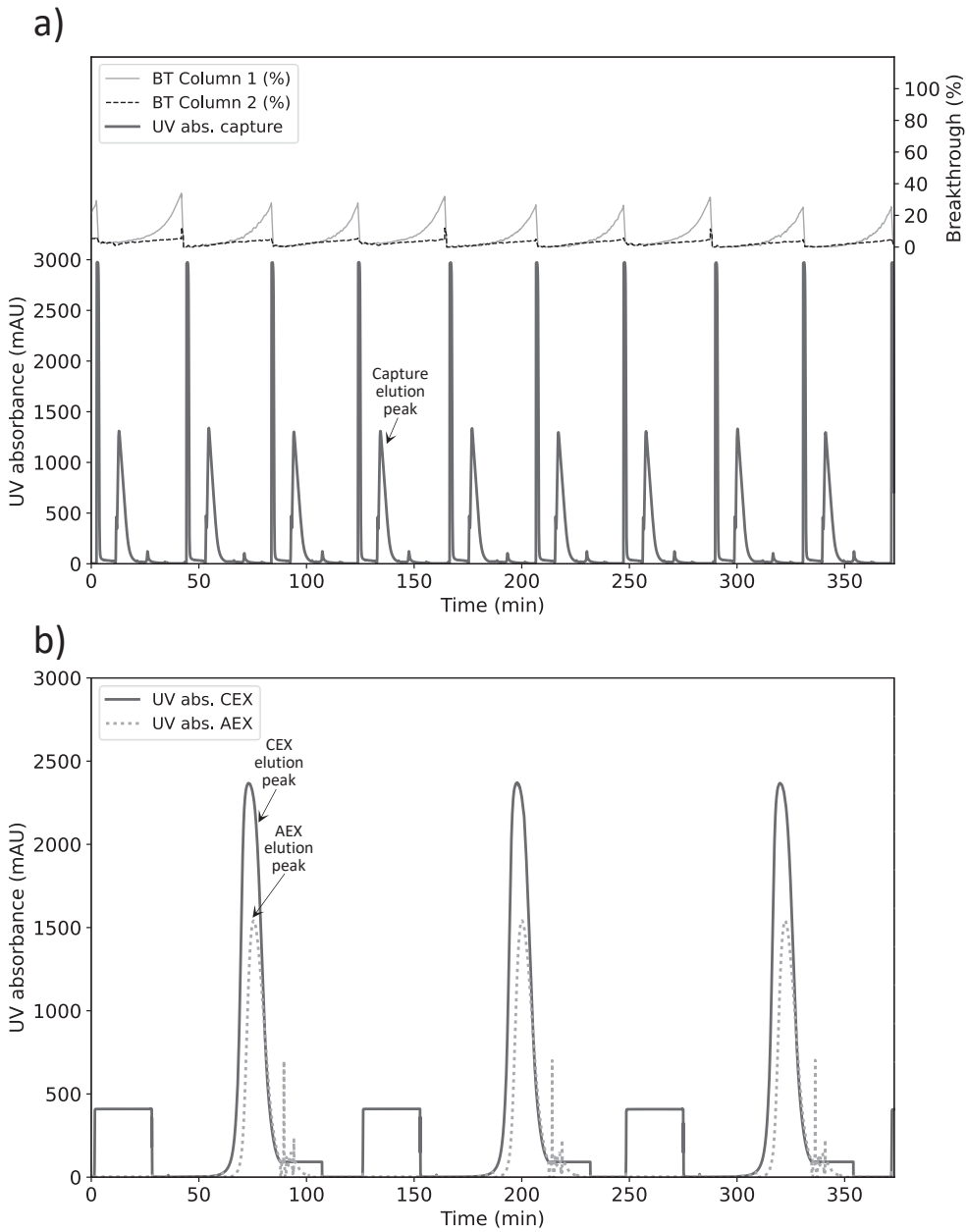

Figure 24. Results of the pilot-scale ICDP described in Paper VII. a) Chromatographic profiles from the capture step in the PCC operation: UV absorbance from the Z_{Ca} protein A column in the recovery phases, and BT curves expressed as percentages of the feed concentration. b) Chromatographic profiles from the polishing steps: UV absorbance from the two polishing steps (CEX and AEX).

The recovery yield was calculated for each process step, showing very high values throughout the pilot-scale run (Figure 25). The yield in the VI, CEX and AEX steps was close to 100% during most of the run. It is especially remarkable that the yield in the CEX step was so high, as aggregate removal in this step usually compromises the yield significantly. The low level of aggregates due to the mild conditions used in the process allowed a more yield-focused design of the CEX step, where product loss could be minimized. The yield of the capture step was about 90% during steady-state operation; the remaining 10% corresponding to the product loss in the BT. The total recovery yield of the downstream process was also about 90%, which is among the highest ever reported for a mAb downstream process: previous values ranging from 80% to 92%.[12,84,105] The bioreactor-based productivity reached approximately 1 g pure mAb/day/L reactor volume during steady-state operation, and thus outperformed the ICB process described in Paper II. This was mainly due to the use of the more productive PCC, instead of the 2-column periodic capture process, and the increase in the recovery yield.

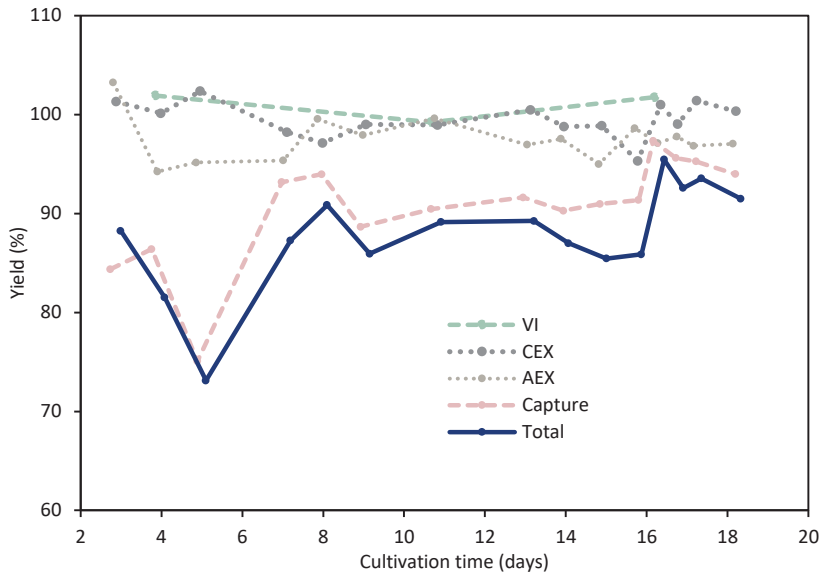

Figure 25. Recovery yield for the different downstream steps in the pilot-scale ICDP described in Paper VII.

Regarding product purity, the downstream process was able to effectively remove host cell proteins and DNA, with logarithmic reduction values of approximately 5.0 and 6.0, respectively, which are comparable to state-of-the-art integrated mAb processes.[84,106] The aggregate concentration in the purified product was insignificant, with values below the detection limit in size-exclusion HPLC analysis. Another important aspect in this process was to ensure the removal of the tri-n-butyl-phosphate added as solvent in the VI step, as it has carcinogenic effects.[107] Complete removal of the solvent was achieved in the CEX and AEX steps, with no detectable solvent in the purified product.

6 Conclusions

ICB is key in reducing the cost of biologics, thus enabling global access to biopharmaceuticals, and improving the flexibility of the processes to meet complex and growing demands. This thesis describes some tools that can be used to design and control ICDPs thus increasing process efficiency. The feasibility of several processes has been demonstrated in several proof-of-concept case studies.

Mechanistic modelling was used as a support tool for process design, control and optimization. The control software Orbit was used to automate and control the processes. It was adapted to enable the control of several systems at the same time and to handle communication between them, allowing the connection of external devices. In addition, several control strategies were developed and implemented in Orbit. The loading of the capture step is crucial as it determines the productivity in an ICDP using periodic chromatography, as shown in Paper III. Therefore, special effort was devoted to developing control strategies for this step. Model-based loading control is a promising approach as the loading operation can be optimized in real time, as shown in Paper II, and it allows process performance indicators to be predicted. Combining this approach with online analytics would also make the process more robust. Another important aspect in the control of such processes is product pooling. An adaptive pooling strategy was developed to efficiently separate mAb monomers from mAb aggregates in the CEX step, as described in Paper II, adjusting the pooling cut-off limits to the feed concentration. Finally, a proportional integral controller was implemented in a UF/DF process to control the permeate pressure and monitor the permeate flow rate.

The design of an ICDP requires a holistic approach, which makes it more complex than the design of separate batch unit operations. Firstly, process integration based on ICS, where the pool from one column is loaded directly onto the next, requires the consideration of several conditions. The column capacity and volume must be sufficient to accommodate the product eluted from the previous step; the flow rates of two consecutive columns must match; the residence time cannot be shorter than a certain predetermined value to ensure good separation; and, when running a UF/DF process at the end of the processing train, the scale of the process must be sufficiently large to supply the UF/DF process. Secondly, continuous downstream processes often require the implementation of multi-column periodic chromatography in the capture step to ensure a continuous stream from the bioreactor system. Process synchronization and cycle time are thus crucial in

multi-column periodic capture. In this work it was shown that the approach used to integrate the periodic capture step with the other steps in the process can have a significant effect on process synchronization and the cycle time, which, in turn, affect the design of the columns. Thirdly, scaling-up is a very important aspect that must be considered in process design, and a method for safe, flexible and easy scale-up was presented in this thesis.

Model-based process optimization can be a powerful design tool. In this work, it was applied to design a PCC process for the purification of mAbs using multi-objective optimization. An optimal set of solutions, called the Pareto front, was obtained, with different values of productivity and resin utilization, resulting in a much more efficient process than batch operation. Flow programming in the loading phase was also used to optimize the process and increase process efficiency. Multi-objective model-based optimization of a mAb capture step was performed to obtain several flow trajectories. A 5-step flow rate trajectory and a linear flow rate trajectory were compared to a loading process with constant flow rate, showing an increase in productivity of up to 12%, and an increase in resin utilization of up to 9%.

The design and control tools developed were applied to implement ICDPs in five different case studies. In Case Study I, four chromatography steps were integrated with a UF/DF step in a single chromatography system to purify and concentrate a recombinant protein, with minimal footprint. Process automation led to a reduction in the total process time from 12.5 h to 7.5 h.

Case Study II involved the scale-down implementation of an ICB process for the production of mAbs, using a 200 mL bioreactor and a compact downstream set-up employing a single chromatography system. The perfusion culture was run for 13 days, and the downstream process was run with a 2-column periodic capture step to allow continuous loading from the harvest vessel. This resulted in a mean bioreactor-based productivity at steady state of 0.6 g/day/L bioreactor volume, and a downstream productivity of 1.8 g/h/L protein A resin.

In Case Study III, a 3-column PCC capture step was integrated in an ICDP for the purification of mAb using a two-system set-up. After model-based process optimization, a resin utilization of 74% and a yield of 78% were obtained. The downstream productivity was 12 g/h/L protein A resin, more than 6 times higher than that obtained in Case Study II. This demonstrates the value of model-based optimization and the use of PCC instead of a 2-column capture step.

In Case Study IV, a pre-capture continuous VI step based on the solvent/detergent method, to avoid pH conditioning, was integrated with a 3-column PCC process for the purification of a recombinant protein. The productivity was twice as high as that in a comparable batch downstream process, and the yield was increased by 28%.

Finally, Case Study V involved an ICB process for the production of pH-sensitive mAbs, with a minimum pH of 5.5 in the whole process. This was achieved using a newly developed calcium-dependent protein A ligand, called Z_{Ca}, which allowed mild elution, in contrast to a traditional mAb production process, where elution is carried out at pH values of 3.2 to 3.5.[29,97] Post-capture solvent/detergent-based VI allowed the mild conditions to be maintained even in this step. The process conditions in the capture, VI and CEX steps were optimized to avoid a conditioning step before loading the CEX column, and to minimize the volume loaded onto that column. The ICDP developed on laboratory scale was scaled up and coupled to a 30 L bioreactor. This pilot-scale process was run for 17 days to demonstrate its feasibility. A total recovery yield of 90% at steady state was obtained, the bioreactor-based productivity was about 1 g/day/L bioreactor, and the downstream productivity was 7.3 g/h/L protein A resin. These are among the highest values of yield and productivity ever reported for a mAb production process. Previously reported recovery yields range from 80 to 92%,[12,84,105] and bioreactor-based productivity ranges from 0.33 to 0.8 g/day/L bioreactor.[12,84,108] This shows that not only is this process suitable for pH-sensitive mAbs, but also has a very competitive performance.

It was shown in this work that an ICDP with a model-based optimal design, not only allows continuous feed from the bioreactor, but also affords a significant increase in productivity, recovery yield and resin utilization, as well as a reduction in the process time, with maintained product quality compared with a traditional batch-based downstream process. The compact design of the process set-up minimizes equipment requirements and thus the footprint of the system. Furthermore, the application of control strategies leads to increased process automation and reduced need for manual intervention. The process set-ups described in the work presented in this thesis can be applied for the production of a large variety of biopharmaceuticals with minimal changes in the process, making them the perfect candidates for use in multi-product and multi-purpose facilities. This approach will also facilitate the more rapid development of biologics due to the possibility of testing numerous drug candidates at the same time, in an automated fashion, and the higher flexibility to adapt to market changes.

7 Future work

Although the work presented in this thesis has hopefully made some contribution to the field of ICDPs, much research remains to be done in this field. Some examples of future development are given below.

Control strategies could be improved by including online analytics through the more extensive use of external PAT devices, for example, by integrating a preparative chromatography system with an HPLC analytical system. This would allow all the necessary analyses to be performed automatically, and the information obtained could be used for feedback control. This approach would increase the robustness of process control to ensure that the critical quality attributes are within acceptable limits, while maintaining the same degree of automation. Most analyses are currently performed manually offline, and are both time- and resource-consuming.

Flow programming has been found to have the potential to increase productivity without significantly increasing the complexity of the process. In this work, flow programming was applied to a batch process, but process efficiency could be increased even further by combining this approach with a multi-column periodic process, such as PCC.

Convective chromatography media, such as membranes and monoliths, offer a promising means of increasing productivity, as they allow much higher flow velocities without compromising yield or purity. They could, for example, be used in a PCC set-up to reduce cycle times to several minutes instead of hours, thus providing a quasi-continuous product output in the eluate stream, allowing steady state to be reached in a much shorter time. They may also prove to be useful in speeding up the development of new biopharmaceuticals as the purification cycles would be shortened.

Finally, in this work, the application of integrated continuous processing was demonstrated for the purification of proteins, mainly monoclonal antibodies, since these currently dominate the biopharmaceutical market. However, next-generation biologics, such as viruses, viral vectors, plasmids and nucleic acids are attracting much interest, and the development of integrated continuous processes for the production of these biopharmaceuticals will be important in the near future.

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THE PRODUCTION OF BIOPHARMACEUTICALS so far has been based on batch processes that are robust and well-known, but very inefficient and inflexible, causing the products to be very expensive and process development to be slow and costly. The production of biopharmaceuticals is thus changing towards integrated continuous biomanufacturing in order to reduce costs and increase flexibility in a constantly changing market. Continuous processing has been successfully implemented in upstream operation through the use of perfusion bioreactors, but the maturity required for the widespread use of integrated continuous biomanufacturing has not yet been achieved in downstream processes.

The research presented in this thesis provides several tools for the design, optimization, control and scale-up of integrated continuous processes for the purification of biopharmaceuticals with the aim of reducing the technological gap in downstream processing. These new tools were successfully tested experimentally on laboratory and pilot scale for the integrated continuous purification of biopharmaceuticals. In all the cases studied, the unit operations in the purification processes were integrated and automated for the application of complex process sequences without manual intervention. The potential of integrated continuous biomanufacturing to reduce production costs and minimize the development time of a biopharmaceutical was demonstrated by the high productivity, high utilization of the equipment and increased automation, allowing the process time to be reduced from days to hours.

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