Integrating high-throughput experimentation with advanced decision-support tools for chromatography process development

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By

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Declaration

I, **Christos Stamatis** confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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To ***Christina Varvogli***

Abstract

The development and commercialisation of a new therapeutic drug is a lengthy and expensive process hindered with uncertainties and high attrition rates. Monoclonal antibodies are a major contributor to the continuous growth of the global biopharmaceutical industry. Chromatography remains the workhorse in antibody purification despite its complex process development and the high operating cost. The research here presents the establishment of an integrated and data-driven decision-support framework in early-stage protein chromatography process development. The key focus of the research is the development of a systematic and rational methodology to automate and accelerate data analysis and decision-making. A novel workflow was developed that combined high-throughput experimentation (HTE) at micro-scale with design of experiments (DoE), multi-variate data analysis, multi-attribute decision-making and a robustness analysis technique to screen and optimise chromatography resins. DoE was linked with an advanced chromatogram analysis method to cope with the large datasets resulting from HTE by automating raw data manipulation. Additionally, the approach offers the ability to correlate the trade-offs between purity and yield with process parameters through a regression analysis. High-throughput purification data were further leveraged using a decision-support tool for the chromatographic purification train linked with a bioprocess economics spreadsheet model. The bioprocess economics model was also used to provide insights regarding the cost-effectiveness of pre-packed chromatography columns as an alternative to conventional self-packed columns for clinical and commercial manufacture. The implementation of the framework demonstrated the synergy of different decision-support tools and allowed for the rapid evaluation of multiple chromatographic purification trains in order to determine the most cost-effective resin sequence and column type considering the whole manufacturing process. Additionally, it is demonstrated that chromatography process development activities could be accelerated by defining platform purification processes and identifying manufacturing bottlenecks fast and with limited feedstock material.

Research Implementation and Impact

The overall outcome of this research is an integrated framework in chromatography process development linking high-throughput purification data with process economics and advanced decision-support tools. The research was performed in collaboration with the industrial sponsor, MedImmune. The Department of Biochemical Engineering at University College London and MedImmune have established a Centre of Excellence in bioprocessing with a number of researchers and industrial and academic supervisors. All the experimental and analytical protocols were performed according to MedImmune's specifications in order to enable the direct introduction of the approach into their process development strategy. MedImmune has recognised the benefits of high-throughput experimentation in bioprocess development. This research provides a framework for the systematic generation, manipulation and analysis of high-throughput purification data. Through the implementation of a framework it was demonstrated that high-throughput data can be further leveraged in decision-support tools to rapidly evaluate different purification strategies, identify process bottlenecks and determine cost-effective chromatography resins across different products.

In this research, emphasis was placed on automation and flexibility through a data-driven decision-making framework. The proposed framework is the consolidation of different tools developed throughout this research: A high-throughput process development workflow, a process economics model in bio-manufacturing and a decision-support tool for the chromatographic purification train. A key benefit of the framework is the flexibility that it provides on different levels. Individual components of the framework can be used independently without compromising the function or the structure of the rest of the framework. Additionally, all its major components offer to the user the ability to define the appropriate inputs and outputs to address a certain purification challenge.

Outside the research scope of this thesis, the process economics model has been used in collaboration with other researchers at UCL. The first example of implementation was

performed with another UCL/MedImmune researcher to simulate the cost of bio-manufacturing in a cash-flow model evaluating the cost of development for new biologics. A different example was offered by another UCL researcher that was able to modify the process economics spreadsheet to simulate the economics of high throughput vaccine manufacturing facilities. Furthermore, UCL has moved forward with the improvement of the process economics model and the development of a simulation engine to evaluate new and disruptive upstream processing technologies for the manufacture of stratified biologics.

Additionally, other UCL/MedImmune researchers currently focusing of the development of scale-down models to mimic and predict the performance of different unit operations at large scale, could benefit from using the process economics model. The integration of process economics with scale-down models as demonstrated in this thesis could provide critical insights for the performance and the cost-effectiveness of a unit operation at commercial scales. Since the development of a working version of the bioprocess economics engine, new UCL researchers have been offered training on the use of the engine and additional training sessions would provide the necessary skills to further develop the engine. Through proper training, current and new researchers could benefit from previous work and accelerate their research through the mitigation of the model development effort.

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Chapter 1. Introduction and research scope

1.1. Introduction

The road, from discovery to development and commercialisation of a new therapeutic drug, is an extremely time-consuming and expensive process, hindered by high risk (Mehta, 2008). There is a continuous effort to develop and establish the necessary methods and tools to accelerate research and development (R&D) activities, achieve an in-depth level of product and process understanding and provide affordable medicines. Monoclonal antibodies (mAbs) have a significant impact on the continuous growth of the biopharmaceutical industry (Aggarwal, 2014). Chromatography is still the preferred choice in antibody purification despite associated challenges in process development and manufacturing (Vunnum et al., 2009). This thesis focuses on the establishment of an integrated framework in early-stage protein chromatography process development linking high-throughput experimentation with advanced decision-making techniques. The overall objective was to provide a data-driven decision-support tool for the rapid manipulation and analysis of high-throughput purification data to evaluate different chromatographic purification strategies and accelerate process development activities.

Despite the obstacles and the complexity, the biopharmaceutical sector is a significant and fast growing segment of the global pharmaceutical market (Walsh, 2010). Over the past decade, the dependence of the pharmaceutical industry on biopharmaceuticals has increased substantially and there is a mutually beneficial relationship between biotech and pharmaceutical companies, with a significant surge in the negotiating power of the former (Thiel 2005; Lawrence & Lahteenmaki, 2014). Global sales of biologics reached $289 billion in 2014 with reports projecting the sales to surpass $400 billion by 2019 (Deloitte Touche Tohmatsu Limited, 2016). Although the total sales are increasing, year by year, the biotech industry suffers from low profitability. Profit is not following the same rising trend with total sales (Lawrence & Lahteenmaki, 2014). It is the high complexity

and the unique level of uncertainty, which govern the biotech industry that inhibit the industry from reaching its full potentials (Pisano, 2006).

Between 2007 and 2012 the best-selling class of biologics, in the US were monoclonal antibodies (mAbs) (Aggrawal, 2008; Aggarwal, 2009; Aggarwal, 2010; Aggarwal, 2012; Aggarwal, 2014), with total US sales, in 2012, of approximately $25 billion. On a global scale the sales of mAbs reached $75 billion in 2013 (Ecker et al., 2015). It is the sales of mAbs that are mainly responsible for the continuous growth of the biopharmaceutical sector (Aggarwal, 2014). Table 1.1 shows the top-ten selling biologics between 2012 and 2015. It should be noted that half of the best-selling biologics every year are mAbs with Humira® from AbbVie (North Chicago, Illinois) ranked number one consistently that four year period (Huggett, 2013; Lawrence & Lahteenmaki, 2014; Morrison & Lahteenmaki, 2015; Morrison & Lahteenmaki, 2016).

Table 1.1: Top-ten selling biologics for the years 2012 – 2015 (Worldwide sales in $billion).

Rank	2012	2013	2014	2015
1	Humira* (9.3)	Humira* (10.7)	Humira* (12.5)	Humira* (13.9)
2	Enbrel (8.0)	Enbrel (8.7)	Sovaldi (10.3)	Harvoni (13.9)
3	Rituxan* (7.1)	Lantus (7.6)	Enbrel (8.9)	Enbrel (9.0)
4	Remicade* (6.6)	Rituxan* (7.5)	Lantus (8.4)	Rituxan* (7.4)
5	Herceptin* (6.2)	Remicade* (7.0)	Rituxan* (7.5)	Lantus (7.1)
6	Avastin* (6.1)	Avastin* (6.8)	Remicade* (7.2)	Avastin* (7.0)
7	Neulasta (4.1)	Herceptin* (6.6)	Avastin* (7.0)	Herceptin* (6.9)
8	Lucentis* (4.0)	Gleevec (4.7)	Herceptin* (6.9)	Remicade* (6.8)
9	Avonex (2.9)	Neulasta (4.4)	Revlimid (5.0)	Prevnar 13 (6.3)
10	Rebif (2.4)	Revlimid (4.3)	Gleevec (4.8)	Revlimid (5.8)

* Monoclonal Antibodies

Despite a slow commercial start, mAbs have managed to overcome significant challenges to become the most important segment of biologic drugs (Reichert et al., 2005; Kozlowski & Swann, 2006). Until 2014, the US Food and Drug Administration (FDA) had approved over 40 mAbs dominating the biopharmaceutical market with 38.5% of the total biologics sales (Aggarwal, 2014). By 2017, the number of mAbs that had been approved and were in review reached 75 while 52 new mAbs were in late stage clinical trials with 23 and 29 for cancer and non-cancer indications, respectively (Antibody

Society, 2017). Although mAbs demonstrate impressive performance in the market in terms of sales and growth rates, there is plenty of room for more innovations and improvements in their development (Davidson & Farid, 2014).

This chapter offers an overview of the development pathway for the commercialisation of a new therapeutic drug to present the key milestones in R&D along with associated costs and timelines (Section 1.2.). Furthermore, Section 1.3., describes the bio-manufacturing process emphasising on unit operations currently employed in the production, harvest, clarification and purification of therapeutic antibodies. Additionally, Section 1.3., presents a summary of single-use technologies available in bio-manufacturing and discusses their benefits, limitations and the major considerations associated with their introduction into a biologics' facility. Subsequently, Section 1.4., describes different approaches used in chromatography process development and provides examples of implementation demonstrating some of the benefits and challenges associated with each method. Finally, Section 1.5., offers an overview of the efforts over the past two decades in the development of computer aided tools able to capture both process and business aspects and enhance decision-making in bio-manufacturing. The overview in Section 1.5., starts by describing early work in bioprocess economics providing examples on the evaluation of single-use technologies in bio-manufacturing. Moreover, published work is presented focusing on different methods to identify and manage uncertainties on both process and business level. Finally, the section concludes with a summary of published work emphasising on the design of cost-effective purification strategies.

1.2. New drug development pathway

The pathway to commercialisation of new therapeutic drugs, either small molecules (pharmaceuticals) or large molecules (biopharmaceuticals), is extremely challenging and highly regulated. In the United States the government authority that regulates and approves therapeutic products is the Food and Drug Administration (FDA). In Europe, the European Medicines Agency (EMA) is responsible for regulation and approval and

in Japan the Pharmaceutical and Medical Devices Agency (PMDA) is the responsible government organisation. In 1990, the regulatory authorities of the United States, Europe and Japan gathered and formed the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The purpose of the ICH is to provide quality, safety and efficacy guidelines and harmonise the regulatory authorities of the US, Europe and Japan, in order to promote the discovery and commercialisation of new therapeutics and improve global health.

1.2.1. From inception to pre-clinical studies

Most commonly, the development of a new therapeutic product is driven by the necessity to treat a disease problem, in which either there is no treatment, or the current treatment is not deemed to be efficient (Mehta, 2008). The purpose of the pre-clinical studies is to specify the pharmacodynamic and pharmacokinetic properties of the candidate drug and moreover, identify toxicity levels and determine a preliminary dose (Mehta, 2008; Mundae & Ostro, 2010). Initially, such studies are conducted in small animals such as rodents and if the results are satisfactory, the studies are continued in larger animals like monkeys to prove safety data (Mehta, 2008; Mundae & Ostro, 2010). It is important to mention that all the experiments must be implemented under current good laboratory practices (cGLP). The guidelines provide an extensive but complex framework on how the studies must be planned, conducted, monitored, analysed and reported (Mehta, 2008; Cevc, 2014).

Subsequently, it is mandatory to submit an investigational new drug application (IND) to the FDA, in order to receive approval to continue the studies in humans (Mehta, 2008; Mundae & Ostro, 2010). The subsequent clinical trials are divided into 4 phases. The first three phases are conducted in order to prove safety and efficacy and acquire regulator's approval to proceed in commercialisation of the therapeutic product, while the fourth phase is conducted after the launch of the product in the market (Mehta, 2008). During the clinical trials, current good clinical practices (cGCP) provide the guidelines

regarding the design and the execution of the studies, as well as monitoring and control of the studies and moreover, in regards with the analysis and report of the data (Mehta, 2008; Cevc, 2014).

1.2.2. From clinical trials to market approval

Clinical trials, phase I

In Phase I of the clinical trials, healthy subjects (20 – 200 people) between the age of 18 and 45, receive a single dose and they are monitored very closely to identify any undesired reactions (Mehta, 2008; Mundae & Ostro, 2010; Cevc, 2014). The dose the subjects receive, is a small portion of the predetermined dose, estimated based on the pre-clinical results, and is increasing gradually until the limit is reached or a complication occurs. Therefore, Phase I is necessary to prove safety and identify a dosage therapeutic window, in which there is a favourable balance between the benefits of the drug and the risk associated with its use. It is worth mentioning that in case of anticancer therapeutics, usually, the studies are conducted with diseased patients unable to respond successfully to other treatments (Mehta, 2008).

Clinical trials, phase II

In Phase II, approximately 400 patients are involved in a randomised, double-blind study. Randomised, because there is no discrimination among the patients and thus, some of them are placed randomly in placebo groups and double-blind because neither the physician, the patients or the company, know which patient belongs in each group. The overall aim is to determine the optimum dose, prove safety and demonstrate preliminary efficacy (Mehta, 2008).

Clinical trials, phase III

Phase III involves a significantly larger group of patients, up to 5000 (Cevc, 2014). The purpose of this phase is to prove efficacy and identify undesired side-effects that were not detected during Phase I & II. The increased number of patients indicates that Phase

III intends to test enough patients in order to conduct an accurate statistical analysis of the results that can represent adequately the projected market share (Mehta, 2008). It is worth to mention that FDA normally requires Phase III to be also a randomised, double-blind study, while with EMA this is not always the case and one such study could be enough to proceed in authorisation (Cevc, 2014).

Positive and promising results from Phase III provide the green light to proceed in the submission of a new drug application (NDA) for small molecules, or a biologics licence application (BLA) for large molecules. It is highly recommended that at the end of each phase, a meeting between the company and the regulators should take place in order to evaluate the results and discuss the progression of the trials, as well as the context and the details that must be included in the NDA/BLA (Mehta, 2008).

Clinical trials, phase IV

Phase IV takes place after the therapeutic drug has launched the market and the goal is to ensure safety and efficacy, as the population under treatment is increasing. Moreover, special subpopulations can be studied to identify any malfunctions and the results must be analysed and reported regularly to the regulators, in order to be aware of any problems with the progression of the treatment (Mehta, 2008).

New therapeutic drug development timelines

The time that is required, from research and discovery to final market approval, of a new therapeutic drug, is approximately 10 – 15 years (Mehta, 2008; Mundae & Ostro, 2010; Cevc, 2014). Discovery and pre-clinical studies are usually extended over a 7 year period, while clinical trials require approximately 6 – 8 years. Additionally, the regulatory agencies need some time (1 – 2 years), first to evaluate the NDA/BLA application and then to inspect the manufacturing facilities in order to ensure that the facility and the process align with the guidelines and the specifications (Mehta, 2008; Cevc, 2014). In other words, the manufacturers must guarantee that the product meets and will continue

to meet the desired and predetermined quality attributes and the regulators must advise and inspect to make sure that the product meets the specifications.

1.2.3 Cost of R&D and attrition rates

Although the time required to commercialise a new therapeutic drug has remained unchanged over the past decade, the same cannot be claimed for the cost of R&D or the success rate of getting new therapeutics to market. In 2013 the public biotech industry had spent $28.5 billion in R&D, a 13% increase compared with 2012 while revenues grew by 7% in the same period (Lawrence & Lahteenmaki, 2014). Several studies have addressed the challenge to estimate the total cost of R&D for a new therapeutic drug and the transition probabilities for each stage in clinical development and market approval. The vast majority of these studies provide an estimate of the capitalised cost of R&D per successful therapeutic drug in order to capture the cost of failed drug candidates and the development timeframe. Although this approach offers a more pragmatic estimate of the total R&D cost, it is subject to the databases used and the assumptions made for the analysis.

For instance, published work from different research teams reported success rates (or transition probabilities) from Phase I to commercialisation within a range of 21.5% to 30.2% (DiMasi et al., 2003; Adams & Brantner, 2006; DiMasi & Grabowski, 2007; Adams & Brantner, 2010). Later, Paul et al. (2011) reported a much lower success rate of 11.7%. Deviations of this magnitude among key estimates like the attrition rates can lead to significant differences in the capitalised cost. Morgan et al. (2011) provided a review study assessing published R&D cost estimates. The authors compared the capitalised R&D cost from thirteen studies and identified a significant variation in the results starting from $160 million and reaching $1.8 billion in 2009 US dollars. Additionally, the authors highlighted the implications that arise from the lack of data transparency rendering it extremely difficult to reproduce the results and assess their accuracy and validity. It was

concluded that despite more than thirty years of research on the subject it is very challenging to identify a benchmark estimate (Morgan et al., 2011).

More recently, new estimates for the capitalised R&D cost and the cumulative success rate demonstrate an even higher cost with similar attrition rates compared with previous studies. The latest estimate of the total post-approval capitalised R&D cost reaches $2.9 billion (2013 US dollars) with transition probabilities of approximately 60%, 36%, 62% and 90% from Phase I to Phase II to Phase III, to NDA/BLA submission and finally to NDA/BLA approval; leading to a success rate of 11.8% (DiMasi et al., 2016).

1.3. Biopharmaceutical manufacturing

The production of a specific therapeutic biological drug requires a very specific cell line, which will provide the product with the desired conformation, at the optimum productivity (Birch & Racher, 2006). The intrinsic variability in bio-manufacturing is observed not only among different products but also between different batches of the same product (Steinmeyer & McCormick, 2008). Therefore, consistency of the product between batches must be ensured and thus, the guidelines from the regulators are extremely strict. Additionally, consistency may be an issue when the manufacturing capacity changes. As the clinical trials progress, a greater population is treated and therefore higher production capacity is necessary to cover the demand. Furthermore, if/when a therapeutic product receives the approval from the regulators to be marketed even higher demands might be required. Hence, efficient scale-up methods are necessary to demonstrate that the product has the same characteristics and the same quality from scale to scale (Werner, 2004).

The long development times and the high costs force biopharmaceutical companies to plan and evaluate their strategy from a very early stage. Additionally, they need to attempt and predict the circumstances that will occur if/when a product reaches the market (Werner, 2004). It is extremely important to determine, from the beginning of a project, the criteria that will dictate if the project should be continued to the next phase

or to be abandoned (Mehta, 2008). Hence, a risk assessment is mandatory to evaluate the potential of the product to reach the market and withstand any competition, determine the balance between the benefits and the risks associated with the patients and estimate the potential market share and reimbursement (Werner, 2004).

A very significant consideration in the biopharmaceutical industry is the accurate prediction of the desired manufacturing capacity. It is extremely difficult to evaluate accurately the market share, especially when it comes to a new type of medicine, for which there is no or little indication on how accepted it will become (Kamarck, 2006). An example is the unexpected success of the fusion protein, Enbrel™; an anti-tumour necrosis factor that treats autoimmune diseases. As soon as the drug was launched on the market in 1998, the demands increased exponentially and within the first 6 months, the US sales surpassed the projected annual global sales, leading to a capacity crisis. To overcome the challenge, contract manufacturers were called to increase capacity and additionally, new facilities had to be acquired to cover demand. Nevertheless, changing capacities and/or expanding to new facilities, requires time and the losses from the potential sales can be significant (Kamarck, 2006). On the other hand, in 1992, three different companies (Deerfield, Bayer and Wyeth now acquired by Pfizer) were manufacturing the same product (Recombinant factor VIII) and they all made major investments to increase their capacity and meet the demands, but soon they all faced overcapacity problems, due to competition (Kamarck, 2006).

Considering the above examples regarding the manufacturing capacity, it is clear that the risks and the uncertainties involved require methodical and precise management in order to gain flexibility. Bio-manufacturing is challenging and it is critical to be able to establish robust and flexible processes, as well as the predictive tools to make initial estimations regarding the facility demands for a portfolio of therapeutic drugs.

1.3.1. Manufacturing processes of monoclonal antibodies

Usually, mAbs are administrated in relatively high doses and the treatment has a prolonged duration. Additionally, the success of mAbs, both in the clinic and the market, means that they are a major contributor of the continuously increasing biopharmaceutical market (Reichert et al., 2005; Shukla & Thommes, 2010).

Until the early 1990s, bio-manufacturing processes varied significantly across the industry (Kelley et al., 2009). The main differences in early bio-manufacturing processes can be observed in the purification train with a diverse chromatography sequence. The existence of different purification strategies can be explained, partially by the antibodies' nature and how it evolved from murine to humanised and fully human. Despite the high degree of homology among the same type of antibodies (in terms of nature), it is extremely difficult to establish a generic process, with a generic sequence of unit operations that will operate under the same conditions. Nevertheless, platform manufacturing processes show significant benefits in terms of process development. Essential efforts have been made, both from industry and academia, to develop platform processes and to identify operating windows, in which different types of products and feed-streams can potentially be operated efficiently with minimum modification and adjustment (Kelley et al., 2009).

1.3.1.1. Upstream processing of mAbs

The large-scale production of mAbs is dominated by mammalian host cell lines and they have been established as the standard production platform, due to their ability to perform post-translational modifications (human-like N-glycosylation) and produce proteins compatible with the human immune system (Farid, 2009; Shukla & Thommes, 2010; Ho et al., 2013). Full length antibodies are glycosylated proteins and although the glycosylated domain does not interact directly with the antigens, it promotes stability. Incorrect glycosylation can result in undesired immune responses (Ho et al., 2013).

Mammalian cell lines

The most commonly used mammalian cell lines are: the Chinese hamster ovary (CHO), murine lymphoid NS0 and Sp2/0, murine hybridoma and human cell lines. Among different human cell lines the human embryonic retinoblast derived cell line (Per.C6) is considered the most promising (Ho et al., 2013). Nevertheless, CHO cell lines are the preferred option since they can produce mAbs with the correct conformation and post-translational modifications and provide higher biocompatibility with the human immune system and, therefore, demonstrate potentially an increased safety and efficacy (Shukla & Thommes, 2010; Ho et al., 2013). Modifications and advances have been made also with mammalian cell lines, to accelerate cell line development and optimisation and increase productivity and yield. Titres of 3 – 5 g/L are considered now typical for mAbs (Farid, 2009) with reports in the order of 10g/L being reported for some cases within the industry (Li et al., 2010). Production capacity and titre are closely related to cost of goods per gram (COG/g). For instance, a 10-fold increase in titre can reduce COG/g by approximately 80% (Werner, 2004). Alldread et al. (2014) compiled a data table summarising the evolution of COG/g over the past five decades. The authors reported an increase in the specific cell line productivity along with an increase in the cell culture duration leading to a significant surge in the cell culture titres. Considering also improvements in downstream processing with the total process yield reaching approximately 70 – 80% compared to 40% reported almost 50 year ago, the COG/g have experienced a significant reduction from 1000s to 10s of $/g. The uncertainties and risk associated with alternative host cell lines in combination with the advantages and the improvements in mammalian cell lines, demonstrate the strong reliance of large-scale bio-manufacturing processes on mammalian cell lines, at least for the foreseeable future (Farid, 2009; Alldread et al., 2014)).

Large scale production strategies: Fed-batch and perfusion culture

Two mammalian cell culture manufacturing strategies dominate the large scale upstream processes; fed-batch and perfusion culture (Lim et al., 2006). In fed-batch mode, first the

culture goes through a batch phase until the carbon source becomes limited and subsequently fresh media is introduced periodically to supplement the required nutrients. The duration of the culture is approximately 2 – 3 weeks. Fed-batch is usually the preferred mode of operation due to its simplicity in operation and control, its robustness and flexibility (Lim et al., 2006). However, large bioreactors are required to meet the demands resulting in high capital investment and large facility footprints (Pollock et al., 2012).

In perfusion mode, the culture starts with a batch phase and during the exponential growth phase, fresh media is added continuously while culture broth containing toxins and inhibitors, are removed continuously. Additionally, a cell retention device is necessary to maintain (or recycle) the cells in the bioreactor. The duration of the cell culture can be prolonged for weeks or even months and the resulted productivity is significantly higher compared with the fed-batch mode. Furthermore, perfusion culture requires smaller bioreactor capacity and thus the capital investment and the size of the facility may be reduced compared with the fed-batch mode (Pollock et al., 2012). However, the operational complexity and the increased risk of equipment failure can discourage inexperienced manufacturers to make a change from fed-batch to perfusion culture (Farid, 2009).

Lim et al. (2006) evaluated the trade-offs in productivity, cost and uncertainty between fed-batch and spin-filter perfusion culture. They concluded the perfusion mode of operation was more cost-effective, demonstrating a significant reduction in capital investment and higher net present value, relative to the fed-batch mode. Nevertheless, when the uncertainties of each mode of operation were considered, perfusion was shown to be incapable of meeting demand. It was also commented that a further decrease in equipment failure rates would be needed, to increase the reward/risk ratio, if perfusion is to become more attractive as an option (Lim et al., 2006).

Alternative tangential flow (ATF) perfusion demonstrates advantages compared with spin-filter perfusion in terms of equipment failure and reliability. The ATF system utilises an external filtration unit to remove the broth and retain the cells, which results in a considerable reduction in the filter fouling. Pollock et al. (2012) evaluated the feasibility of fed-batch, spin-filter perfusion and ATF perfusion culture strategies under uncertainty. They concluded that ATF perfusion was the most cost-effective option even under uncertainty, demonstrating approximately a 20% decrease in the COG/g, for all the investigated titres and across different scales of production (Pollock et al., 2012) for single product facilities.

Alternative cell lines

Significant efforts have been made to introduce microbial cell lines for the production of mAbs, due to their significant advantages regarding productivity, yield, cell density, as well as simpler culturing media requirements (Farid, 2009; Spadiut et al., 2014). Currently, microbial cell lines such as yeast and bacteria are utilised for the production of antibody fragments (Fabs), which are not glycosylated proteins and thus, do not require the advanced post-translational machinery of mammalian cells (Spadiut et al., 2014). Furthermore, eukaryotic microbial cells like *Pichia pastoris* have been genetically modified in order to perform human-like N-glycosylation (Li et al., 2006).

1.3.1.2. Downstream processing of mAbs

Harvest and clarification technologies

In mammalian cell culture, mAbs are extracellular products, secreted into the cell culture broth and the first step in the downstream process is to separate the liquid from the solid phase (Shukla et al., 2007). Mammalian cells are sensitive to hydrodynamic forces and cell breakage is a common phenomenon. It is desirable to minimise cell disruption and prevent the release of impurities like proteases and other host cell proteins (HCPs), which can potentially affect the stability of the product. Moreover, holding times play a

significant role in product stability and in microbial contamination of the culture media (Shukla & Kandula, 2009).

In large scale production of mAbs, centrifugation is typically the preferred harvest choice. Initially, centrifugation was inflexible and scalability was hindered by inadequate predictive models that lacked the ability to mimic the shear stresses occurred during large scale operations (Shukla & Kandula, 2009). Moreover, it was believed that large scale centrifuges require a substantial capital investment and therefore, often microfiltration was the preferred option (Low et al., 2007). Microfiltration is an alternative that offers a significant advantage over centrifugation by requiring fewer additional filtration steps in order to achieve an acceptable clarification level at which product purification can proceed. However, membrane fouling is an important consideration especially with large culture volumes and high cell concentrations (Zhao et al., 2000; Charcosset, 2006).

Centrifugation can handle large culture volumes and high cell concentrations and the development of scale down models and devices helped to overcome some important drawbacks. Continuous disk-stack centrifuges are most commonly utilised for harvest, providing the advantage of low residence times. Maybury et al. (1998) developed a scale down set-up to evaluate the performance of centrifugation and managed to reduce the required separation area and the volume of the centrifuge, enabling the use of less feed material. The study demonstrated an experimental set-up with interlocking inserts that can provide adequate information in early process development and has the ability to reduce substantially pilot-scale studies. Additionally, in another study, Maybury et al. (2000) reported a bench-top method to mimic shear stresses in laboratory scale centrifuges and predict the performance of large scale centrifuges. Hutchinson et al. (2006) developed an ultra scale-down method using a rotating disc device to predict the performance of industrial scale centrifuges using millilitre quantities of material. More recently, a capillary shear devise first developed by Westoby et al. (2011) was integrated

in an automated high-throughput experimental method in order to mimic the shear stresses observed at industrial scale disk-stack centrifuges (Joseph et al., 2016).

The use of centrifugation as the harvesting method implies the use of additional filtration units to remove completely cells and cell debris that would compromise the subsequent purification steps. Usually, dead-end depth filtration follows centrifugation to achieve the desired level of clarification (Shukla et al., 2007; Shukla & Kandula, 2009). Depth-filters have the ability to retain particles throughout the filter matrix and not only on the surface of the membrane, providing a larger surface area for separation. It is a common practice to use filter aids like diatomaceous earth, in order to reduce compressibility of the formed cake on the surface of the filter (Shukla et al., 2007; Shukla & Kandula, 2009). Furthermore, charged matrices have been evaluated as an efficient primary removal of HCPs during mAb purification (Yigzaw et al., 2006). Most commonly depth filters are single-use and thus no cleaning is required resulting in less validation requirements and faster turnaround times. Process development and filter capacity studies usually take place at constant flow requiring a substantial amount of feedstock material and time. Joseph et al. (2017) correlated constant flow with constant pressure operation providing an alternative approach in filter capacity quantification reducing time and materials requirements.

Dead-end microfiltration membranes with an absolute pore size (0.2 – 1 μm) are utilised for final clarification and particle removal prior to chromatographic purification. It is possible to combine the depth filter with an absolute filter attached on the bottom. Strategies utilising multiple depth filters for harvest and clarification tend not to be so robust and cost-effective at large scale and thus not favoured (Shukla & Kandula, 2009).

Purification technologies

Purification and polishing steps are major contributors to the COG/g and significant efforts have been made to improve productivity and yield. Manil et al. (1986) investigated the performance in terms of yield and purity of several purification techniques for mouse

mAbs and they concluded that Protein A chromatography was the best performing option. Precipitation was one of the first techniques used to purify mAbs and there are studies suggesting the re-introduction of such an approach in antibody purification to replace traditional chromatography (Alahari, 2009; Ma et al., 2010; Oelmeier et al., 2013).

Capture step in the purification sequence

Nowadays, the majority of the purification schemes employ Protein A affinity chromatography as the first purification step (Shukla et al., 2007). Protein A is a polypeptide originally found in the cell wall of *Staphylococcus aureus*, that has a high binding specificity for the fragment crystallisable (Fc) domain of antibodies (Vunnum et al., 2009). Due to the physicochemical stability of Protein A, the ease of process development and the exceptional purification factors that can be achieved, Protein A chromatography is considered the workhorse of antibody capture and purification processes (Shukla et al., 2007; Vunnum et al., 2009). The duty of Protein A chromatography as the capture step in the purification sequence is to remove high molecular weight (HMW) species and degradation products (LMW) (product-related impurities), HCPs, DNA, potential adventitious viruses and cell culture media components (process-related impurities) (Vunnum et al., 2009). Moreover, it offers significant reduction in the volume that has to be processed downstream by concentrating the product (Kelley et al., 2009; Vunnum et al., 2009).

Protein A chromatography operates in bind-and-elute (BE) mode, meaning that the target mAb interacts strongly and binds to Protein A, during loading, while HCPs and other impurities flow through due to their weaker interactions. Initially, the column is equilibrated with the loading buffer to achieve the appropriate environment and promote product binding. Subsequently, the clarified feed is loaded into the column, typically at a constant flow rate. In general the loading step is considered the rate-limiting step as it is desired to achieve the greatest possible dynamic binding capacity, which typically varies

among different mAbs (Vunnum et al., 2009). Ghosh et at, (2004) demonstrated a loading strategy involving a high flow rate followed by a lower flow rate and they concluded that their strategy can offer higher throughput, compared with the typical single flow rate strategy, and therefore mitigate the required loading time.

During loading of the product, nonspecific binding occurs due to electrostatic and/or hydrophobic interactions between impurities, ligand and product. Hence, it is favourable to introduce a washing step before elution in order to remove weakly bound impurities and provide a ‘’cleaner’’ eluate. The pH of the washing buffer has to be between the loading pH (neutral) and the elution pH (acidic), to promote impurities removal, while keeping the product bound (Vunnum et al., 2009). If a single washing step is incapable of reducing the concentration of impurities, then a second step is usually employed, using additives such as salt, detergent and/or a solvent. Subsequently, pH is reduced to an acceptable level to promote product elution while avoiding aggregation and precipitation (Vunnum et al., 2009). Different mAbs have different optimum elution pH values, usually between 3 and 4.5. It is critical to maintain the pH at a level sufficient to provide high product yield and avoid destabilisation. Additives such as NaCl, urea, ethylene glycol are used to minimise interactions between the product and the ligand and permit the operation at higher pH and thus avoid the formation of aggregates (Vunnum et al., 2009).

Then a stripping step usually takes place at pH 2.5 – 3 to remove strongly bound product and impurities. Subsequently, regeneration of the column is required to prepare the column for further runs. More often, chaotropes like urea and guanidine hydrochloride or low concentrations of sodium hydroxide (NaOH) are used for column regeneration (Vunnum et al., 2009). Traditionally, Protein A resins are sensitive under strong alkaline conditions that, in general, offer better column sanitisation. However, due to the low cost of NaOH and its efficiency to clean and regenerate the column and prevent any microbial growth, efforts have been made to develop resins resistant to alkaline treatments, such as MabSelect SuRe^{TM} (GE Healthcare, Uppsala, Sweden).

Wang et al. (2013) investigated the performance of MabSelect^{TM} (GE Healthcare, Uppsala, Sweden) after cleaning and regenerating the column with NaOH, sodium sulphate (Na_2SO_4) and benzyl alcohol at different concentrations. They reported an increase in the number of cycles from 30 to 55 using their cleaning strategy over the cleaning strategy proposed by the manufacturer, without compromising the performance of the column in terms of dynamic binding capacity (DBC), yield and Protein A leaching. In another study, Jiang et al. (2009) compared two Protein A resins under three different cleaning strategies, using NaOH and sodium chloride (NaCl) at different concentrations, with and without a stripping step before regeneration. They reported that 50mM NaOH with 0.5M NaCl, without a striping step, was the most efficient strategy for both resins and moreover that the addition of protective additives like sucrose, xylitol and ethylene glycol reduced sufficiently the decline in binding capacity and provided greater stability against the strong alkaline conditions.

With increasing titres and bioreactor volumes, the number of chromatography cycles needed to process a batch is expected to increase significantly (Lain et al., 2009). Therefore, column re-use plays a significant role, especially in Protein A chromatography, in which the cost of the resin is substantially higher compared to other resins, like ion exchangers. Kelley (2007) demonstrated a two-column very large-scale purification process, utilising a Protein A capture step followed by an anion exchange chromatography (AEX) column and reported that Protein A unit operation accounts for half of the total downstream process cost, with the cost of the resin being the major contributor.

Protein A chromatography is associated with several limitations including the cost of the resin, number of cycles, low throughput, Protein A leaching and aggregation and/or precipitation due to the low elution pH values (Vunnum et al., 2009). Therefore, alternative purification technologies are being developed and evaluated, in order to replace Protein A chromatography and mitigate the associated drawbacks.

Cation exchange (CEX) chromatography is a key alternative and has been applied as a capture step in manufacturing of Synagis® by MedImmune (Gaithersburg, Maryland) and Humira® by AbbVie (North Chicago, Illinois) (Chon & Zarbis-Papastroitsis, 2011). Miesegaes et al. (2012) investigated the performance of five different CEX resins for the capture chromatography step at loading capacities similar to Protein A resins (25g/L and 40g/L). They demonstrated a significant trade-off between purity and yield leading to infeasible windows of operation where high yield results in low purity and vice versa. Moreover, they mentioned that the operating conditions must be specified for each mAb to obtain optimum efficiency. The authors concluded that CEX chromatography is a promising alternative to replace Protein A for the capture chromatography step mainly due to the economic benefits it offers.

More advanced CEX resins have been developed with high DBC values up to 100g mAb/L of resin to cope with the increased upstream titres (Chon & Zarbis-Papastroitsis, 2011). Lain et al., (2009) evaluated two CEX resins for the capture step and reported binding capacities of 75 – 100g/L. Further investigation showed high yield (>97%) and purity (~99%) and up to 95% removal of HCPs. Other alternatives include mixed-mode resins that take advantage of both electrostatic and hydrophobic interactions. Pezzini et al. (2011) evaluated four mixed-mode resins and concluded that all of the tested resins were capable of replacing Protein A capture step and provide sufficient HCP clearance.

Polishing steps in the purification sequence

Downstream the capture chromatography step, two polishing steps are usually employed to remove remaining product- and process-related impurities. It is common practice to utilise two tandem ion exchange (IEX) chromatography columns post Protein A chromatography to achieve the desired level of purity and product quality (Ghose et al., 2009). IEX chromatography takes advantage of the electrostatic interactions between the product and the resin. Antibodies, as all proteins, have a net charge that enables them to interact with the charged groups on the IEX resin. The isoelectric point (pI) of

the mAb under consideration determines the mode of operation for each type of the IEX resin. Anion exchange (AEX) resins are positively charged and thus bind anions while CEX resins are negatively charged and hence bind cations (Carta & Jungbauer, 2010)

- *AEX chromatography*

Murine mAbs usually have a more acidic pI than fully human mAbs and therefore, AEX chromatography is operated in BE mode. However, human mAbs have higher pIs and thus bind very weakly on AEX resins, which promotes a flow-through (FT) mode of operation (Gagnon, 2012). The increasing interest in human mAbs due to the low likelihood of immunogenicity, has established FT as the selected mode of operation for AEX chromatography (Arunakumari & Wang, 2009). The major advantage of operating in FT mode is that the AEX resin binds impurities, which constitute the smallest fraction of the product stream and therefore, high load challenges can be applied, increasing throughput and improving processing time (Kelley et al., 2008).

Membrane AEX chromatography has attracted much attention as an alternative to AEX packed bed chromatography. Although membranes have lower binding capacity, their operation in FT mode minimising this drawback, since only trace levels of contaminants have to be retained (Zhou & Tressel, 2006). Additionally, conventional packed-bed AEX chromatography usually operates at linear velocities of 100 – 150 cm/hr, requiring large column diameters to cope with pressure drop limitations of the resin (Lim et al., 2007). Moreover, the transport phenomena involved with porous beads are more complex compared with membranes, where pore diffusion is not required and thus processing time can be mitigated (Lim et al., 2007). Another advantage is that membrane chromatography uses disposable single-use membranes and thus no clean-in-place (CIP) step is required. This is also beneficial from a validation point of view. Membrane chromatography offers significantly reduced buffer consumption, while providing very high yields (98 – 99.9%) and acceptable levels of virus removal (Zhou & Tressel, 2005).

Knudsen et al. (2001) compared the Q-Sepharose® Fast Flow AEX resin (GE Healthcare, Uppsala, Sweden) with Q membranes (Sartorius & Pall Biopharmaceuticals) and demonstrated that flow rate had an insignificant effect on the shape of the DNA breakthrough curve, providing the advantage of high throughput. Additionally, they compared the Sartobind® Q15 membrane (Sartorius, Göttingen, Germany) with the Q-Sepharose® FF, in terms of HCP clearance and virus removal and concluded that HCP clearance was similar while virus removal was lower for the membrane, presumably because of membrane fouling.

Kelley et al. (2008b) established an alternative mode of packed-bed AEX chromatography, known as weak partitioning chromatography (WPC). The difference stems from the distribution coefficient (Kp), which is the proportion of the amount of product bound to the stationary phase (AEX resin) over the amount of product that flows through without binding. In BE mode usually Kp is higher than 100 and in FT mode Kp is often lower than 0.1, while in WPC the distribution coefficient is between 0.1 and 20 and preferably between 1 and 3. By manipulating the buffer's pH and ionic strength, the authors identified the optimum Kp, which promotes strong binding of the impurities, while providing high product yield. By increasing Kp more product is bound on the resin, however once equilibrium is reached, no more product is able to bind. Consequently, high load challenges (>250g/L) are preferred in order to minimise product losses and increase recovery (Kelley et al., 2008). Additionally, the authors suggest that a wash step, after the load phase is completed, is necessary to recover bound product and increase yield. WPC demonstrates efficient removal of HCPs, HMW species and leached Protein A, while achieving high product recovery (>95%). The major disadvantage is that specific tuning is required for each mAb, to identify the optimum operating conditions (Kelley et al., 2008). However, the increased performance of WPC makes possible the establishment of a two-column purification process and thus offers significant economic benefits (Kelley, 2007; Kelley et al., 2008).

- *CEX chromatography*

Another polishing step, frequently used in the chromatographic purification of mAbs, is CEX chromatography, most commonly operated in BE mode. Binding capacity is a major factor in the selection of the appropriate CEX resin. CEX chromatography has the ability to remove HCPs, HMW species, degradation products and leached Protein A (Ghose et al., 2009). Staby et al. (2006) compared the DBC values of several strong and weak CEX resins, at the same operating conditions and reported high DBCs, up to 100g/L, for two Fractogel® EMD resins from Merck Millipore. In a subsequent study, the same resins were compared in terms of HCP clearance and it was reported that both resins were capable of achieving a logarithm reduction value for HCPs around 3. Moreover, the authors estimated the optimum pH to maximise DBC and concluded that pH 6.0 provide the best DBCs for both resins; 76g/L and 86g/L for the Fractogel® EMD SO_3^- & Fractogel® EMD SE Hicap, respectively (Stein & Kiesewetter, 2007).

In CEX chromatography, two operating parameters that affect significantly the binding capacity of the resins are pH and conductivity (Harinarayan et al., 2006; Liu et al., 2010). Harinarayan et al. (2006) evaluated the effect of pH and conductivity on two CEX resins and reported unprecedented behaviour in which DBC rose with conductivity, at all pH values. Initially, as conductivity and/or pH increase, DBC also increases. However, after a certain point any additional increase in pH or conductivity has a negative effect on DBC. The latter is the anticipated behaviour and the research team explained the initial behaviour based on the concept that by increasing pH or conductivity, protein-protein exclusion is mitigated and therefore binding capacity is improved.

CEX membrane chromatography has been evaluated as an alternative column type due to the benefits noted earlier regarding membrane columns. Knudsen et al. (2001) compared packed-bed and membrane CEX chromatography in BE mode. They reported almost constant DBC for the membrane at increasing flow rates, in contrast with packed-bed, where DBC decreases as flow rate increases. They concluded that the low binding capacity of membranes is a major disadvantage compared to packed-bed columns.

- *Alternatives to IEX chromatography as polishing steps*

Hydrophobic interaction chromatography (HIC) and hydroxyapatite (HA) chromatography as well as mixed-mode chromatography have been utilised for the purification of mAbs (Liu et al., 2010; Gagnon, 2012). HIC depends on the hydrophobic interactions between regions of the antibody and the hydrophobic ligands of the resin. The milder conditions applied in HIC make the method more favourable than reverse phase chromatography where product denaturation could be an issue. Furthermore, HIC is preferred when HCPs and HMW species removal is the major objective. On the other hand, IEX chromatography has a superior ability to remove leached Protein A and DNA (Ghose et al., 2009).

HA chromatography offers both positively and negatively charged binding sites and it has the ability to remove leached Protein A, HCPs, HMW species and DNA. Charge distribution and strength is affected by pH and conductivity and the isoelectric point of the mAb has a significant influence on the selection of the operation conditions. Therefore, a substantial disadvantage associated with HA chromatography is the need to determine the operating conditions specifically for each mAb (Ghose et al., 2009). An example of HIC and HA chromatography application is provided by Amgen (Thousand Oaks, California), which has adopted a flexible platform, involving HIC, HA and IEX chromatography, to process different products (Shukla et al., 2007).

As described earlier, mixed-mode chromatography employs both electrostatic and hydrophobic interactions and provides great selectivity (Liu et al., 2010). Chen et al. (2010) compared a multi-modal, strong AEX resin with six conventional AEX resins and demonstrated a superior reduction of HMW species with comparable levels of HCP reduction. However, product recovery is significantly higher for the conventional AEX resins. In another study, Gao et al. (2013) evaluated the ability of the same multi-modal, strong AEX resin to remove aggregates, HCPs and DNA and reported that it is possible to use the resin as a sole polishing step after Protein A chromatography.

Virus inactivation/removal

The purification sequence in the downstream process is designed to remove product- and process-related impurities and provide a stable and potent product that meets predetermined quality criteria. Although the commonly used purification sequence starting with Protein A chromatography and followed by CEX and AEX chromatography, is qualified to provide a pure product, regulators also require two distinct, orthogonal virus clearance/inactivation steps (ICH Q5, 1999). The acidic elution conditions in Protein A chromatography promotes the inactivation of enveloped viruses. However, normally a dedicated virus inactivation step follows Protein A chromatography and the analysis of the inactivation efficiency is determined for each step individually (Vunnum et al., 2009). Furthermore, a virus removal step is placed after the last polishing step, before final concentration and formulation of the product. Typically, a 20nm virus filtration step is preferred due to its robustness to remove most of the viral contaminants (Zhou, 2009).

1.3.2. Disposable chromatographic purification of biopharmaceuticals

Traditionally, manufacturing facilities for biologics operate in batch mode incorporating mainly reusable stainless steel equipment leading to high capital investment and facility footprint. Additionally, extensive requirements in terms of time and materials for cleaning and preparation of each unit operation along with CIP and sterilisation-in-place (SIP) validation are needed. Disposable, single-use technologies (SUT) present an alternative approach especially with improvements in cell culture titres that can exceed 5g/L (Shukla & Gottschalk, 2013). SUT in bio-manufacturing have been available since the 1970s, however they were limited to filters and liquid hold bags for the first couple of decades (Laukel et al., 2011; Lopes, 2015). Disposable cell culture flasks have been extensively used, however, they have been restricted only to inoculum expansion. The WAVE Bioreactor™ system (GE Healthcare, Uppsala, Sweden) offered the first single-use platform for inoculum preparation suitable for large-scale manufacturing or as a replacement of the production bioreactor when cell culture volumes are low enough (Haldankar et al., 2006). Single-use stirred-tank bioreactors became available in 2004

with relatively low capacity of 250L, which a few years later improved to 2000L (Shukla & Gottschalk, 2013; Lopes, 2015).

Advances in SUT in downstream processing have not fallen behind compared with upstream. In early 1990s the first disposable membrane chromatography column was introduced by Sartorius. Furthermore, filtration operations utilised disposable filters and membranes for many years however, the lack of single-use skids was hindering the manufacturers from leveraging their true benefits. Significant milestones in the area have been achieved with the commercialisation of disposable depth filtration, tangential flow filtration and chromatography skids (Laukel et al., 2011). These single-use skids utilise disposable parts that come in contact with buffers and product thus preventing the contact with fixed parts of the equipment and avoiding any cleaning activities that otherwise would be required (Shukla & Gottschalk, 2013). A unit operation that still poses a great challenge to switch to single-use is centrifugation, with only limited examples on the market and none of them based on disk-stack centrifuges (Shukla & Gottschalk, 2013).

Adopting SUT offers significant benefits: reduction in capital investment, increased facility flexibility, reduced turnaround times between batches and campaigns and mitigation of cross-contamination risk (Shukla & Gottschalk, 2013; Lopes, 2015). Commonly used fixed process equipment in GMP manufacturing require cleaning after each batch. These cleaning activities increase the manufacturing cost and require validation studies to prove their effectiveness. Additional utilities are necessary to support CIP and SIP leading to large facility footprints and high demands in terms of capital investment. Multi-product facilities and new-build facilities have the potential to benefit the most from adopting SUT. Fewer fixed process equipment would lead to less construction and installation work thus accelerating the timeframe for the completion of the facility. Moreover, with less fixed equipment in the facility it is possible to address capacity changes faster avoiding over- or under-production (Lopes, 2015).

On the other hand different challenges arise with the introduction of SUT in bio-manufacturing. A rather obvious limitation is the manufacturing capacity of SUT with the largest available stirred tank bioreactor reaching 2000L (Lopes, 2015) and the biggest chromatography column of 60cm in diameter (Grier and Yakubu, 2016). Equipment size restrictions such as these could require a large number of batches for a single campaign and/or significant improvements in the performance of the process. For instance, improvements in cell line development and cell culture performance to increase the titre could lead to lower cell culture volumes and potentially to smaller bioreactors thus enabling the option of disposables. Additionally, high capacity chromatography resins would require smaller columns and the introduction of membrane chromatography that could replace the traditional packed-bed column could all make any size limitations obsolete.

Another challenge the biologics manufacturers are facing is the lack of standardisation of disposable components rendering it difficult to inter-change parts or even connect equipment from different vendors. The absence of regulatory guidelines that could provide a level of clarity regarding the materials that could be used by suppliers of disposables and the level and the quality of the certification they need to provide enhances the complexity of the decision to introduce SUT (Shukla & Gottschalk, 2013; Lopes, 2015). A key concern as expressed by manufacturers is the lack of sufficient validation studies by the suppliers to determine the level of risk associated with leachables and extractables that arise with the use of plastic, disposable equipment. An additional consideration could include the increased dependence of manufacturers on suppliers not only in terms of quality assurance but also in terms of availability, consistency and punctuality (Ding & Martin, 2008).

Although for the majority of the unit operations used in bio-manufacturing there is an SUT option available, the same cannot be claimed for ancillary process control instruments like sensors and monitoring devices. This issue is highlighted even more when a start to finish single-use process is considered where connectivity issues become

more apparent. Furthermore, the lack of single-use process control instruments leads to a decrease in automation that requires an increase in trained personnel (Lopes, 2015).

From an environmental perspective, studies have demonstrated that single-use facilities offer a lower CO_2 footprint compared with traditional facilities due to the reduction in heat consumption that comes with cleaning activities. However, the increased amount of solid waste that comes with disposables adds an additional consideration regarding the establishment of the appropriate methods and protocols for solid waste management (Lopes, 2015).

As discussed earlier, manufacturing of mAbs and antibody-related products is commonly based on a platform process involving 2 – 3 distinct chromatography steps in the purification sequence (Kelley et al., 2009). Typically, reusable, self-packed chromatography columns are utilised and packed with the appropriate resins that can be operated for multiple cycles. The main drawbacks in manufacturing regarding self-packed columns are the need for a packing/unpacking system, the materials (WFI, buffers etc.) and the personnel to prepare the column for operation and storage as well as the time requirements to perform qualification tests and documentation activities that are required to ensure the desired column packing quality. As mentioned earlier, membrane chromatography is an alternative, plug-and-play technology offering comparable purification performance, reduction in materials and a decrease in capital expenditures compared to packed-bed chromatography columns (Knudsen et al., 2001; Zhou & Tressel, 2005; Lim, et al., 2007; Jacquemart et al., 2016).

Another available option is the utilisation of disposable pre-packed chromatography columns, which offer the advantage of eliminating the need for packing/unpacking activities and hence the need for a packing system. Pre-packed chromatography columns have been available for over a decade and were primarily used at laboratory scale for bioprocess development. Scharl et al. (2016) collected a large dataset over a period of ten years from test runs with small-scale pre-packed columns considering a

significant number of commercially available chromatography resins covering all steps in the purification sequence and their analysis concluded that pre-packed columns offer consistently good packing quality.

Although pre-packed columns have been extensively used at small-scale, larger columns have become available only recently that could fit into pilot and commercial facilities. Grier and Yakubu (2016) summarised potential merits and drawbacks with pre-packed columns in GMP and non-GMP facilities and compared their performance with conventional self-packed columns. Potential benefits that have not been mentioned earlier could include simplified technology transfer activities and column packing consistency across different manufacturing sites. On the other hand, potential disadvantages could be the lead time to obtain pre-packed columns and the loss of column packing know-how. Their evaluation showed minor dissimilarities in packing qualification between data provided by the vendors and tests runs they performed post-shipment. Additionally, a slightly better purification performance and a lower pressure drop for linear velocities above 300cm/h were demonstrated for self-packed columns. Their evaluation concluded that these differences between pre-packed and self-packed columns could be attributed to differences in packing methods and materials.

1.4. Process development approaches in chromatographic purification of biopharmaceuticals

The increasing demands for mAbs, combined with intellectual property issues (expiration of patent(s)) and the need to be the first-in-market and acquire an advantage over other competitors, make it essential for process development to be rapid, efficient and off the critical path. Additionally, the advances in upstream processes have shifted the manufacturing bottleneck to downstream processes (Nfor et al., 2009). Initially, the traditional approach to the development of purification processes, involved the application of prior knowledge and the execution of an extensive number of experiments at bench scale. However, several restrictions such as limited or no previous knowledge,

the ability to screen only one set of operating conditions at a time, the relatively large amounts of product required and the high cost of the necessary laboratory equipment, prevent the in-depth evaluation of all the necessary operating parameters. As a result, pilot scale studies were delayed until sufficient data were gathered through clinical trials. A major drawback then is the incomplete determination of the design space and the regulatory inflexibility, coupled with the adoption of suboptimal processes (Chhatre & Titchener-Hooker, 2008).

Nowadays, the regulators, through Quality by Design (QbD) and Process Analytical Technology (PAT) initiatives, prompt manufacturers to accommodate a more systematic approach in process development and optimisation in order to explore the whole design space, define its boundaries and determine the impact of critical process parameters (CPPs) on critical quality attributes (CQAs). Currently, different strategies for the development of purification processes are available including heuristic or knowledge-based methods, algorithmic- and model-based methods, high-throughput experimental and hybrid methods combining different approaches (Nfor et al., 2008).

1.4.1. Knowledge-based methods

The heuristics approach is based on leveraging prior knowledge from experts on a topic of interest. Usually prior knowledge is captured through a set of rules-of-thumb that can guide process synthesis and design (Nfor et al., 2009). Wheelwright (1989) discussed different design methods based on heuristics from copying an already existing process to the development of computer-aided expert systems that rationalise that prior knowledge. Asenjo et al. (1989) developed a prototype expert system summarising a set of 65 rules-of-thumb to synthesise and select complete downstream processes in bio-manufacturing. The authors demonstrated the efficient synthesis of primary recovery steps in downstream processing and highlighted the need for additional information regarding the physicochemical properties of the protein of interest and its impurities to make decisions on high resolution chromatography steps. Leser and Asenjo (1992)

showed that the integration to an expert system of a database with comprehensive information on the major mixture components identified in upstream processing offers a more accurate selection of purification processes. Further developments in expert systems introduced more accurate databases, improved tool structure and flexibility and mathematical correlations to model more accurately different downstream processing trains (Leser & Asenjo, 1994; Lienqueo et al., 1996; Leser et al., 1996; Lienqueo & Asenjo, 2000).

Heuristics-based methods can be used for rapid evaluation of alternative bio-manufacturing strategies and have been proven to provide essential insights for the design of a purification process when limited data on the protein of interest and its impurities are available. Furthermore, with such an approach complex process synthesis and design tasks can be organised into more manageable operations. On the other hand, adopting solely a heuristics approach could lead to sub-optimal manufacturing processes and thus further evaluation should be performed to increase the level of process understanding and optimise the process (Nfor et al., 2008; Nfor et al., 2009; Hanke & Ottens, 2014).

1.4.2. Algorithmic- and model-based methods

Throughout the bio-manufacturing process there is a tendency to progressively replace empirical correlations with mechanistic models in order to provide a higher level of process understanding (Hanke & Ottens, 2014). Mechanistic models are derived from fundamental (or first) principles (conservation of mass, heat and momentum) and thus have the potential to describe more accurately the phenomena occurring in any system. The chemical industry has a long history and sufficient databases on thermodynamic properties exist to support the use of mechanistic models. In contrast, the biotechnology industry lacks the advanced models, software tools and simulation packages to improve and assist in process synthesis, design and optimisation mainly due to the complex nature of biomolecules (Nfor et al., 2009).

Significant research has been done since the late 1960s in the area of mathematical modelling for packed-bed adsorption operations (Bellot & Condoret, 1991; Bellot & Condoret, 1993). A comprehensive overview has been given by Ruthven (1984) classifying different modelling approaches into three main categories; equilibrium, plate and rate models. Mathematical models based on the equilibrium theory make the assumption of rapid equilibrium achieved between the stationary and the mobile phase neglecting the effects of mass transfer resistance and axial dispersion. Models based on the plate theory adopt a different approach by dividing the column into a series of theoretical plates within which equilibrium has been achieved. The empirical nature of plate models cannot relate them to first principles (Gu et al., 1993). Nevertheless, their application has been demonstrated on the separation of multicomponent systems (Gu et al., 1993; Guiochon, 2002).

Rate models are expressed mathematically through a set of differential equations that describe the phenomena acquiring between the stationary and mobile phase for each component (Gu et al., 1990). Among rate models, the general rate model is considered one of the most comprehensive that attempts to capture the effect of all possible contributions such as axial dispersion, intra-particle diffusion, external firm mass transfer resistance and adsorption/desorption kinetics. The complexity and non-linearity of the general rate model have posed great challenges in solving the system of the differential equations required to describe the operation and efficient algorithms are necessary to provide a solution (Gu et al., 1990; Gu et al., 1993; Guiochon, 2002). Gu et al. (1990) proposed a numerical procedure to solve the general rate model using the finite element and the orthogonal collocation methods. Later the authors extended their work with the addition of second order kinetics and size exclusion effects in their model (Gu et al., 1993).

Other studies have used the general rate model or a variation of it to address various aspects of the chromatographic performance. Degerman et al. (2006) used the general rate model with Langmuir isotherm kinetics and a modulator to optimise the separation

of an IgG from bovine serum albumin. Their model was able to simulate real gradient elution profiles using a constraint on the slope of the gradient embedded in their optimisation procedure, thus avoiding making assumptions on ideal gradients. The authors demonstrated their approach using six decision variables with a fixed bed-height and confirmed graphically the success of their optimisation method. Gerontas et al. (2010) integrated scale-down experimental data with general rate modelling to scale-up and predict the chromatographic separation at manufacturing scale. The general rate model with Langmuir kinetics and a mobile phase modulator was used and solved using the finite element method. Additionally, the authors demonstrated the use of genetic algorithms to calibrate model parameters and showed the validity and applicability of their approach using three different CEX resins. Boushaba et al. (2011) used the same modelling approach described by Gerontas et al. (2010) to evaluate the effect of fouling in the chromatographic performance. The authors demonstrated the use of windows of operation to visualise the trade-offs between the effort and the benefits of feed clarification prior to chromatography.

A systematic approach to model chromatography operations has been developed by Chan et al. (2008). Their approach consists of three main parts. First a methodology was proposed to determine the feed concentration and identify significant components in the feed mixture that are required for the development of an accurate model. Then, the second part involves the estimation of model parameters for different rate models. The authors identified two models that have been studied extensively; the equilibrium-dispersive and the general rate model and employed them to demonstrate their approach. The general rate model offers a more accurate description of the separation process however at a high computational cost. In contrast, the equilibrium-dispersive model makes further assumptions to simplify the general rate model thus mitigating the time required to solve it with potential consequences on the accuracy of the model. At the third and last part the authors utilised the fractionation diagram method to compare the accuracy of the mechanistic models they included. Three case-studies were

presented to illustrate the applicability and usefulness of their approach. The fractionation diagram method was employed due to its sensitivity to capture efficiently the trade-offs between purity and recovery.

Origin of the fractionation diagram approach

The final output of a chromatography cycle is a chromatogram, which presents how the concentration of the eluate changes against volume or time. However, it is difficult to extract straightforward relationships that can correlate performance with operating conditions and moreover, this task becomes more difficult when product and impurities concentrations are expressed in different units and when inadequate data are available (Ngiam et al., 2001). To address this issue, Ngiam et al. (2001) proposed a method to manipulate the information provided by chromatograms and construct graphical representations to visualise the trade-offs between purity and product recovery. Originally, the graphical method was developed by Richardson et al. (1990) to correlate product recovery and purity in protein precipitation and furthermore, to optimise the precipitation conditions and identify the precipitant cut points. Later a similar graphical method was developed to optimise high-performance tangential flow filtration operations (Reis & Saksena, 1997).

Examples of implementation of the fractionation diagram approach

The fractionation diagram approach in chromatography development was first used by Ngiam et al. (2001) with a hypothetical three component mixture. The authors utilised a mathematical model of size exclusion chromatography (SEC) to describe and simulate the separation of the desired component at different flow rates of the mobile phase. The resulting chromatograms where then translated into fractionation diagrams and maximum purification factor (PF) versus recovery diagrams were generated to visualise the effect of flow rate on the chromatographic performance. In the same study, the team conducted experiments with an IEX column to evaluate the removal of endotoxin and plasmid DNA. The fractionation approach was used to identify the collection points on

the chromatogram that will provide the desired level of purity and yield. In a subsequent study, the same approach was employed to optimise a two-column purification sequence (HIC followed by SEC). The authors identified the need to perform an integrated optimisation approach and develop the purification sequence as a whole and not as individual steps, which may lead to a sub-optimal result. Initially, the mobile phase flow rate was chosen as the investigated variable and how it affects HIC performance in terms of purity and yield. Subsequently, the effect of each HIC flow rate on the following SEC step, was evaluated in order to make decisions regarding the operating flow rate of the HIC step. The authors concluded that SEC is strongly affected by HIC (Ngiam et al., 2003).

Salisbury et al. (2006) considered the need to optimise the chromatographic purification sequence as a whole, utilised the fractionation diagram approach and introduced 2D windows of operation, capturing the trade-offs between productivity and purity. To generate a window of operation, initially a range of product breakthrough levels and operating flow rates have to be chosen based on the desired level of resolution. Then the time to achieve each breakthrough level was determined and the elution profiles were generated for each flow rate/breakthrough level combination. Subsequently, PF versus recovery diagrams were created for each combination of flow rate and breakthrough level and purity was estimated at the desired level of product recovery. Finally, productivity was calculated for each flow rate and breakthrough level combination. A window of operation for the first chromatography step was created and a feasible region within the window of operation was identified for the operation of the next chromatography step.

The above studies demonstrate that the fractionation diagram approach is a powerful tool in purification process development and optimisation. The method offers an efficient solution to the issues arising from the insufficient approaches on how to extract rapidly useful information from a plethora of chromatograms. Moreover, it considers the importance of optimising each chromatography step as part of the whole purification sequence and not individually. Incorporation of the fractionation diagram approach with

other techniques has been proven to be an excellent tool in process development that can help make feasible decisions.

1.4.3. High-throughput experimentation and design of experiments

Although mechanistic models offer a high level of process understanding, there are challenges that arise from the selection of the appropriate modelling approach, to the related experimental effort to determine model parameters, to the high computational cost associated with some models (e.g. general rate model). Additionally, the inherent complexity of biopharmaceuticals and their manufacturing processes, increase the need for the development of novel tools and techniques to enhance the efforts towards accelerating bioprocess development while gaining an in-depth understanding of the process (Hanke & Ottens, 2014).

High-throughput experimentation has emerged as a useful tool in purification bioprocess development and is characterised by miniaturisation, automation and parallelisation (Bhambure et al., 2011).

- Miniaturisation due to the very small quantities of materials required to run the experiments, which are usually an order of magnitude lower than the requirements for small scale studies
- Automation due to the ability to incorporate automated liquid-handling platforms
- Parallelisation because a set of experimental runs can be executed simultaneously

The major advantage with high-throughput experimentation is the ability to explore a large combination of operating parameters with minimal feedstock requirements. Additionally, pilot scale studies can be more targeted and designed to explore further only the conditions that appear the most promising, minimising cost and time requirements. Despite the significant merits of high-throughput experimentation techniques, there are important concerns that have to be addressed regarding the design

of the experiments and the organisation and manipulation of the resulting information (Chhatre & Titchener-Hooker, 2008; Nfor et al., 2009).

1.4.3.1. High-throughput screening techniques in chromatography

Currently, different high-throughput screening techniques are employed in the development of chromatographic purification processes. Chhatre and Titchener-Hooker (2008) proposed a route to chromatography process development using micro-scale chromatography and provided a comprehensive overview of the different formats of micro-scale chromatography. The authors identified three different formats most commonly used: 1) microliter batch incubation, 2) micropipette chromatography tips and 3) miniature pre-packed columns. These micro-scale chromatography devices can be operated manually or automatically and can generate quantitative and qualitative data (Chhatre & Titchener-Hooker, 2008). The ability to operate these devices using a robotic workstation provides many advantages such as, increased productivity, consistency and accuracy. However, automated liquid-handling devices such as Freedom EVO® by Tecan require proper training and time to develop the appropriate operating protocols. Those protocols are especially important in platform processes where specific unit operations are employed and therefore the same protocol, for example for Protein A chromatography, can be used multiple times (Chhatre & Titchener-Hooker, 2008). Lacki (2012) evaluated the limitations and the advantages of the three micro-scale formats available and provided guidelines based on published high-throughput screening purification data, setting out which format is more appropriate for a given kind of experimental goal.

The implementation of the first micro-scale format utilising the microliter batch incubation technique most commonly takes place using 96-well filter plates loaded with chromatography resins. Manually loaded 96-well plates have been used to identify the best performing chromatographic purification sequence for recombinant proteins (Rege et al., 2006). A plethora of different studies have been published utilising 96-well filter plates and automated liquid handling systems, to investigate different types of protein

chromatographic purification (Kramarczyk et al., 2008; Kelley et al., 2008; Kelley et al., 2008b) and to identify the accuracy and consistency of automated liquid landing systems (Coffman et al., 2008). Additional studies have been conducted using 96-well filter plates to determine the dynamic binding capacity (Bergander et al., 2008; Carta, 2012).

The demonstration of the second micro-scale format using pre-packed micropipette chromatography tips has been less extensively reported, compared to microliter batch incubation. Wenger et al. (2007) evaluated the purification of virus-like-particles from yeast lysate and developed an operating protocol using an automated robotic workstation. Chhatre et al. (2009) used the same micropipette chromatography tips format and a 2-level full factorial design of experiments to evaluate the effects of pH and salt concentration in the recovery and purification of polyclonal antibodies by a mixed-mode cation exchange resin. The authors identified the challenges associated with scale-up of chromatography processes based on the HTE results and highlighted the increasing necessity for faster analytical methods to cope with the large datasets that result from HTE.

The third and last micro-scale technique available incorporates miniature packed-bed chromatography columns with bed volumes of 100, 200 and 600µl. The miniature columns are a close representation of a packed-bed chromatography column, in terms of geometry and flow characteristics. Wierling et al. (2007) recognised the necessity for more rapid analytical tools and utilised these miniature columns coupled with an automated liquid handling system and an analytical system to evaluate the ability of four different chromatography resins to purify monoclonal antibodies from host cell proteins. In a different study, miniature chromatography columns were used to conduct breakthrough and elution experiments and to screen different resins and operating conditions. The authors concluded that there was a good agreement between micro-scale and laboratory scale data when comparing breakthrough curves and an acceptable representation of purification performance. Finally, the authors highlighted the important role HTE has in chromatography process development and mentioned the potential

benefits that could be achieved by integrating HTE with model-based methods for the rapid evaluation of model parameters (Wiendahl et al., 2008).

These examples all evaluate the performance of a single purification step. Treier et al. (2012b) demonstrated the ability to develop the whole purification sequence and highlighted the analytical challenges that arise from HTE. Welsh et al. (2014) incorporated 96-well filter plates for initial screening of binding conditions, followed by miniature columns to evaluate impurities removal capabilities. The authors demonstrated the applicability of their strategy with proof of concept examples and confirmed the HTE results with small-scale experiments.

HTE offers benefits not only in terms of materials and time consumption but also in terms of the in-depth level of process understanding that can be achieved from very early stage in process development. Process limitations and manufacturing bottlenecks can be identified and alternatives can be rapidly evaluated with limited resources and the results could be used to drive and accelerate scale-up studies. The potentially vast amounts of data that can result from HTE require the appropriate methods and tools in order to fully leverage the information provided and support decision-making in bio-manufacturing.

1.4.3.2. Design of experiments in chromatography

Micro-scale, HTE techniques available for the chromatographic purification of biopharmaceuticals have increased the impetus for methodical experimental planning in order to maintain the number of experiments to a manageable level both in terms of experimental and analytical work while gaining the desired level of process understanding. The classical experimental approach evaluates the impact of each factor (or variable parameter) individually by keeping all the other independent variables constant. This approach is also known as one-factor-at-a-time (OFAT) and usually requires extensive experimentation and increased effort in data analysis and visualisation. The limitations of the OFAT approach become apparent even with relative simple systems where only a few factors have an influence. For instance, for a system

with four factors evaluated at four different levels each, the resulted number of experiments is 4^4=256, excluding replicates necessary to evaluate experimental errors and lack of fit of the proposed model. Moreover, the investigation of each factor independently hinders the identification of interactions among factors thus providing a limited level of confidence on the resulted model (Lazic, 2004).

Design of experiments (DoE) is an alternative approach that investigates multiple factors simultaneously and provides empirical correlations that link factors with responses (Kumar et al., 2013). A comprehensive overview of the statistics involved in DoE as well as a review and discussion on the different designs that have been developed with applications in Chemical Engineering is given by Lazic (2004). Ferreira et al. (2007) identified chemometric tools commonly employed for the development of chromatographic systems. They reported that two-level full factorial, central composite, Box-Behnken, Doehlert and mixture design of experiments were among those more frequently used. Different types of experimental designs in DoE have been discussed also by Hibbert (2012). The author identified different designs in the DoE arsenal and provided a concise description summarising advantages, and limitations for each of them. Additionally the author offered recommendations on the procedure of reporting studies incorporating DoE, mentioning the inadequate description by many authors in their publications of the DoE methodology they followed. Furthermore, it was highlighted that DoE is a well-established chemometric tool with a list of different software packages to assist in implementation and analysis. Hence, there is no need to repeat its full history in every study but rather focus on the description of the details in the development of the DoE, the reasons behind the selection of a specific design by a researcher and the methodology that was followed to identify the appropriate model to describe the system.

Following Hibbert's (2012) paradigm, Kumar et al. (2013) used the microliter batch incubation technique with a 96-well filter plate loaded with a CEX resin to purify a therapeutic protein and they evaluated different design of experiments. The authors proposed a systematic approach for optimum model selection. Their approach

investigates different combinations of model parameters using a stepwise regression analysis and identifies the optimal model through a series of comparisons among key statistics of different models to describe a given system. Xu et al. (2012) followed the QbD paradigm in the development of a CEX chromatography step to separate a mAb from its aggregates. The authors used DoE to screen different CEX resins and develop a pH-conductivity hybrid elution profile. However, limited information was provided on the methodology that was followed to select their DoE method and model parameters that describe the chromatographic purification.

1.4.4. Hybrid methods

Another option in chromatography process development is the combination of different approaches that were discussed above (Nfor et al., 2008; Nfor et al., 2009; Hanke & Ottens, 2014). Susanto et al. (2009) integrated HTE with genetic algorithms to optimise chromatography operations. Their approach combined an automated liquid handling system with software tools to manipulate raw data and perform the experimental design using a genetic algorithm. The authors demonstrated their fully automated platform using both the microliter batch incubation and the miniature pre-packed chromatography columns technique. The use of genetic algorithms in combination with HTE has also been discussed by Treier et al. (2012) to optimise a CEX chromatography step. The authors developed an automated protocol to prepare chromatography buffers based on conditions dictated by their platform and compared their approach with DoE and response surface analysis. They concluded that in case of multiple optimal solutions their approach to incorporate a genetic algorithm for experimental planning and analysis provided better correlations. However, in case of a single optimum the authors mentioned that it would require four times more experimental runs than using the DoE approach.

An alternative methodology was presented by Osberghaus et al. (2012) linking mechanistic modelling with HTE. Miniature pre-packed chromatography columns were

operated with an automated liquid handling system and a series of experiments were performed to determine model parameters. The authors calibrated a lumped transport-dispersive model to make scale-up predictions and reported an acceptable agreement between the predicted performance and the experimentally achieved with a laboratory scale chromatography column. Traylor et al. (2014) incorporated microliter batch incubation with 96-well filter plates operated on an automated liquid handling system with a multi-channel robotic arm and adjusted the pore diffusion model assuming a Langmuir isotherm to describe a multi-addition batch uptake.

Another hybrid approach in chromatography process development was demonstrated by Nfor et al. (2011). The authors integrated HTE with model-based optimisation using a combination of the equilibrium-transport-dispersive and the liquid-film linear driving force models. HTE was implemented using the microliter batch incubation technique on an automated liquid handling system to acquire model parameters to describe the chromatographic operation. A multi-objective optimisation was performed considering quality and performance attributes along with process economics metrics related only to chromatography and Pareto frontier graphs were generated to visualise the trade-offs among different attributes. The authors utilised the weighted sum method to compare three different mixed-mode resins under their respective optimal operating conditions that were screened and optimised using their proposed hybrid approach for the purification of a particular mixture.

The QbD paradigm in chromatography process development has also been demonstrated by Bhambure and Rathore (2013). The authors established a High-Throughput Process Development (HTPD) platform combining DoE with the microliter batch incubation technique using prefilled 96-well filter plates. The experimental plan of their HTPD platform was operated manually leading to the introduction of correction factors to minimise experimental errors. Moreover, the authors reported a difference in the step recovery between their HTPD platform and laboratory-scale systems possible due to non-specific binding of the protein on the bottom of the filter plates, reporting the

high hydrophobicity of the protein as the cause of this behaviour. Overall, the study demonstrated the applicability and usefulness of their HTPD platform highlighting the enormous benefits in terms of time and feedstock material required compared with traditional, laboratory scale experimentation.

More recently, Liu et al. (2017) developed a decision-support framework for the optimum resin selection and operation of a two-step chromatographic purification train. The proposed hybrid approach was developed using miniature, pre-packed chromatography columns on an automated liquid handling system. High-throughput purification data generated for each resin candidate were integrated into a multi-objective, mixed-integer nonlinear programming model and used Pareto frontiers to visualise the trade-offs between purity and yield. A key advantage of the suggested decision-support framework is the integration of two steps in the purification train highlighting the importance of considering the whole purification train when developing individual chromatographic operations. Although such a holistic approach offers critical insights regarding the interdependencies among individual chromatography steps it comes with an increased experimental effort when screening multiple chromatography resin sequences and multiple process parameters at a wide range.

HTE has emerged as a powerful tool in chromatography process development. There is an increasing trend to develop hybrid approaches and incorporate HTE into platforms (or workflows) that combine different tools and methods forming a HTPD strategy (Bhambure & Rathore, 2013). Hybrid methods offer the advantage of linking different approaches emphasising on the merits while mitigating the limitations that each method offers when used individually (Hanke & Ottens, 2014). Regulatory agencies through the QbD initiative suggest the manufacturers to adopt a HTPD strategy in order to accelerate process development from a very early stage in a product's lifecycle and achieve a high level of product and process understanding.

1.5. Process economics modelling in bio-manufacturing

The high cost of R&D, combined with the increased necessity to provide affordable new biologics, is driving the biotech sector to identify solutions that can mitigate the clinical and commercial manufacturing cost. The design and optimisation of bio-manufacturing facilities for therapeutic antibodies have been evaluated in several studies. The Department of Biochemical Engineering at University College London has a key focus on the development of decision-support tools to address process (e.g. Farid et al., 2005; Farid et al., 2007; Simaria et al., 2012; Allmendinger et al., 2014; Yang et al., 2014) and business (e.g. Rajapakse et al., 2005; George & Farid, 2008) aspects in bio-manufacturing. One key performance metric used by a plethora of studies is the cost of goods (COG) that provides a sum of direct (or running) and indirect (or overhead) manufacturing costs.

Farid et al. (2000) developed a prototype hierarchical tool to model process and business aspects in bio-manufacturing. The authors provided a breakdown of COG that reflects the cost associated with cGMP bio-manufacturing facilities and demonstrated the implementation and functionality of their tool through a case study evaluating different stainless steel and single-use manufacturing strategies. Mustafa et al. (2004) followed the same modelling approach and used COG as the key metric in decision-making between packed-bed chromatography and expanded bed adsorption. Later Mustafa et al. (2006) presented a framework to address both business and process aspects related to retrofitting using discrete event simulations.

The continuously increasing demands for higher annual outputs have a positive effect on the COG per gram of product produced (COG/g) and therefore, as the annual production capacity rises, COG/g reduces (Farid, 2007). At small scales the contribution of capital investment and labour cost in COG is typically greater compared with the cost of materials. However, the proportion of indirect and direct costs is reversed, as scale increases. Moreover, as the scale and/or the titre increase, upstream processes tend to provide a smaller portion of the COG compared to downstream processes (Farid, 2009).

It has been suggested that the proportion of upstream over downstream cost changes from 55/45 to 30/70, as titre increases ten-fold (Sommerfeld & Strube, 2005). The trend shows that downstream processes play an important role in the economic feasibility of the whole process and hence significant challenges have to be addressed. Innovations and further improvements in the existing technologies are required to minimise the cost of downstream processes and by extension the overall cost of manufacturing.

Single-use technologies (SUT) are considered an alternative in order to reduce the cost of manufacturing. Some advantages and disadvantages associated with SUT were discussed earlier in Section 1.3.2. Novais et al. (2001) developed a process economics model to evaluate and compare conventional (fixed) and single-use bio-manufacturing facilities using the net present value (NPV) as the key performance metric. The authors estimated the capital investment for each facility following principles discussed in chemical engineering textbooks (Peters & Timmerhaus, 1991) by using a method initially proposed by Lang (1948). Their evaluation was demonstrated through a case study concluding that the cost of consumables increases substantially in disposable-based facilities while on the other hand the capital investment reduces significantly.

Other examples on the economic evaluation of SUT have been published using commercially available computer aided tools. Sinclair and Monge (2002) used a spreadsheet-based COG model (BioPharm Services Ltd) and discrete-event modelling to compare the cost-effectiveness of disposable bags over stainless steel vessels throughout the bio-manufacturing process. The authors considered a 2000L manufacturing capacity and demonstrated a reduction in capital investment of 20% and an increase in throughput of 7% resulting in 8% drop in COG for facilities utilising disposable bags over stainless steel vessels. In a following study, Sinclair and Monge (2005) illustrated the design of a concept bio-manufacturing multi-product facility based on SUT. Their concept facility was based on a 1000L bioreactor for the production of material for late stage clinical trials. The authors reported a 40% decrease in capital

investment and a 17% drop in COG for their concept facility compared with a traditional facility of the same capacity.

1.5.1. Managing risk and uncertainty in bio-manufacturing

Most of the examples discussed above regarding the economic evaluation of bio-manufacturing facilities used a knowledge-based and deterministic approach in the design of the process. However, the complex nature of biomolecules and their manufacturing processes introduce deviations in the behaviour of a process for different products and even between batches of the same product. Additional uncertainties regarding the regulatory approval of a new product and its commercial success, all create a complex environment that requires the appropriate tools to identify the optimum manufacturing strategy and enhance decision-making. In order to attempt to address these challenges and consider risks associated with manufacturing and commercialisation of new biopharmaceuticals, Farid et al. (2005) extended a prototype hierarchical decision-support tool (Farid et al., 2000) and used stochastic analysis to evaluate different manufacturing strategies for early stage clinical manufacturing. The authors initially determined critical technical and market uncertainties using one-way sensitivity analysis and then used Monte Carlo simulations to further evaluate their impact on COG and project throughput. A case study was presented to demonstrate the application of their prototype tool comparing different manufacturing technology options (stainless steel, disposable and hybrid facility configuration).

Farid et al. (2007) identified the lack of commercially available software tools in bioprocessing that can integrate process design calculations with logistical activities (e.g. resource allocation between competing tasks) and incorporate risks associated with all relevant activities. The authors used a task-oriented computer-aided tool combining the above features through a case study comparing traditional, stainless steel with disposable facilities in terms of COG/g and annual project throughput. A key process parameter that introduces a significant variability in COG and throughput is the

fermentation titre (Farid et al., 2007). Subsequent studies have illustrated a mixed integer linear programming approach to optimise medium term (1 – 3 years) planning of bio-manufacturing facilities considering a distribution of potential titre values through Monte Carlo analysis (Lakhdar et al., 2006; Lakhdar & Papageorgiou, 2008).

The integration of stochastic simulations with multivariate data analysis and visualisation techniques to address technology transfer challenges was presented by Stonier et al. (2013). Technology transfer activities related with the scale-up of manufacturing processes from pilot scale to late phase clinical trials and commercial facilities are typical based on limited information rendering very difficult to perform properly facility fit assessments. Stonier et al. (2013) identified though discussions with industrial experts in technology transfer activities significant process parameters that are most likely to cause process deviations at larger scale and used triangular distributions in their Monte Carlo analysis to evaluate their impact on key metrics (e.g. COG and product mass loss). Results from these stochastic simulations were leveraged using principal component analysis and clustering algorithms to reduce the dimensions of the process design space and promote visualisation. Finally, the authors demonstrated a novel visualisation technique using co-ordinate plots with actual process related data rather than principal components scores to address facility fit issues in bio-manufacturing facilities. Yang et al. (2014) improved upon previous work by Stonier et al. (2013) and used a data-driven, discrete simulation tool and a decision tree technique to leverage the large datasets from Monte Carlo simulations. The authors demonstrated a case-study to investigate the impact of process deviations on the product mass loss and provided solutions to debottleneck the manufacturing process based on their decision tree analysis.

Another prototype framework was developed by Rajapakse et al. (2005) to assist in decision-making in portfolio management for the development of new biopharmaceuticals. The authors evaluated a portfolio with three different mAbs entering clinical trials and estimated the portfolio's NPV through a 20-year period comparing in-house (early and late build) with contract manufacturing. Critical technical and

commercial uncertainties were identified through one-way sensitivity analysis and their impact was further investigated using Monte Carlo simulations. A different application of this prototype tool in portfolio management was demonstrated by Rajapakse et al. (2006) to evaluate the rewards and risks related with different portfolios. The authors integrated the efficient frontier approach in portfolio management initially proposed by Harry Markowitz (1991) with Monte Carlo analysis. The authors used NPV of each portfolio as the metric for reward and its standard deviation as the risk metric and demonstrated the functionality of their approach comparing different drug portfolios for a hypothetical biopharmaceutical company.

A holistic approach was presented by George and Farid (2008) to address simultaneously R&D portfolio management and bio-manufacturing capacity planning decisions. The authors developed a framework to evaluate different portfolio strategies by leveraging Bayesian networks and evolutionary algorithms to search efficiently a vast decision space and identify optimal solutions. Their approach was demonstrated with a case study discussing the alternative strategies a hypothetical biopharmaceutical company could consider when developing a portfolio under technical and commercial uncertainties. Two conflicting objectives were considered in the evaluation: to maximise the NPV and the probability of the NPV being positive. In general terms, their holistic approach provided insights on various aspects that need to be considered in portfolio management such as which drug candidates should be included in a portfolio and their priority in the development sequence along with scheduling of the related development activities and finally the decision to include (or not) third parties into the venture (e.g. contract research and manufacturing organisations).

An additional study used the same framework and evaluated the impact of portfolio size and cash flow restrictions on the decision space. It was indicated that cash flow constraints could lead to a reduction in the expected rewards or the success probability of a strategy. Moreover, decisions regarding scheduling and third party collaborations are significantly influenced by the size of a portfolio (George & Farid, 2008b).

The majority of the studies presented so far provide a framework that considers usually one or two criteria in the decision-making process thus excluding potentially important attributes. To address this limitation Farid et al. (2005b) developed a multi-attribute decision-making (MADM) approach that considers qualitative and quantitative attributes in the evaluation of different bio-manufacturing strategies. One type of classification of different MADM methods is based on the dimensions in which the attributes are analysed. For instance, multi-dimensional methods evaluate each attribute considering its actual value and are typically used for initial screening of alternatives by excluding those that do not meet certain criteria (Farid et al., 2005). On the other hand, methods that involve a single dimension are more preferred when trade-offs among attributes require evaluation.

Farid et al. (2005b) recognised the simplicity to formulate and utilise the simple additive weighting technique (also known as weighted sum method) and extended it to introduce uncertainty in the weight coefficient assigned to each attribute as well as the attributes. Hence, instead of using constant weights for the attributes in their model, the authors considered a distribution of values evaluated through Monte Carlo simulations. The proposed probabilistic additive weighting method capturing financial and operational attributes was demonstrated through a case study that was initially investigated by Farid et al. (2005) comparing stainless steel, disposable and hybrid facilities for manufacturing material for late phase clinical trials. The authors evaluated a range of operational to financial ratios and compared the aggregated scores for each ratio among the three different manufacturing strategies in order to provide a better understanding between trade-offs and their impact in decision-making.

The simple additive weighting technique has also been used in a decision-support framework to evaluate strategies for achieving bio-manufacturing capacity at commercial-scale (George et al., 2007). A hypothetical biopharmaceutical company with three drugs with different development and market characteristics was used as an example to illustrate the implementation of the approach. Options involving third parties

(contract manufacturers or other partners) and/or investment in building new commercial facilities were considered and uncertainties associated with each option were determined. The authors included financial and operational attributes and evaluated different options based on their average aggregated score at different operational to financial ratios. Hence, highlighting the importance of considering multi-criteria in decision-making especially in the presence of conflicting attributes under uncertainty.

A significant number of studies discussed throughout this chapter have employed Monte Carlo simulations in their analysis to introduce and evaluate uncertainty. Monte Carlo is a numerical method that attempts to describe the function of a complex system that does not follow a predefined pattern but rather a stochastic (Landau & Binder, 2009). Thus, the method has been widely accepted and used to provide solutions to very challenging "problems". Nevertheless the development of the appropriate algorithm can be challenging; from the selection of the correct sampling method for random numbers to the determination of the convergence criteria (Ballio & Guadagnini, 2004; Landau & Binder, 2009).

1.5.2. Design, economic evaluation and optimisation of chromatographic separation of biopharmaceuticals

Significant work has been published investigating different methods in the design and optimisation of the chromatographic purification train. As cell culture titres increase the manufacturing bottleneck tends to shift from upstream to downstream processing (Sommerfeld & Strube, 2005). Therefore, optimal design of the purification train requires the consideration of multiple criteria including product quality, productivity and cost-effectiveness along with additional restrictions associated with product, process, facility and business aspects.

Process development and optimisation of chromatography unit operations require numerous decisions to be made: from the selection of the appropriate chromatography resin type, its position in the purification train, the operating conditions and the size of

the column(s) to scheduling of shift patterns, monitoring and control of the operation. The traditional approach in decision-making relied heavily on prior knowledge and experience of process engineers thus lacking a systematic framework to aid design and optimisation often leading to sub-optimal solutions. Joseph et al. (2006) developed a model-based framework to assist in the systematic design of the chromatographic separation of biopharmaceuticals. The authors evaluated different strategies comparing chromatography column sizes and operating conditions assessing resin degradation, lifetime and compression effects on productivity, COG and facility fit restrictions. An example case study was presented assuming a given manufacturing process to demonstrate their approach using windows of operation to visualise trade-offs under constraints among key performance metrics for the capture step in the purification train utilising an affinity (recombinant Protein A) resin.

The use of mechanistic models in an integrated process synthesis and optimisation framework has been presented by (Nfor et al., 2013). The authors used HTE to screen different chromatography resins and obtain model parameters and utilised Pareto frontiers to visualise the trade-offs of different optimal solutions considering purity and purification cost as the two decision-making attributes.

Purification sizing strategies have also been discussed by Stonier et al. (2012). A decision-support tool was developed to integrate process and business aspects in antibody manufacturing and identify the optimum sizing strategy for the purification sequence. All three chromatography steps were modelled in a multi-product bio-manufacturing facility consisting of 3 x 2000L bioreactors operated in a staggered mode with two downstream processing (DSP) suites. The authors evaluated the effects of increasing cell culture titre on the chromatographic purification train and assessed the robustness of the process to titre fluctuations. Pareto (or efficient) frontier graphs were used to visualise the set of optimal solutions provided by their database-driven, discrete-event simulation tool to visualise the trade-offs between COG and time for different solutions. It was demonstrated that as titre increases from 1g/L to 10g/L the COG/g of

the DSP reduces by approximately 50% and it was highlighted that the affinity (Protein A) chromatography step was the main bottleneck in DSP with high cost of consumables and relative low binding capacity (Stonier et al., 2012).

Another approach has been presented considering also the effect of different impurities loads of the harvested feed material on the design and optimisation of the purification train (Simaria et al., 2012). The proposed framework was based on previous work by Farid et al. (2007) and linked with a meta-heuristic optimisation approach using genetic algorithms to identify the optimum purification train configuration. A set of heuristics (rules-of-thumb) were proposed after discussions with industrial experts to rationalise the capabilities and restrictions of different chromatography resin types. A case study inspired from the biopharmaceutical industry investigated a portfolio of mAbs at different stages in their respective lifecycle with different product and capacity specifications. The overall objective of the case study was to identify the optimal upstream processing (USP) to DSP ratio, determine the sequence of the chromatographic purification train and the sizing strategy for each chromatography step to minimise the COG/g for each product in the portfolio (Simaria et al., 2012).

Furthermore, it was demonstrated through a case study the ability of evolutionary algorithms to identify global optimal solutions even when considering conflicting attributes. The value and benefits of evolutionary algorithms in bioprocess optimisation become more apparent when evaluating very large decision spaces. Allmendinger et al. (2013) formulated a single-objective, multi-constrained optimisation problem using evolutionary algorithms performing Monte Carlo trials to account for uncertainty in cell culture titre. Optimisation involved only the sizing strategy of the chromatographic purification train considering a process economics model developed by Farid et al. (2007). The authors demonstrated their approach with an industrially relevant case study for the manufacture of a mAb at commercial scale optimising bed-height, diameter, number of columns in parallel and number of cycles per batch for each chromatography

step in the purification sequence using the COG/g as the key performance metric to identify the optimum sizing strategy.

A following study improved upon previous work (by Allmendinger et al., 2013) and developed an evolutionary multi-objective optimisation algorithm. The authors included chromatography resin utilisation, impurities reduction capabilities, COG/g and its robustness as the four objectives in their optimisation approach. Additionally, uncertainty was introduced in several process parameters with emphasis in the purification sequence and It was highlighted the importance of including uncertainty in design and optimisation to avoid sub-optimal manufacturing strategies. The authors demonstrated their approach evaluating a bio-manufacturing process for a single mAb and compared their results with a typical platform purification strategy from the industry and reported a reduction in COG/g up to 10% (Allmendinger et al., 2014).

An alternative to evolutionary algorithms was presented by Liu et al. (2013). A mixed-integer nonlinear programming (MINLP) approach was developed to identify the optimum chromatographic purification train sizing strategy with increasing cell culture titres. The COG/g was used as the single objective of optimisation for a biopharmaceutical company manufacturing a single mAb at commercial scale. Results from their approach suggested that for low titres, single USP and DSP trains with a single chromatography column for each step, offer the lowest COG/g values without any product loss. However, for higher titre values (>6g/L) it was presented that increased number of USP trains (bioreactors working in staggered mode) and parallel chromatography columns were necessary to avoid product loss and minimise COG/g (Liu et al., 2013). Subsequently, Liu et al. (2014) formulated a mixed-integer linear fractional programming (MILFP) model to optimise the chromatography sizing strategy along with the resin sequence and mitigate the computational cost of their MINLP approach. The authors followed an industrial relevant case study initially presented by Simaria et al. (2012) considering a single mAb instead of a portfolio of three products and discussed the advantages in computational speed of their MILFP approach.

Furthermore, Liu et al. (2015) improved upon previous work to consider more chromatography resin candidates in their optimisation approach along with continuous values for the bed-height of the chromatography columns in the purification train.

1.6. Research scope and thesis layout

The biopharmaceutical industry is a fast growing segment of the global pharmaceutical market and projections indicate the continuously increasing contribution of biopharmaceuticals in global sales. Nevertheless, the high level of uncertainty that governs the industry along with increased pressure from regulatory agencies and the necessity to commercialise affordable therapeutic drugs all create enormous challenges. The high R&D cost and time requirements combined with low success rates are driving the industry to develop cost-effective bio-manufacturing processes while demonstrating an in-depth level of product and process understanding.

The continuously increasing annual product demands for mAbs combined with improvements in cell culture titres tend to shift the manufacturing bottleneck from upstream to downstream processing. Chromatography remains the workhorse in antibody purification despite the high cost and complex process development. The complexity in the development of chromatographic separation processes stems from the large number of factors that can potentially have a significant impact on the performance of the process mainly due to the complex nature of biomolecules. Additionally, process design and optimisation should evaluate the complete bio-manufacturing process considering both operational and financial criteria. The traditional approach in chromatography process development involves extensive experimentation at small and pilot scale to gain the required level of process understanding. Additionally, it relies heavily on prior knowledge and experience of process engineers. This approach usually requires substantial amount of feed material often leading to the postponement of process development activities until late-stage clinical trials where a lot of decisions have already been made regarding the manufacturing process.

Hence, in this thesis the research focus is the development of decision-support tools to assist in chromatography process development for the purification of therapeutic antibodies. Considering some of the drivers identified above, the overall aim of this research project is to establish a systematic methodology to rationalise and accelerate resin screening, selection and optimisation at early-stage process development. Additionally, emphasis is placed on evaluating the cost-effectiveness of a chromatographic purification train using high-throughput screening data in order to determine its commercial feasibility.

Chapter 1 of the thesis discussed the development pathway of a new therapeutic drug, the associated R&D cost and the uncertainties related with R&D and commercialisation. Furthermore, key aspects in bio-manufacturing focusing on the chromatographic purification were presented and a series of different approaches in chromatography process development were discussed. Finally, alternative methodologies for the design and optimisation of chromatographic purification trains were identified.

Chapter 2 outlines materials, equipment and computer software tools that were used throughout this research. Additionally, the experimental and data analysis methodologies are presented. The integration of the fractionation diagram approach with design of experiments is presented along with a decision-support tool to assist in chromatography resin comparison and selection procedure for a single chromatography step. A robustness analysis technique is presented to identify windows of operation considering multiple attributes under uncertainty. Chapter 2 continues with the description of a process economics model developed to capture technical and financial differences between self-packed and pre-packed chromatography columns. Finally, a decision-support tool for the chromatographic purification train is presented to link high-throughput purification data with process economics modelling.

Chapter 3 demonstrates the implementation of a novel High Throughput Process Development (HTPD) workflow integrating a scale-down chromatography

experimentation strategy with an advanced chromatogram evaluation method, multivariate data analysis and decision-making and robustness modelling techniques. This new approach enables efficient evaluation of vast amounts of data generated through high-throughput experimentation, achieves a fair comparison among different chromatography resins and identifies windows of operation that can meet quality and performance criteria under uncertainty.

Chapter 4 presents a case study to investigate the economics of pre-packed chromatography columns in antibody purification as an alternative to self-packed columns. Single-Use technologies in antibody purification demonstrate significant benefits over conventional self-packed columns with the majority of the studies focusing on the operational and financial evaluation of membrane chromatography columns. On the other hand, there are limited published studies on the evaluation of pre-packed chromatography columns. Hence, a series of hypothetical scenarios are analysed to identify advantages and limitations of pre-packed columns from a process economics perspective at various stages in the drug development pathway. Stochastic simulations are performed to evaluate the uncertainty in process parameters and other model assumptions and identify their impact on key performance metrics.

Chapter 5 demonstrates an integrated framework consolidating the HTPD workflow, the process economics model and the decision-support tool for the chromatographic purification train. The objective is to enable the evaluation of the performance of different resin candidates considering the implications on the complete manufacturing process. Chapter 6 is an overview of the current validation approach in bio-manufacturing discussing elements in Quality by Design relevant to the methodologies and the tools presented in the context of this research. Finally, Chapter 7 provides concluding remarks from the whole research, outlines the overall benefits of the proposed methods and tools, discusses their current limitations and offers recommendations regarding future work.

Chapter 2. Materials and methods

2.1. Materials, laboratory equipment and software tools

All chemical reagents used to prepare chromatography buffers were provided by J.T. Baker (Avantor Performance Materials Inc., Pennsylvania, U.S.A). All weight measurements were performed using a BP 3100 S balance (Sartorius, Surrey, UK) while pH and conductivity were measured using a PHM220 Lab pH meter and a CDM230 conductivity meter (Radiometer Analytical, Colorado, U.S.A), respectively. Antibody solutions were concentrated using a tangential flow filtration system (Pall Life Science, Portsmouth, UK) with a 30kDa ultrafiltration membrane (Ultracel®, Merck Millipore, Hertfordshire, UK). High-throughput experimentation was performed using an automated liquid handling system, Freedom EVO® 200 (Tecan, Mannedorf, Switzerland). Small-scale experiments were conducted using an AKTA™ Avant 25 chromatography system (GE Healthcare Life Sciences, Uppsala, Sweden).

Samples, throughout the experimental work, were collected in 2mL Masterblock® 96 deep well plates (Greiner Bio-One, Stonehouse, UK) and total protein concentration was measured using an Xpose™ UV/VIS spectrophotometer at a wavelength of 280nm (Trinean, Ghent, Belgium). The Xpose™ system was operated manually analysing 16 samples at a time. Sample composition in terms of aggregates, product and fragments was determined using an Acquity UPLC™ system (Waters, Milford, Massachusetts) with a size exclusion chromatography column (BEH200 with 200A, D=4.6mm, L=150mm and 1.7μm bead size). The Acquity UPLC™ system can analyse eight 96-deep well plates without any intervention, with a sample volume of 5μL and a flowrate of 0.4mL/min. Feed antibody solution samples were analysed prior to the analysis of the experimentation samples to determine the initial composition of the mixture.

The automated liquid handing system was programmed, operated and monitored using the corresponding software package (Freedom EVOware®, Tecan Group Ltd., Mannedorf, Switzerland). Additional software tools that were used in data handling,

manipulation and analysis throughout the research involve: MS Excel 2010 (Microsoft) including the SOLVER optimisation tool and the data analysis tool-pack add-ins along with @RISK 6.5 (Palisade Corporation, West Drayton, UK). The SOLVER optimisation tool add-in offers three optimisation engines: a Simplex LP engine for linear problems, a GRG Nonlinear engine for non-linear and smooth problems and an Evolutionary engine for non-linear and non-smooth problems. Additionally, Visual Basic for Applications (VBA) was used to automate and accelerate different analytical procedures. Finally, JMP Pro 11 (SAS Institute Inc., Marlow, UK) was used for the design and analysis of the experiments.

2.2. Experimental and data analysis methodology

2.2.1. Design of experiments

Screening experimentation was based on definitive screening designs while for the optimisation purposes central composite designs were used. Central composite designs have been widely employed in optimisation of chromatography systems (Ferreira et al., 2007; Hibbert, 2012; Kumar et al., 2013). On the other hand, definitive screening designs were introduced in 2011 (Jones & Nachtsheim, 2011) and yet have limited published examples in bioprocessing. Jones and Nachtsheim (2011) identified limitations of resolution III and IV fractional factorial designs used for early-stage screening experimentation and proposed a new class of screening designs known as definitive screening designs.

The proposed new class of screening designs estimates main effects completely independent of two-factor interactions. Moreover, the evaluation of each factor at three levels offers the ability to estimate quadratic effects orthogonal to main effects and not completely confounded with second order interactions. Tai et al. (2015) implemented a hybrid approach combining HTE using an ambr250™ system with a 10-factor definitive screening design for the characterisation of fermentation processes for therapeutic proteins. According to Jones and Nachtsheim (2011) a definitive screening design with

10 factors results in 21 experimental runs. Tai et al. (2015) augmented that DoE space with 3 additional control runs at the centre point and reported the ability of their model to capture efficiently main and quadratic effects along with higher order interactions.

The construction and the evaluation of the DoE space were performed in JMP Pro 11 which offers a definitive screening design platform. Insights gained through screening experimentation were leveraged to develop the DoE space for the subsequent optimisation study. The resin comparison and selection methodology described in Section 2.2.3.3., determines the set-point of process parameters for each resin to meet quality and performance targets. These set-point values were used as the mid-values when designing the optimisation experiments.

Having completed the experimental runs the DoE responses were estimated in MS Excel and imported into JMP to enable the initiation of the DoE analysis. Using the Model Fit platform in JMP, a stepwise regression fitting approach was followed considering all possible main and quadratic effects and second order interactions. The stepwise regression platform in JMP can be tailored to follow a similar methodology to that demonstrated by Kumar et al. (2013) to identify the appropriate empirical model avoiding under- or over-fitting issues using a series of standard statistical criteria (e.g. Akaike information criterion (AIC), Bayesian information criterion (BIC), R^2, adjusted R^2 and p-value threshold (<0.05)).

2.2.2. Experimental set-up

For the estimation of the $DBC_{10\%}$, the flow-through volume was fractionated into fractions of equal volume (200µL) and collected in 96-deep well plates for further analysis to determine the protein concentration in each fraction. To evaluate selectivity and recovery, the elution pool was fractionated and each fraction (200µL) was analysed to determine its total protein concentration and purity. Several steps were involved in the experimental set-up to investigate the dynamic binding capacity as well as the capability for purification and recovery.

The process cycle started with equilibration of the chromatography resins at the appropriate pH and conductivity conditions. Then to evaluate the binding capacity, each resin was challenged at a high load mass. For the stepwise elution experiments, equilibration was followed by loading of the product to a specific load challenge expressed as a fraction of the maximum $DBC_{10\%}$. Product and impurities were eluted with increasing conductivity of the mobile phase, followed by a high pH – high conductivity step to clean and sanitise the chromatography resins. The same protocols were used for the purposes of subsequent steps in the HTPD workflow, to optimise the process parameters for the selected chromatography resin as well as for the model verification experiments at bench-scale. The only differences in the protocols were the volume of the fractions that was collected (300µL and 1.7mL) and the volume of the chromatography column (600µL and 4mL) for the optimisation and model verification studies at bench-scale, respectively.

2.2.3. Data analysis methodology

2.2.3.1. Dynamic Binding Capacity at 10% breakthrough

The $DBC_{10\%}$ was estimated using Eq.2.1 (Carta and Jungbauer, 2010), where $V_{10\%}$ is the cumulative flow-through volume (L) at 10% breakthrough, C_{Feed} is the protein concentration of the feed material (g/L) and V_{column} is the volume of the column (L). $DBC_{10\%}$ was calculated at every set of operating conditions as dictated by the DoE for screening and optimisation.

$$\mathrm{DBC}_{10\%}\ (\mathrm{g/L}) = \frac{\mathrm{V}_{10\%} * \mathrm{C}_{\mathrm{Feed}}}{\mathrm{V}_{\mathrm{column}}} \qquad (\mathrm{Eq.\ 2.1})$$

2.2.3.2. Integration of fractionation diagram approach and design of experiments

The execution of the experimental plan for every resin combined with the fractionation of the elution pool generated thousands of samples and hundreds of different chromatograms, posing a significant challenge in terms of sample and data analysis. To overcome the data analysis difficulties, the fractionation diagram approach was used to

rapidly analyse the resulted chromatograms (Ngiam et al., 2001 & 2003; Salisbury et at., 2006).

The fractionation diagram approach offers a quantitative relationship between purification and recovery. Any changes to the operating conditions such as pH or linear velocity can potentially lead to a shift to the relative position of the peaks of the product and its impurities in a chromatogram and hence to a different purification-recovery trade-off. It is worth highlighting that the Y axis (Eq.2.2) in the fractionation diagram expresses the amount of product (M_P) collected over the total amount of product eluted, while the X axis (Eq.2.3) displays the cumulative mass fraction of the total material (M_T) eluted. In Eq.2.2 and Eq.2.3, "F" represents the total number of fractions, "f" is the fraction index (e.g. the third fraction) and "n" is the number of fractions collected in the elution pool. Therefore, any product loss during binding and wash steps in a typical chromatography cycle operating in bind-and-elute mode is ignored. Additionally, under strong binding conditions it is possible not to be able to recover the entire product during the elution step in a cycle. This issue becomes more apparent when the fractionation diagram method is used to screen a wide range of process parameters.

$$Y = \frac{\sum_{f}^{f+n} M_{P,f}}{\sum_{f=1}^{F} M_{P,f}} \qquad \forall\, n \in \mathbb{N}, \qquad n \leq F - 1 \qquad (Eq.\ 2.2)$$

$$X = \frac{\sum_{f}^{f+n} M_{T,f}}{\sum_{f=1}^{F} M_{T,f}} \qquad \forall\, n \in \mathbb{N}, \qquad n \leq F - 1 \qquad (Eq.\ 2.3)$$

Hence, the distance between two points on the Y axis on the fractionation diagram ($Y_{f+n} - Y_f$) in this context represents $Yield_{Elution}$ instead of yield (Figure 2.1d). $Yield_{Elution}$ changes from 0% to 100% regardless of the operating conditions thus ignoring any product loss. Yield can be estimated simply as the ratio of total amount of material collected in the elution pool over the total amount of material that was loaded onto the chromatography resin (Eq.2.4). To estimate yield, the total amount of material loaded is calculated as the product of $DBC_{10\%}$ and the volume of the chromatography column. The

amount of product collected in the elution pool is estimated by multiplying the desired level of $Yield_{Elution}$ with the total amount of product eluted $\left(\sum_{f=1}^{F} M_{P,f}\right)$. At a certain value of $Yield_{Elution}$ there is a maximum purification factor, thus a maximum purity value (Figure 2.1e). Purity is estimated as the product of the purification factor and the initial feed purity (Figure 2.1f).

$$\text{Yield}\ (\%) = \frac{\text{Yield}_{\text{Elution}} * \sum_{f=1}^{F} M_{P,f}}{\text{DBC}_{10\%} * V_{\text{column}}} \qquad \text{(Eq. 2.4)}$$

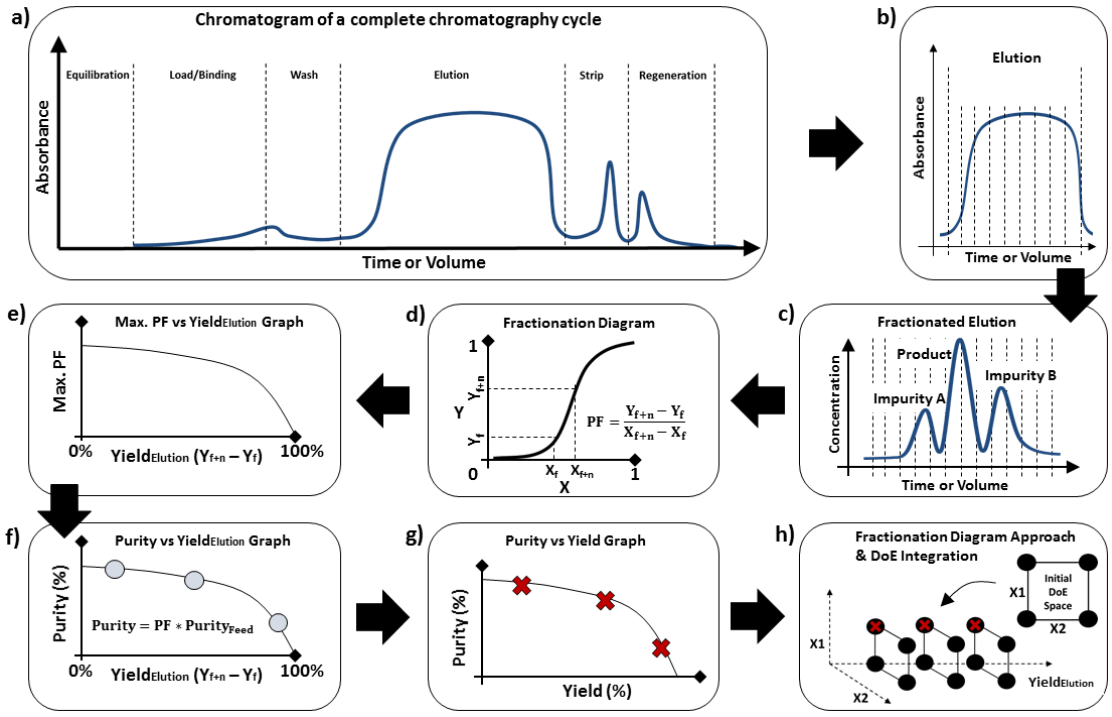


Figure 2.1: Schematic illustration of the Fractionation Diagram methodology. a) Typical chromatogram of a complete chromatography cycle, b) Elution part of a chromatogram, c) Fractionated elution chromatogram, d) Fractionation diagram plotting the cumulative product fraction against the cumulative fraction of the total protein, e) maximum purification factor against elution yield graph, f) purity against elution yield graph, g) purity against yield graph, h) schematic illustration of the DoE – Fractionation Diagram approach integration. Steps a) through f) presented as described by Ngiam et al. (2001) and steps g) & h) show the extension to the method as proposed here to account for potential product loss and integrate the method with DoE

In cases where there is no product loss $\left(\sum_{f=1}^{F} M_{P,f} = M_{P,IN} = \text{DBC}_{10\%} * V_{\text{column}}\right)$ yield would be equal to the $Yield_{Elution}$. The final step in the approach plots purity against yield (Figure 2.1g). Additionally, every purity-yield pair corresponds to a set of $Y_{f+n} - Y_f$ and $X_{f+n} - X_f$ values that define the cut-points on the respective chromatogram and thus

determine the volume of the elution pool that delivers that purity-yield pair. The difference with the method initially proposed by Ngiam et al. (2001) is that using the current terminology the final graph would be purity against $Yield_{Elution}$ (Figure 2.1f) thus potentially overestimating the recovery capabilities of a resin at a certain set of operating conditions. Using the Purity versus Yield graph (Figure 2.1g) as discussed here, a series of purity-yield pairs can be selected as dictated by the DoE space thus integrating the fractionation diagram approach with DoE (Figure 2.1h). Figure 2.1 shows a simplified example of the integration of the fractionation diagram approach with a two factor, two level DoE. The steps a) to f) in Figure 2.1 illustrate the fractionation diagram approach as described by Ngiam et al. (2001) while steps g) and h) visualise the integration of purity-yield pairs with DoE using the $Yield_{Elution}$ term as an additional factor in the DoE.

The integration of the fractionation diagram approach with DoE requires meticulous calculations to estimate the DoE responses (e.g. purity and yield). Considering also the large number of chromatograms resulted from a resin screening study the raw data manipulation becomes extremely challenging and prone to error. Nevertheless, the well-defined mathematical structure of the fractionation diagram approach allows for the development of a tool to automate the analysis. Here, for the purpose of this research, the fractionation diagram approach was programmed in MS Excel. The GRG Nonlinear engine of SOLVER (optimisation tool add-in) was called through VBA to estimate the purification factors at different $Yield_{Elution}$ values (0 – 100%). Furthermore, a procedure was written in VBA to automate the analysis of multiple chromatograms and for multiple resins.

2.2.3.3. A decision-support tool for resin comparison and selection for a single chromatography step

Statistical analysis of the DoE space for each resin candidate develops a regression model that describes each attribute (or DoE response) as a function of the process parameters (or DoE factors). Apart from screening for significant process parameters, the purpose of screening different chromatography resins is also to compare them and

identify those with the greatest performance. To standardise and automate the resin comparison and selection procedure a decision-support tool was developed in MS Excel using the SOLVER optimisation tool add-in with the evolutionary engine activated. Given the non-linear and non-smooth structure of the resin selection tool the only feasible option is the evolutionary engine. The evolutionary engine receives variable inputs and searches different combinations of them to achieve the objective function while satisfying a set of constraints. A short script was written in VBA calling SOLVER to accelerate the resin comparison and selection process. A generalised schematic of the structure of the resin selection decision-support tool is presented in Figure 2.2a.

A record of resin candidates included in the study is created. The resin selection tool identifies each resin using an index (i) assigned to it in order to recognise its associated fixed and variable parameters. The mean value (μ) of the DoE factors investigated in the screening study are used as the variable inputs in the tool. Fixed inputs include the limits of the DoE factors and the regression coefficients of model parameters describing DoE responses. Additional assumptions required for the calculation of key metrics included in the resin selection tool are considered as fixed inputs. For instance, to calculate productivity it is necessary to estimate the processing time thus assuming a column bed-height and the number of column volumes for each step in a chromatography cycle. Furthermore, threshold values are assigned for the decision attributes (e.g. purity and yield) included in the resin selection tool along with their respective weight coefficient. The weight coefficient of each attribute defines its relative importance among the other attributes and therefore, dictates its priority order in the tool. It should be noted that the user has the flexibility to define any metric as a decision attribute and determine its relative importance and its threshold value. Therefore, the resin selection tool is not restricted in using fixed decision attributes but provides the framework to the user to introduce its own metrics depending on the purification problem.

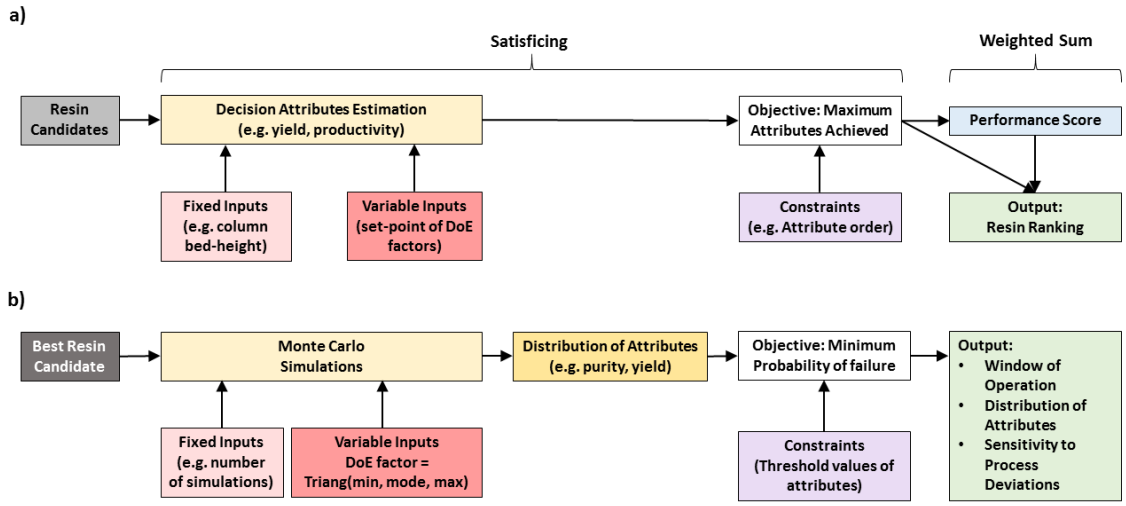


Figure 2.2: Schematic illustration of the decision-making components of the HTPD workflow. a) Structure of the resin selection tool used to screen different resin candidates and determine the best candidate, b) Robustness analysis methodology to identify a window of operation under uncertainty

The resin selection tool operates in two steps. The first step identifies decision attributes (e.g. yield, purity) and their relative significance and prioritises them in descending order. Thus the most important attribute is evaluated first while the least significant attribute is considered last. The evolutionary engine in SOLVER is searching for the set-point of process parameters (variable inputs) to achieve the threshold value for the first decision attribute. Once this is achieved the search continues with the second decision attribute while still satisfying the previous attribute. The procedure continues for all decision attributes included in the analysis with the last attribute requiring the satisfaction of all previous attributes. This approach of satisfying criteria also known as satisficing can potentially exclude resin candidates that meet a significant number of criteria but however failed to reach an important decision criterion that was ranked high in the priority order. Hence, it is crucial that the appropriate weight coefficients are assigned to the decision attributes in order to capture specific requirements from the resin candidates and avoid selecting resins that meet less significant attributes.

The evolutionary engine runs with the objective to maximise the number of decision attributes achieved in their respective priority order for each resin candidate while managing to meet the constraints. A set of constraints for each resin candidate in the

resin selection tool include the limits within process parameters (variable inputs) are allowed to vary. The lower and upper limits of variable inputs are dictated by the DoE and considered as fixed inputs. The role of the constraints is to ensure that variable inputs fall within the acceptable range and set the threshold value for each decision attribute.

A number of resin candidates could potentially achieve all decision attributes due to either relatively low threshold values or their adequate performance. Additionally, resin selection would be difficult when resin candidates perform very similarly. To address these issues a second step in the resin selection tool is used to assign a score to each resin candidate that successfully meets all decision attributes. A performance score is estimated for each successful resin candidate using the weighted sum method combining all the attributes used in the first step of the tool. In Eq.2.5, w_j is the weight coefficient of each attribute (j), "J" is the total number of attributes and $K_{i,j}$ is the normalised value of attribute (j) for resin (i). In case an attribute needs to be maximised in Eq.2.5.1 the worst and best normalised values correspond to the minimum and maximum values of the attribute, respectively. On the other hand, in case an attribute needs to be minimised, worst and best refer to the maximum and minimum value, respectively. The term $k_{i,j}$ in Eq.2.5.1 represents the actual value achieved of attribute (j) for resin (i). Finally the sum of the weight coefficients should be equal to the unit.

$$\text{Performance Score}_i = \sum_{j=1}^{J} w_j * K_{i,j} \qquad \text{(Eq. 2.5)}$$

where:

$$K_{i,j} = \frac{k_{i,j} - k_{i,j\ \text{worst}}}{k_{i,j\ \text{best}} - k_{i,j\ \text{worst}}} \qquad \text{(Eq. 2.5.1)}$$

$$\sum_{j=1}^{J} w_j = 1 \qquad \text{(Eq. 2.5.2)}$$

The final decision regarding the selection of the most suitable resin candidate(s) is performed by identifying the candidate that reached the highest number of decision attributes and achieved the maximum performance score, in this order. Hence, the comparison based on the performance score is only taking place when more than one resin candidates achieve the same maximum number of decision criteria.

2.2.3.4. Window of operation and robustness analysis

The resin selection tool suggests the most suitable resin candidate(s) and also provides the set-point values for the process parameters included in the regression model that describes the factors-response relationship. These set-point values were used as the centre point in the subsequent central composite designs to optimise process parameters and identify a window of operation. Response surface analysis of the DoE space as constructed by the central composite designs promoted the development of set of MLR equations that describe the relationship between factors and responses. The appropriate MLR model for each response is identified using the stepwise regression approach through the Model Fit platform in JMP with a minimum BIC model selection criterion, backward elimination and a p-value threshold of 0.05.

To identify a window of operation that satisfies a number of quality and performance attributes a stochastic simulations engine was built in MS Excel (Figure 2.2b). Each process parameter was expressed using a triangular distribution defined by its mode, minimum and maximum values representing the set-point and its limits. The resulting probabilistic process parameters were then used to estimate each DoE response (e.g. purity, yield) using the MLR correlations developed through the optimisation study for the selected resin candidate. A series of Monte Carlo simulations were performed by varying the set-point and its deviation for each process parameter and deriving distributions for each DoE response.

An optimisation was set up to determine the optimal set-point and range that minimised the objective function. This was achieved using the evolutionary engine provided by the

SOLVER optimisation tool add-in in MS Excel. It should be noted that although the smoothness of the problem allows for the use of the GRG Nonlinear engine in SOLVER, the evolutionary engine was much faster and easier to tune for the given optimisation problem. SOLVER was set to receive the set-point and its deviation for each process parameter (DoE factors) as decision variables. The objective function was defined as minimisation of the probability of process failure. In this context process failure was considered the probability of failing to meet the threshold value for any of the quality and performance attributes. For instance, assuming two attributes: purity and yield the probability of process failure is defined as (Ross, 2009):

$$\mathrm{P(fail)} \equiv \mathrm{P(A \cup B)} = \mathrm{P(A)} + \mathrm{P(B)} - \mathrm{P(A \cap B)}$$

where:

$$\mathrm{P(A)} = \frac{\sum_1^{\mathrm{N}} \mathrm{E(A)}}{\mathrm{N}}, \qquad \mathrm{P(B)} = \frac{\sum_1^{\mathrm{N}} \mathrm{E(B)}}{\mathrm{N}}$$

E(A): the event where purity fails to meet the threshold purity value

E(B): the event where yield fails to meet the threshold yield value

P(A): the probability of failing to meet the threshold purity value

P(B): the probability of failing to meet the threshold yield value

P(A∩B): the probability of failing both purity and yield threshold values

N: Total number of simulations (sample space)

A potential window of operation is defined by a set-point value and an acceptable range for each process parameter (DoE factor) in order to minimise the probability of process failure. The robustness of the optimal solution as identified by SOLVER was further analysed by examining its associated Monte Carlo simulation dataset to determine the parameters with the greatest impact on each attribute and visualise its distribution using @RISK.

2.2.4. Bench-scale model verification

To verify the predictive ability of the model, targeted experimentation within the suggested window of operation was performed at bench-scale using a self-packed chromatography column with a bed-height of 20cm and a diameter of 0.5cm. The sequence and configuration of the chromatography cycle was similar to that used at micro-scale with the only difference being the gradient elution profile. Experimentation related to the HTPD workflow was conducted with a stepwise gradient elution profile due to limitations related with the liquid handling system. On the other hand, experimentation at bench-scale was performed by applying a linear conductivity gradient profile so as to enable a more accurate determination of the conductivity range where product is collected in the elution pool. Experimentally achieved values (or actual values) were plotted against predicted values for each attribute to visualise the accuracy of the predictions. Additionally, statistical tests (F-test and t-test) were performed to address the hypothesis that actual and predicted values are all from the same distribution comparing the mean and standard deviation for each dataset.

2.3. A process economics model for antibody bio-manufacturing facilities

2.3.1. Process economics model structure

A process economics spreadsheet model was developed in MS Excel based on previous work conducted at the Advanced Centre for Biochemical Engineering at University College London (Farid et al., 2007; Simaria et al., 2012). A schematic of the information flow in the process economics model is presented in Figure 2.3.

The model receives a series of inputs form three different sources. The first source is the user which needs to build a scenario for evaluation by determining model parameters such as the annual product demand and cell culture titre. The second source is a list of assumptions related to the operation of different process steps and the facility. Additional, assumptions include process economic factors used to estimate manufacturing costs that are not directly captured by the model. For instance, the cost of equipment maintenance can be estimated based on the fixed capital investment. The

third and final source of inputs is a cost database with process equipment sizes and prices. Moreover, a list of chemical reagents and consumables (e.g. filters) is included in the database. The default values used in the cost database and the model assumptions can be found in the Appendix (Table A.1 and Table A.2).

The user has the option to change any of the values in the list of assumptions and the cost database. Throughout this research a number of different assumptions were challenged. In order to avoid extensive user intervention into the model, VBA was used to automate and accelerate iterative procedures. As soon as the user has determined the required scenario inputs the model performs a series of mass balance and equipment sizing calculations across all unit operations. Individual unit operations are sized based on their performance and the product load that has to be processed per batch. A more detailed description of the model calculations is discussed in the next Section of this chapter (Section 2.3.2.) and process equipment sizing formulas can be found in the Appendix (Table A.3).

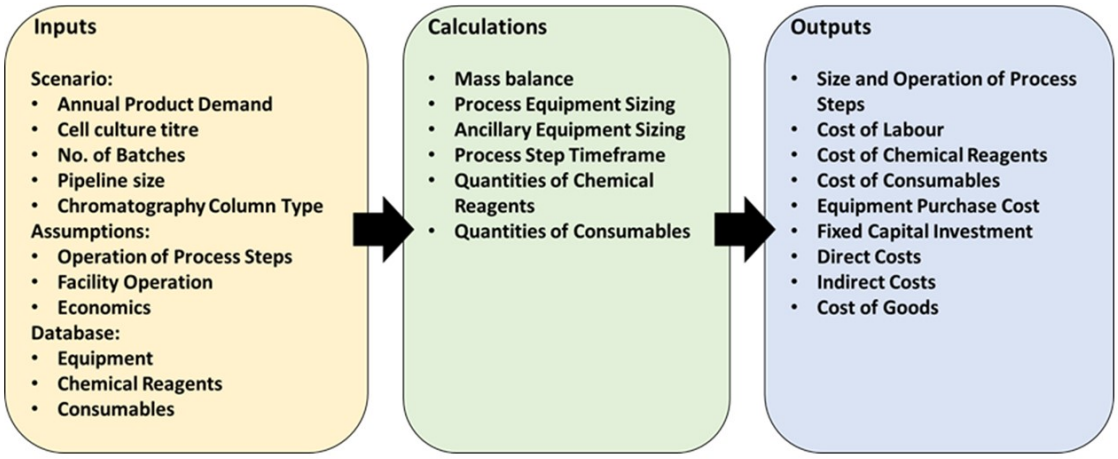


Figure 2.3: Schematic of the information flow in the spreadsheet process economics model developed to evaluate the economics of pre-packed chromatography columns

The output of the process economics model is a record containing the results regarding the size, the duration and the cost of each unit operation as well as a breakdown of the cost of goods. A level of flexibility in synthesizing the desired process sequence is offered focusing on the purification train. For each chromatography step (capture, intermediate

purification and polishing) the model offers two options: self-packed (SP) or pre-packed (PP) columns.

2.3.2. Design and process economics calculations in the model

The main objectives of the process economics model developed here is the evaluation of pre-packed chromatography columns as an alternative to self-packed columns and the integration of high-throughput purification data with process economics. Hence, a relative simplistic approach was followed for sizing of the unit operations in the process sequence. The equipment sizing calculations begin by identifying the working volume of the production bioreactor using the annual product demand, the cell culture titre, the total yield of downstream processing and the number of batches.

The number of seed bioreactors required and their respective working volume is estimated based on the inoculation ratio. For the primary recovery it is assumed that a disc-stack centrifuge is used to harvest the cell culture broth and separate liquid from solid phase followed by a series of depth filters for further clarification of the supernatant. For the purposes of this research it is assumed that the performance of the primary recovery steps is satisfactory regardless of the cell culture titre and the solids load. The size of the centrifuge is estimated through the working volume of the bioreactor and the desired time window for processing that volume. The total depth filtration area is calculated by dividing the volume of the supernatant by the capacity of the depth filter.

The next step in the bio-manufacturing process sequence traditionally is a chromatography step for which design calculations are based on the mass load the unit operation has to process per batch. For any given set of: number of columns in parallel, number of cycles per batch, bed-height and dynamic binding capacity the process economics model estimates the required column diameter. The available (actual) diameters for self-packed and pre-packed chromatography columns are stored in the database used in the model. Then the model identifies the actual diameter closest to the required diameter to process a given mass load and recalculates the number of cycles

to satisfy mass balance based on the actual diameter. Additionally, it might be necessary to tune the bed-height within an acceptable range to correct for the new number of cycles and diameter. The algorithm that was used to size chromatography columns, either self-packed or pre-packed, can be visualised in Figure A.1, of the Appendix.

Inactivation of potential enveloped viruses takes place at low pH in a stirred tank that receives the total eluate volume from the first chromatography step. It is common practice in the mAb purification field to have the low pH virus inactivation step after the capture chromatography step, which involves typically addition of acid after the low pH elution step. The required working volume of the low pH inactivation tank is estimated by adding the volume of the acidic and the neutralisation buffers to the total Protein A eluate volume. The final antibody solution is then purified by one or two sequential polishing chromatography steps sized by applying the same design methodology as described for the first chromatography step. It should be highlighted that in case a chromatography step was operated in flow-through mode the size of the column was estimated considering the loading capacity rather than the binding capacity since the impurities bind to the resin and the product flows through.

The last two steps in antibody manufacture is a virus filtration step followed by the final concentration and formulation step. For the virus filtration step the total required membrane area is estimated based on the mass load per batch for the unit operation and the capacity of the membrane. To calculate the required total area of the ultrafiltration membrane the assumption is made that the product can be concentrated and exchange buffers in a two-step operation. This assumption holds when the required concentration factor and the hold-up volume of the UFDF system are low enough. When these assumptions fail a four-step procedure is implemented where the antibody solution is first concentrated to an intermediate level, then buffer exchange takes place followed by an over-concentration step above the desired final concentration. The final step in the procedure would involve the recovery of the hold-up volume of the system to retrieve any product left in the system and maximise yield (Rao et al., 2012). For the purpose of this

study the first approach is followed assuming a low hold-up volume and a maximum concentration factor of 10 to achieve the desired final concentration.

The process economics model considers additionally support equipment used throughout the bio-manufacturing process such as stainless steel hold-tanks for the cell culture media, buffers and the product. For instance, a chromatography step operating in bind-and-elute mode typically requires five different buffer solutions for equilibration, wash, elution, strip and regeneration of a chromatography resin plus a hold-tank for the eluted product. The working volumes for all these hold-tanks can be estimated based on assumptions regarding the number of column volumes needed and the calculated chromatography column volume provided by the process economics model.

Through mass balance and design calculations for each unit operation the volume of the product and all related buffers for each process step is estimated thus providing the size and the number of the necessary hold-tanks. The requirements for cleaning-in-place (CIP) buffer, water for injection (WFI) and process water (PW) are estimated assuming a diameter to height ratio for the hold-tanks, a CIP buffer flowrate per diameter and a duration for the WFI and PW rinse steps. To estimate the cost of each hold-tank the 6/10 approach was used (Peters & Timmerhaus, 1991).

The term COG is defined as the sum of direct and indirect costs associated with manufacturing. A COG breakdown is presented in Table 2.5. Direct costs account for the cost of staff and materials. Materials include consumables and chemical reagents used for different buffers and media. The cost of the staff was estimated based on the utilisation of the staff resources rather than an annual-salary per staff (Farid et al., 2007). The annual cost of direct labour is calculated as the product of the number of operators, an hourly wage per operator, the number of batches and the batch time required for each unit operation. The cost of additional personnel for supervision, management and QCQA was estimated based on the direct labour cost (Farid et al., 2007; Simaria et al., 2012).

The total cost of labour is the sum of all costs related to the staff involved in manufacturing.

The cost of chemical reagents involves the cost for WFI, PW and chemical reagents for buffers and media. Consumables include chromatography resins, pre-packed chromatography columns and filters/membranes (depth filters, virus filtration membranes, UFDF membranes). Other consumables include 0.45μm guard filters for the hold-tanks. The cost of these guard filters was estimated based on the 6/10 rule. Moreover, an additional cost to account for miscellaneous materials such as QCQA consumables and chemical reagents and personal protective equipment (PPE) is estimated through the direct cost of chemical reagents and consumables.

Indirect costs include the depreciation of the fixed capital investment (FCI), the cost of general utilities and the cost to maintain and insure the facility as well as local taxes (Farid et al., 2007; Simaria et al., 2012). The FCI is estimated based on the Lang factorial method by multiplying the total cost of purchased equipment with a number corresponding to the type of the facility of interest (Eq.2.6). Novais et al. (2001) suggested a Lang factor of 8.13 for conventional (fixed, stainless steel equipment) bio-manufacturing facilities. The depreciation of the FCI is calculated assuming a 10-year lifespan for the fixed equipment installed in the facility.

The term general utilities is used to include the cost of monitoring and running a manufacturing facility. For instance, general utilities would account for HVAC systems used to maintain the clean status required in different rooms in the facility and is expressed in monetary units per square meter of the facility (Farid et al., 2007). Farid (2001) reported after discussion with industrial experts a value of 300 £/m^2. Using the Chemical Engineering index for the years 2000 (CE_{2000} = 394.1) and 2016 (CE_{2016} = 541.7) it can be estimated an updated value of approximately 400 £/m^2. In order to calculate the cost of general utilities it is required to estimate the size of the facility.

George (2008) suggested a function after analysing data on different facility sizes and their corresponding FCI to estimate the size of a facility based on its FCI (Eq.2.7). Additionally, the annual cost to maintain and insure the facility and cover obligations regarding local taxes related to the facility is estimated based on the FCI. Indirect cost or overheads will also occur regardless of the operational status of the facility. In case of multiproduct facilities these overheads can be distributed across all products (n_P) manufactured annually. As mentioned, the COG is the sum of direct and indirect cost associated with manufacturing and the value of COG/g is estimated by dividing the COG with the annual product demand.

Table 2.1: A breakdown of the cost of goods for a bio-manufacturing facility

Cost Category	Sub-category	Equation
Direct	Chemical Reagents	f(utilisation)
	Consumables	f(utilisation)
	Miscellaneous Materials	$0.1 * (\text{Reagents} + \text{Consumables})$
	Direct Labour	f(utilisation)
	Supervisors	$0.2 * \text{Direct Labour}$
	QCQA	$1 * \text{Direct Labour}$
	Management	$1 * \text{Direct Labour}$
Indirect	Depreciation	$0.1 * \text{FCI}$
	Maintenance	$0.1 * \text{FCI}$
	Insurance	$0.01 * \text{FCI}$
	Local Taxes	$0.02 * \text{FCI}$
	General Utilities	$400\$/\text{m}^2 * \text{Facility Footprint } (\text{m}^2)$
COG		$\text{Direct} + \text{Indirect/pipeline}$
COG/g		$\frac{\text{COG}}{\text{Annual Product Demand}}$

$$\text{FCI} = \text{Lang Factor} * \sum_{\substack{\text{Unit} \\ \text{Operations}}} (\text{Process \& Ancillary Equipment cost}) \qquad (\text{Eq. 2.6})$$

$$\text{Facility Footprint } (\text{m}^2) = 0.093 * 1730 * \left(\frac{\text{FCI}}{10^6}\right)^{0.9136} \quad \text{(Eq. 2.7)}$$

2.4. A decision-support tool for the chromatographic purification train

2.4.1. Structure and functionality of the tool

To provide a greater flexibility and detail in the design of the chromatographic purification train a decision-support tool was developed in MS Excel as an extension on the process economics model (Figure 2.4). The tool receives the target product profile as a user specified input. Additionally, the tool offers to the user several options in the design of a purification train. For instance, the user can specify the mode of operation and type of chromatography resin for each step or allow for any mode and type to be used at any step. A restriction that the tool sets by default is the orthogonality in resin selection, constraining any type of resin to be used only once in the purification train. The last input the tool receives is a list of chromatography resins with their respective properties and purification performance. That resin list acts as a library that the tool can refer to in order to iterate through different purification trains.

Each resin candidate in the library is assigned an index (i) used by the tool to recognise it. Key information each resin candidate should provide include the dynamic binding capacity, yield, maximum linear velocity, resin lifespan and price as well as the ability to separate a product from its impurities. Moreover, essential information would be the position in the purification train at which a resin can provide the given performance for a given product. Due to the structure of the tool it is possible to evaluate the same resin multiple times using different values for its purification performance depending on the conditions. For instance, with reference to the fractionation diagram approach a number of purity-yield pairs can be selected and added to the resin list as alternative performances of the same resin. Although this approach could increase substantially the computational cost it offers the ability to evaluate in more detail a resin by leveraging experimental data.

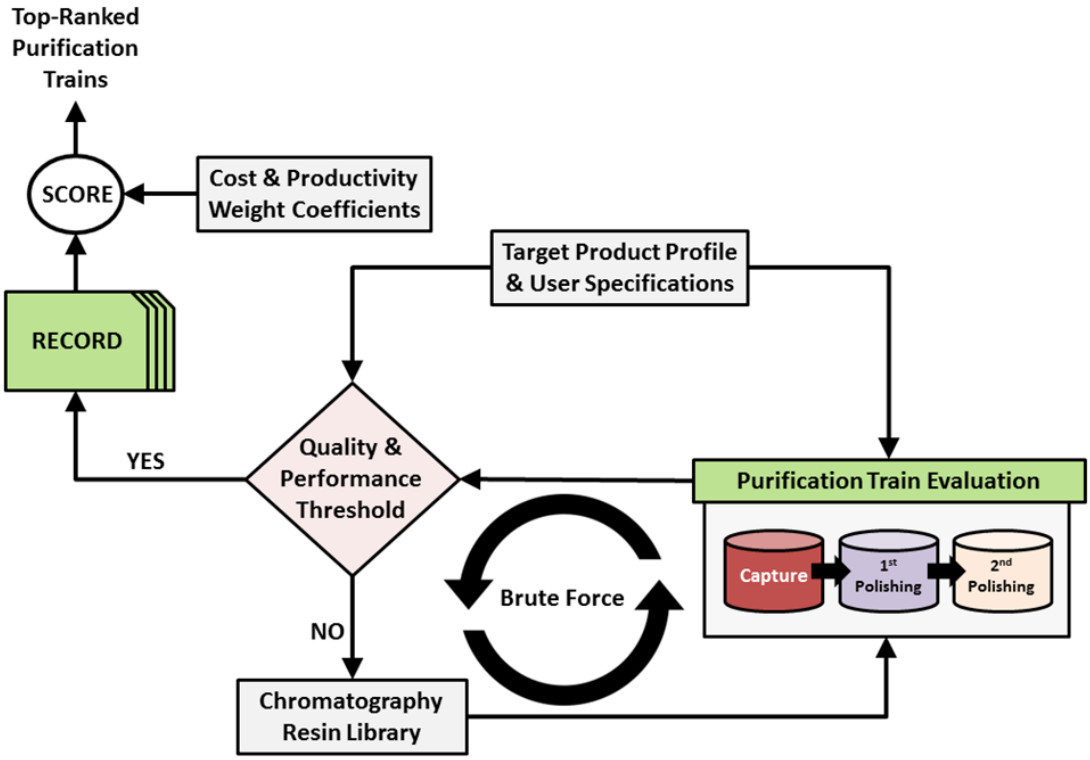


Figure 2.4. A schematic illustration of the structure of a decision-support tool for the chromatographic purification train

Having defined the inputs, the tool is using a brute force search algorithm written and executed in VBA to search among all possible resin sequences and identify those that meet all the specifications defined by the user. The successful purification trains are then recorded and compared with each other using the weighted-sum method considering two attributes with opposite objectives. The score function is given in Eq.2.5. The weight coefficients in the score function (Eq.2.5.2) are also user specified to assign the desired level of significance to each attribute. The tool offers the flexibility to the user to define the appropriate attributes to compare the performance of different purification trains. Additionally, metrics that are calculated and considered as restrictions in the tool include the yield of the purification train (Eq.2.8) along with the reduction capabilities for HCPs (Eq.2.9), HMW (Eq.2.10) and LMW species (Eq.2.11). In equations Eq.2.8 – 2.11, "i" indicates a resin in a purification train of three chromatography steps, LRV stands for the logarithmic reduction value and R_{HMW}/R_{LMW} indicate the percentage removal of HMW/LMW species.

$$\text{Train Yield} = \prod_{i=1}^{3} \text{Yield}_i \geq \text{Target train yield} \quad \text{(Eq. 2.8)}$$

$$\text{HCPs}_{OUT} = \text{HCPs}_{IN} * 10^{-\sum_{i=1}^{3} \text{LRV}_i} \leq \text{HCPs}_{Target} \quad \text{(Eq. 2.9)}$$

$$\text{HMW}_{OUT} = \text{HMW}_{IN} * \prod_{i=1}^{3} (1 - \text{R}_{HMW})_i \leq \text{HMW}_{Target} \quad \text{(Eq. 2.10)}$$

$$\text{LMW}_{OUT} = \text{LMW}_{IN} * \prod_{i=1}^{3} (1 - \text{R}_{LMW})_i \leq \text{LMW}_{Target} \quad \text{(Eq. 2.11)}$$

The overall outcome of the tool is a list of descending score order with all the purification trains that met the target product profile and all the user specified restrictions. Then the process economics model offers the option to import a subset of the successful purification trains and evaluate the complete manufacturing process for each of them. Information related to each chromatography resin in all suggested purification trains is received by the process economics model to adjust its assumptions and database and re-synthesise the process sequence in order to reflect the changes from the default purification train.

Chapter 3. High throughput process development workflow with advanced decision-support for antibody purification

3.1. Introduction

The development of a chromatographic purification process in bio-manufacturing is highly challenging and complex. The large number of parameters that could potentially have a significant impact on the chromatographic separation performance requires extensive experimentation and usually a substantial amount of clarified material in order to optimise the operating conditions (Coffman et al., 2008). An additional consideration especially at early-stage process development could be the number of different drug candidates under development along with the uncertainty around their commercialisation that could limit even further allocated resources and shorten the available development timeline.

Due to the complex nature of mAbs and their impurities, a sequence of chromatography steps is typically designed and developed to achieve the desired level of product quality. For each step in the purification train there are several commercially available options regarding the selection of a chromatography resin. A crucial decision at early-stage process development is the selection of the appropriate chromatography resin for each step in the correct order in the purification train. Resin selection is a rather complex task considering different resins with a wide operating range and number of process parameters for screening. An additional complication might be that different resins can be operated under different conditions thus intensifying substantially laboratory activities for experimentation (e.g. buffer preparation). The impetus for the development of a systematic methodology to address the challenges involved in optimal chromatography resin selection was discussed by Rathore (2001). Although the concept of a rational and systematic resin selection was highlighted and demonstrated for an AEX chromatography step, there were still unresolved issues mainly around the experimental design.

High-throughput experimentation (HTE) has emerged as a powerful technique to accelerate process development with relatively small amounts of feedstock material (Chhatre & Titchener-Hooker, 2008). The integration of HTE with other methods such as DoE and multi-variate data analysis has led to the establishment of hybrid approaches in the development and optimisation of chromatographic separation processes (Susanto et al., 2009; Bhambure & Rathore, 2013; Traylor et al., 2014). Previous studies have focused mainly on individual techniques with limited examples of complete workflows integrating scale-down experimentation with DoE, MVDA and decision-making tools. In addition, a further challenge often associated with HTE is how best to leverage the large amounts of raw data generated and to evaluate trade-offs in the datasets to make decisions on optimal strategies. The work here focuses on the establishment of a high-throughput process development (HTPD) workflow that combines a variety of different methods in a semi-automated manner so as to streamline screening of different cation exchange (CEX) chromatography resins and optimisation of process parameters under uncertainty.

The overall aim of this research is to identify the appropriate combination of experimental approaches and decision-support techniques, in order to provide a consistent methodology for rapid generation and analysis of chromatographic purification data. A single chromatography step is investigated rather than the complete purification train in order to provide a detailed investigation of process parameters that could impact the performance of each resin candidate. The proposed HTPD workflow suggests the use of miniature pre-packed chromatography columns coupled on an automated liquid handling system, for the rapid generation of purification data. Additionally, the integration of DoE with the fractionation diagram method (Ngiam et al., 2001; Ngiam et al., 2003; Salisbury et al., 2006) was introduced to cope with the vast amounts of data generated through HTE. Furthermore, a combination of multivariate data analysis, multi-criteria decision-making and robustness analysis was established to define a potential window

of operation for the chromatographic purification of a highly aggregated antibody solution.

The chapter is structured as follows. Section 3.2 focuses on describing the individual experimental, data analysis and decision-making methods used in the HTPD workflow. An example of implementing the HTPD workflow is demonstrated in Section 3.3 to facilitate resin screening and process parameter optimisation for bind-and-elute ion-exchange chromatography used for intermediate antibody purification.

3.2. Materials and methods

A case study was formulated in collaboration with MedImmune to demonstrate the HTPD workflow, using a bispecific monoclonal antibody with high aggregate concentration post Protein A chromatography. The bispecific antibody was produced in Chinese hamster ovary (CHO) cells and cell culture material was clarified and partially purified by MedImmune. The cell culture broth was clarified by a series of depth filters and the clarified material was further processed using a Protein A chromatography column followed by a low pH hold to inactivate any enveloped viruses. The preparation of the feedstock material for the investigation of the CEX chromatography step was completed with the neutralisation of the low pH antibody solution. Miniature chromatography columns, pre-packed with 200µL and 600µL of resin (Atoll GmbH, Weingarten, Germany) were used in the screening study. Eight CEX resins were included: Poros™ XS and Poros™ HS50 (Life Technologies, California, U.S.A), Toyopearl® GigaCap S-650(S) and Toyopearl® GigaCap CM-650(M) (Tosoh Biosciences, Redditch UK), Capto™ S Impact (GE Healthcare Life Sciences, Buckinghamshire, UK), Fractogel® EMD COO^- (M) and Eshmuno® CPX (EMD Merck Millipore, Darmstadt, Germany) and UNOsphere™ Rapid S (Bio-Rad Laboratories, California, U.S.A).

The main steps involved in the HTPD workflow are outlined in Figure 3.1. The design and execution cycle is the central component of the workflow that combines DoE, the fractionation diagram method, high-throughput experimentation and multivariate data

analysis. A key output of the design and execution cycle is the regression models (i.e. binding and elution models) which can be imported into decision-support tools for further evaluation. Starting with a screening study and regression models are used in the resin selection tool to determine the best resin candidate. On the other hand, an optimisation study leverages binding and elution regression models using a robustness analysis tool to identify a window of operation under uncertainty in process parameters. Experimentation is performed using miniature pre-packed chromatography columns of 200µL and 600µL for screening and optimisation studies, respectively.

It is essential to gather relevant information regarding the product and the process before proceeding into any experimental work. For instance, information on previously tested CEX chromatography resins, potentially significant process parameters and ranges, level and composition of impurities as well as properties related to the molecule under investigation could all provide critical insights and hence promote and improve the design of the experiments. After discussion with the purification process development team and the industrial supervisors of the project in MedImmune (Granta Park, Cambridge, UK), eight CEX chromatography resins (as named above) were chosen. Due to the high aggregate concentration (~20%) of the antibody solution post Protein A chromatography, the primary consideration was the identification of resin candidates that could remove a sufficient amount of high molecular weight (HMW) species.

Additional information required is the mode of operation of the selected resin candidates. Given the high isoelectric point (>8.5) of the particular bispecific mAb, CEX chromatography resins were operated in bind-and-elute mode. A typical chromatography cycle operating in bind-and-elute mode involves six main steps: equilibration, load, wash, elution, strip and resin regeneration. The two steps investigated here were the load (or binding) step and the elution step. The operation conditions for resin equilibration and wash were dictated by the operating conditions for binding and elution, respectively, while a universal protocol was used to strip and regenerate all CEX resins. Binding and elution conditions were evaluated through two different sets of DoE.

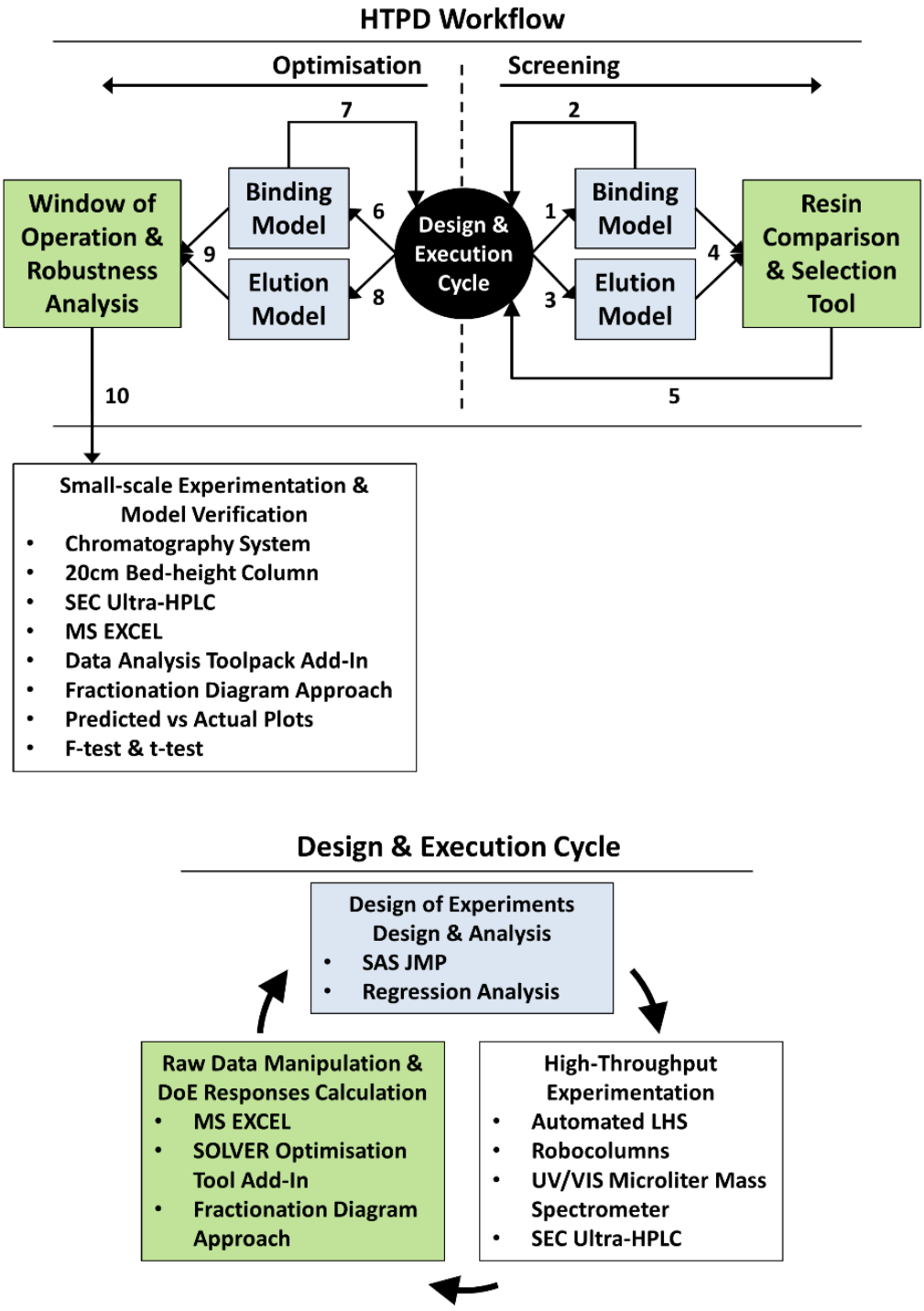


Figure 3.1: Schematic illustration of the proposed High-Throughput Process Development workflow for a chromatography operation in bind-and-elute mode. The Design & Execution Cycle consist the central component of the workflow starting with the design of the DoE space. High-throughput experimentation follows to investigate the suggested DoE space. After sample analysis, DoE responses are estimated and imported into the DoE table to initiate regression analysis. Completion of the cycle results in regression models estimating DoE responses as functions of DoE factors. Finally the regression models are imported into the decision-making components of the workflow allowing for the screening and selection of the best chromatography resin candidate and optimise its operating conditions under uncertainty

3.3. Results and discussion

3.3.1. Design of experiments

The HTPD workflow starts with the construction of the DoE space to investigate the binding conditions for each CEX resin. A definitive screening design was created in JMP with four continuous factors (process parameters) and a single response (Table 3.1). The conditions of the mobile phase (pH and conductivity), the linear velocity and the concentration of the feed were the four factors considered in the DoE with $DBC_{10\%}$ as the only response to be maximised. Linear velocity was adjusted in order to keep the residence time between two scales constant. At micro-scale the bed-height was 1cm and 3cm for a 200μL and a 600μL miniature chromatography column, respectively while a typical bed-height at pilot- and large-scale would be around 20cm. Thus operating a 20cm bed-height column at a linear velocity of 300cm/h would result in a residence time of 4min. The corresponding linear velocity at micro-scale can be estimated by keeping constant the residence time. For this example, using a 600μL column the linear velocity would be 45cm/h.

The definitive screening design platform in JMP Pro 11 created a design of nine runs with four factors to screen in order to maximise the $DBC_{10\%}$ for each CEX resin. Breakthrough curves resulted from the DoE runs were analysed in MS Excel to calculate the $DBC_{10\%}$ at each set of operating conditions and for each CEX resin. $DBC_{10\%}$ values were imported in JMP and through a stepwise regression analysis, a MLR regression model was developed estimating $DBC_{10\%}$ as a function of the DoE factors and for each CEX resin. Each model demonstrated an R^2 and adjusted R^2 value above 80% and a p-value less than 0.05 through analysis of variance (ANOVA) indicating that variability of the response from its mean value is not due to chance.

A set of operating conditions that seemed to favour every CEX resin was identified in order to simplify subsequent experimentation to evaluate the elution operating conditions. Three process parameters were selected as the DoE factors (elution pH,

elution linear velocity and load challenge) in the elution definitive screening design created to screen for maximum purity and yield for each CEX resin. The conductivity gradient profile of the mobile phase during elution was kept the same throughout the experimental runs and for all CEX resins. A stepwise elution profile over 20 column volumes (CVs) from 0mM to 450mM with a 50mM step of sodium chloride (NaCl) was applied.

Table 3.1: Design of Experiments used in the HTPD workflow for screening and optimisation of CEX chromatographic separation for a bispecific antibody

DoE	Binding	Elution
Definitive Screening Design **8 CEX Chromatography Resins** **9+1 Experimental Runs / Resin** **200µL Robocolumns**	**Factors (Range)** • Load pH (4.5 – 7.5) • Load Conductivity (0 – 150mM) • Load Linear Velocity (300 – 500cm/hr) • Feed Concentration (4 – 12g/L) **Responses (Objective)** • $DBC_{10\%}$ (Maximise)	**Factors (Range)** • Elution pH (5.0 – 7.0) • Elution Linear Velocity (300 – 500cm/hr) • Load Challenge (30 – 90g/L) • $Yield_{Elution}$ (50 – 100%) **Responses (Objective)** • Purity (Maximise) • Yield (Maximise)
Central Composite Design **1 CEX Chromatography Resins** **17+1 Experimental Runs** **600µL Robocolumns**	**Factors (Range)** • Load pH (4.5 – 5.5) • Load Conductivity (0 – 75mM) • Load Linear Velocity (350 – 500cm/hr) **Responses (Objective)** • $DBC_{10\%}$ (Maximise)	**Factors (Range)** • Elution pH (4.5 – 5.5) • Elution Linear Velocity (300 – 500cm/hr) • Load Challenge (40 – 120g/L of resin) • $Yield_{Elution}$ (50 – 100%) **Responses (Objective)** • Purity (Maximise) • Yield (Maximise) • Elution Pool Volume (Minimise)

The term load challenge expresses the binding capacity of a CEX resin as a fraction of its $DBC_{10\%}$. Additionally, the product recovery term, $Yield_{Elution}$, was considered as the fourth factor in the elution definitive screening design as discussed in Chapter 2, Section 2.2.3.2., in order to achieve the integration of DoE and the fractionation diagram approach. Hence the resulted DoE space consisted of four factors leading to nine experimental runs investigating two responses. Two CEX resins demonstrated the lowest $DBC_{10\%}$ and therefore load challenge was not considered as a factor in their elution definitive screening design. The evaluation of all three DoE responses ($DBC_{10\%}$, purity and yield) was achieved using the resin selection tool.

3.3.2. Resin comparison and selection

The structure and the main components of the resin selection tool are illustrated in Figure 2.2. Additionally, Table 3.2 provides a summary of its main components used in the case study. To initiate the comparison and selection protocol it is necessary to define the desired decision attributes along with their relative importance. As discussed above, due to the high concentration of HMW species it was crucial to identify CEX resin candidates that had high HMW removal capabilities (over 70%). Therefore, HMW removal (Eq.3.1) was selected as the first decision attribute and it was assigned the maximum factor of significance (significance = 1). High HMW removal may result in product loss in cases with poor selectivity and peak resolution; thus, a second metric was introduced to evaluate the purity. For the purposes of the screening study it is equally important to identify the best resin candidate and determine how the rest of the candidates compare. Therefore, instead of comparing the resin candidates using the purity of the bispecific antibody a different metric was introduced to capture the change in purity relative to the theoretical maximum improvement. Equation 3.2 describes that change in purity which was given a significance of 0.7. The third decision attribute considered was the step yield (Eq.2.4) followed by productivity (Eq.3.3) assigned a relative significance of 0.5 and 0.3, respectively. The determination of the factor of significance for each decision attribute was achieved empirically after discussions with members of the purification team at

MedImmune in Cambridge, UK. Using the factors of significance, the weight coefficient for each decision attribute can be calculated as demonstrated in the Appendix

$$\text{HMW Removal (\%)} = 1 - \left(\frac{\text{HMW}_{\text{Elution pool}}}{\text{HMW}_{\text{Feed}}}\right) \quad \text{(Eq. 3.1)}$$

$$\text{Purity Change (\%)} = \frac{\text{Purity}_{\text{Elution pool}} - \text{Purity}_{\text{Feed}}}{1 - \text{Purity}_{\text{Feed}}} \quad \text{(Eq. 3.2)}$$

$$\text{Productivity (g/L/hr)} = \frac{\text{LC} * \text{Yield}}{\text{t}_{\text{CYC}}} \quad \text{(Eq. 3.3)}$$

where:

$$\text{t}_{\text{CYC}}\ \text{(hours)} = \left(\frac{\frac{\text{LC}}{\text{C}_{\text{Feed}}} + \text{CV}_{\text{Equilibration}}}{\text{u}_{\text{Load}}} + \frac{\text{CV}_{\text{Wash}} + \text{CV}_{\text{Elution}}}{\text{u}_{\text{Elution}}} + \frac{\text{CV}_{\text{Strip \& Regen.}}}{\text{u}_{\text{Strip \& Regen.}}}\right) * \text{H}$$

$\text{HMW}_{\text{Elution pool}}$: Mass of HMW species collected in the elution pool (g)

HMW_{Feed}: Initial mass content of HMW species in the feed stream (g)

t_{CYC}: Processing time for a single chromatography cycle (hours)

LC: Load challenge (g/L of resin)

u: Linear velocity (cm/h)

CV: Number of column volumes

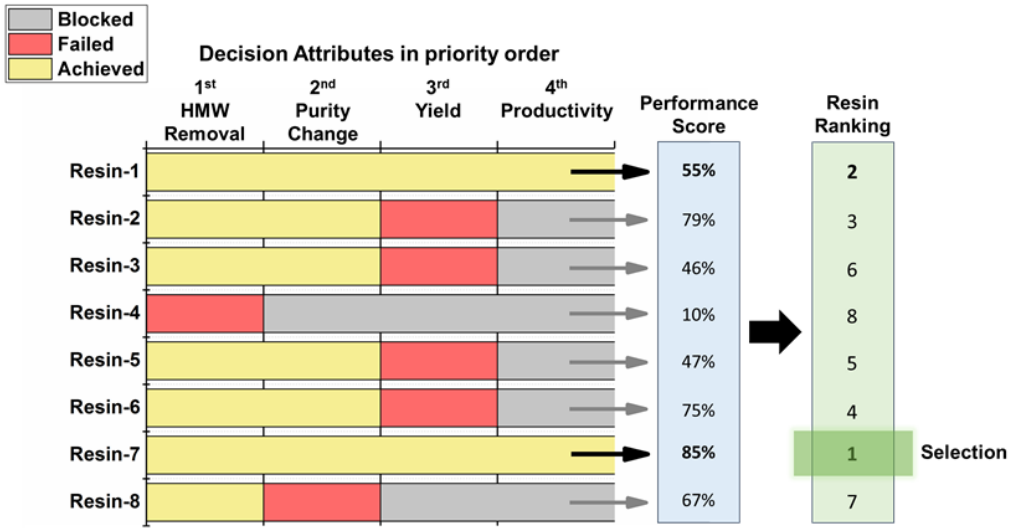
H: Column bed-height (cm)

Table 3.2: Case study formulation of the decision-making components in the HTPD workflow

HTPD workflow step	Variables	Fixed Inputs	Constraints	Objective	Outcome
Resin Comparison & Selection Tool	Set point of process parameters (DoE factors)	• ML Regression coefficients • DoE factors' limits • Weight of Decision Attributes: ○ $W_{HMW\ removal}$ = 0.4 ○ $W_{Purity\ change}$ = 0.3 ○ W_{Yield} = 0.2 ○ $W_{Productivity}$ = 0.1	• $DBC_{10\%}$ > Load Challenge • Low limits < Variables < Upper limits • Attribute targets in priority order: **1.** HMW removal ≥ 70% **2.** Purity change ≥ 50% **3.** Yield ≥ 50% **4.** Productivity ≥ 25 g/L/h	**Maximum:** Number of achieved Decision Attributes in priority order	• **Resin Ranking** • **Set-point of process parameters** • **Selected Resin**
Window of Operation & Robustness Analysis	Set point & acceptable range of process parameters (DoE factors)	• ML Regression coefficients • DoE factors' limits • Number of Monte Carlo runs = 10000	Attributes' threshold values: • $DBC_{10\%}$ ≥ 110g/L • Purity ≥ 91% • Yield ≥ 75% • Pool volume ≤ 5CV	**Minimum:** Probability of process failure P(fail)	• **Window of Operation** • **Distribution of attributes** • **Sensitivity to process deviations**

The resin selection tool receives user specifications and first it calculates the weight coefficient for each attribute based on their relative significance and organises accordingly the order that each attribute is evaluated. Then an evolutionary engine (SOLVER Optimisation tool, MS Excel) searches for the set-point of process parameters for each CEX to achieve threshold values of the decision attributes as shown in Table 3.2. The final decision was based on the weighted sum method assigning a performance score (Eq.2.5) to each CEX resin based on the value it achieved for each decision attribute (Equations: 2.4, 3.1, 3.2 and 3.3). The CEX resin that met the maximum number of decision attributes and achieved the highest performance score (in that order) was selected as the most suitable candidate to undergo further experimentation to optimise its operating conditions. An illustration of the final outcome of the tool for the bispecific mAb case study is presented in Figure 3.2.

Two CEX resin candidates managed to achieve all four decision criteria in their respective order. It should be noted that the yield requirement was dropped to a low level in order to allow a high HMW removal. Due to high aggregate concentration and poor peak resolution achieved with the 200μL chromatography columns a substantial reduction of HMW species was only achieved through decreased product recovery. Although limitations were observed regarding the use of the 200μL miniature columns (e.g. flowrate limitations of the robotic arm and poor peak resolution due to the small bed-height), they were universal for all CEX resins. Therefore, for comparability purposes the 200μL chromatography columns were considered sufficient providing the advantage of low material consumption especially when multiple CEX resins are screened.



CEX Chromatography Resin	R1	R2	R3	R4	R5	R6	R7	R8
pH - Load	4.9	4.5	4.8	4.8	4.9	5.6	4.9	4.5
Conductivity - Load (mM)	54	36	3	60	101	4	35	0
Linear Velocity - Load (cm/h)	402	366	418	395	388	409	421	300
Feed Concentration (g/L)	12.0	9.3	8.8	7.2	5.2	7.2	12.0	4.0
pH – Elution	5.5	6.9	7.0	5.0	6.2	5.8	5.1	5.0
Linear Velocity - Elution (cm/h)	450	368	300	448	354	334	498	300
Load Challenge (g/L)	80	45	31	35	35	30	90	30

Figure 3.2: A visualisation of the resin selection tool developed to identify CEX resins that meet the desired decision attributes. User specifications define the priority order of the decision attributes. Achieved attributes are determined using the satisficing method. Each resin candidate is assigned a performance score combining their respective attribute normalised values. Ranking is performed considering first the resin candidates with the highest number of achieved attributes and then their performance score. The set-point of each process parameter is presented in the table. CEX resin in order of appearance 1 – 8: Poros XS, Poros HS 50, Toyopearl GigaCap S 650 (S), Toyopearl GigaCap CM 650 (M), Capto S Impact, Fractogel COO (M), Eshmuno CPX and UNOsphere Rapid S. Regression correlations used by the resin selection tool were developed through DoE analysis. Experimentation was performed using 200µL miniature pre-packed chromatography columns operated with a Tecan Freedom EVO® 200.

Between the two successful CEX resin candidates the performance score achieved by Resin-7 was approximately 85% compared to 55% achieved by Resin-1. Hence, Resin-7 was determined as the top-ranked CEX resin that was selected to undergo further experimentation to optimise its operating conditions. Figure 3.2 also provides a ranking of all the CEX resins included in screening and the set-points of the operating parameters. Given the configuration of the resin selection tool for the particular bispecific mAb, the vast majority of the CEX resins managed to remove HMW species and increase

purity. However, a low level of yield was observed throughout all resin candidates that partially rendered them incapable of reaching the productivity target.

3.3.4. Window of operation

Leveraging the CEX resin screening results summarised in Figure 3.2, the subsequent optimisation experiments were designed for the selected Resin-7 (Table 3.1). It should be noted that binding conditions were optimised considering only to maximise $DBC_{10\%}$. On the other hand, the elution pool volume that was collected was introduced as the third DoE response after purity and yield due to its impact on productivity. A stepwise regression analysis identified a model to describe each of the four DoE responses as a function of the DoE factors. Figure 3.3 shows the prediction profilers of each DoE response along with a model-fit summary.

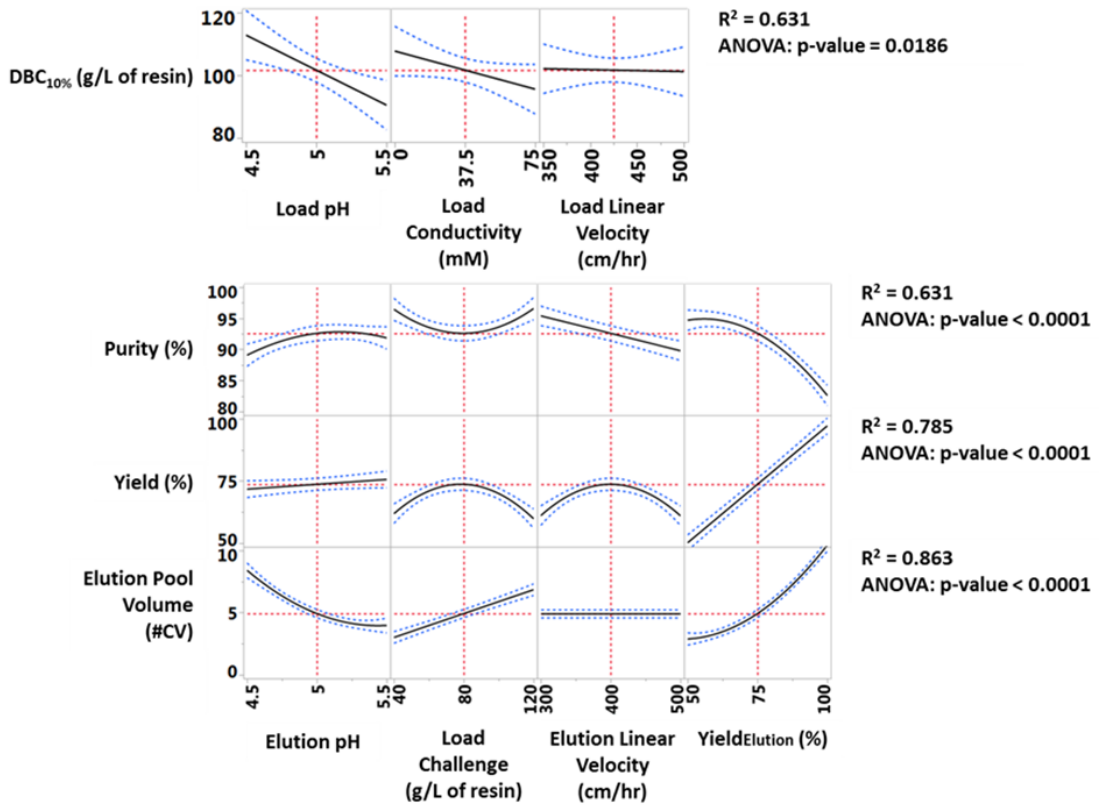


Figure 3.3: Prediction profilers generated using JMP Pro 11 to visualise each attribute (DoE response) as a function of process parameters (DoE factors) for CEX Resin – 7. Solid lines represent the average response and dotted lines indicate the confidence interval at a 95% confidence level. DoE was executed using 600µL miniature pre-packed chromatography columns operated with a Tecan Freedom EVO® 200.

Instead of using the prediction profilers to identify a sweet spot, the MLR coefficients were linked into a stochastic simulation engine evaluating simultaneously the set-point and the acceptable range of each process parameter. Through a series of Monte Carlo simulations a window of operation was determined where the probability of failing to meet the threshold values of any attribute is minimised. The contour plots in Figure 3.4 demonstrate the areas where all attributes are satisfied (white areas) operating under no deviations from the set-point. Additionally, black shaded circular areas present the window of operation considering a level of uncertainty for each process parameter.

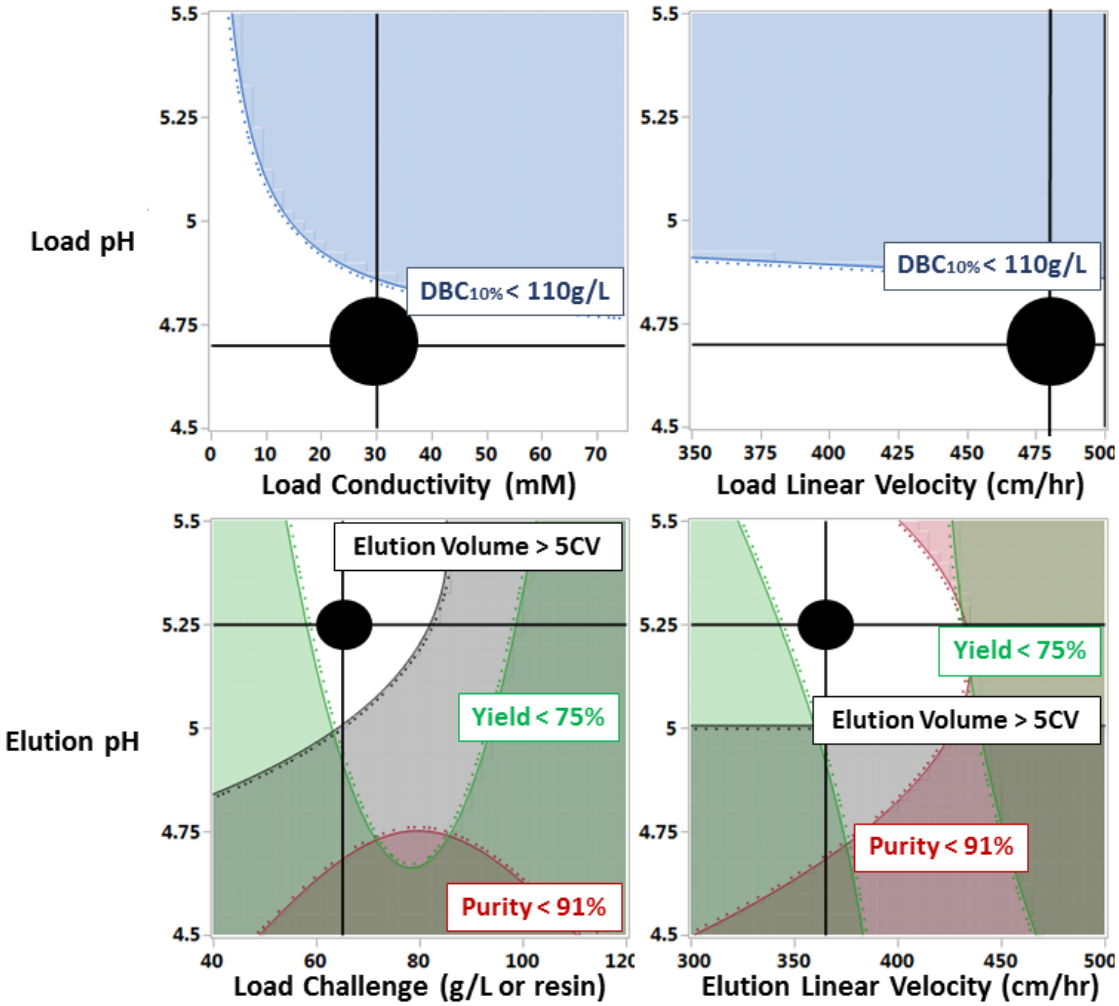


Figure 3.4: Window of operation under uncertainty for CEX Resin – 7. White (blank) areas demonstrate the operating space under no process deviations from the set-point. Black areas define a window of operation that satisfies the attributes under uncertainty by minimising the probability of failing any of the threshold values of the attributes. Number of Monte Carlo simulations per iteration = 10000.

Typically, a window of operation would focus on defining the boundaries of an operating space and determine its centre point as the sweet-spot due to its greatest distance from

the boundaries and thus potentially increasing the robustness of a process. Additionally, stochastic simulations could be performed on the sweet-spot once it has been identified to determine its robustness. Although this approach helps to define a set-point for each process parameter, it neglects to determine an acceptable range within which each process parameter is allowed to vary without compromising any quality and performance attributes. Moreover, iterative calculations would be necessary to assess the acceptable range. Additionally, when multiple parameters are investigated a series of contour plots would be necessary to describe a window of operation especially when parameter interactions have a significant impact on the attributes. Using the methodology as described in Section 2.2.3.4., it is possible to determine an acceptable operating range for each process parameter along with its set-point. Thus providing a greater understanding regarding the impact of process parameters on different attributes and hence guide subsequent experimentation at larger scales.

Using the Monte Carlo simulations dataset that resulted in a minimum probability of process failure a distribution for each attribute was created and a tornado plot was drawn to evaluate the significance of each process parameter (Figure 3.5). The process parameter with the most significant impact on $DBC_{10\%}$ was identified as the load (binding) pH, while all operating parameters demonstrated an impact on purity and yield. It should be noted that it can be expected that the linear velocity of the mobile phase during elution would have no effect on the volume of the elution pool as it can be seen in Figures 3.3 – 3.5. Furthermore, the benefits of using the 600µL miniature chromatography columns should be highlighted as it was possible to achieve a greater peak resolution compared with the 200µL columns. That led to a more realistic approximation of the trade-offs between purity and yield.

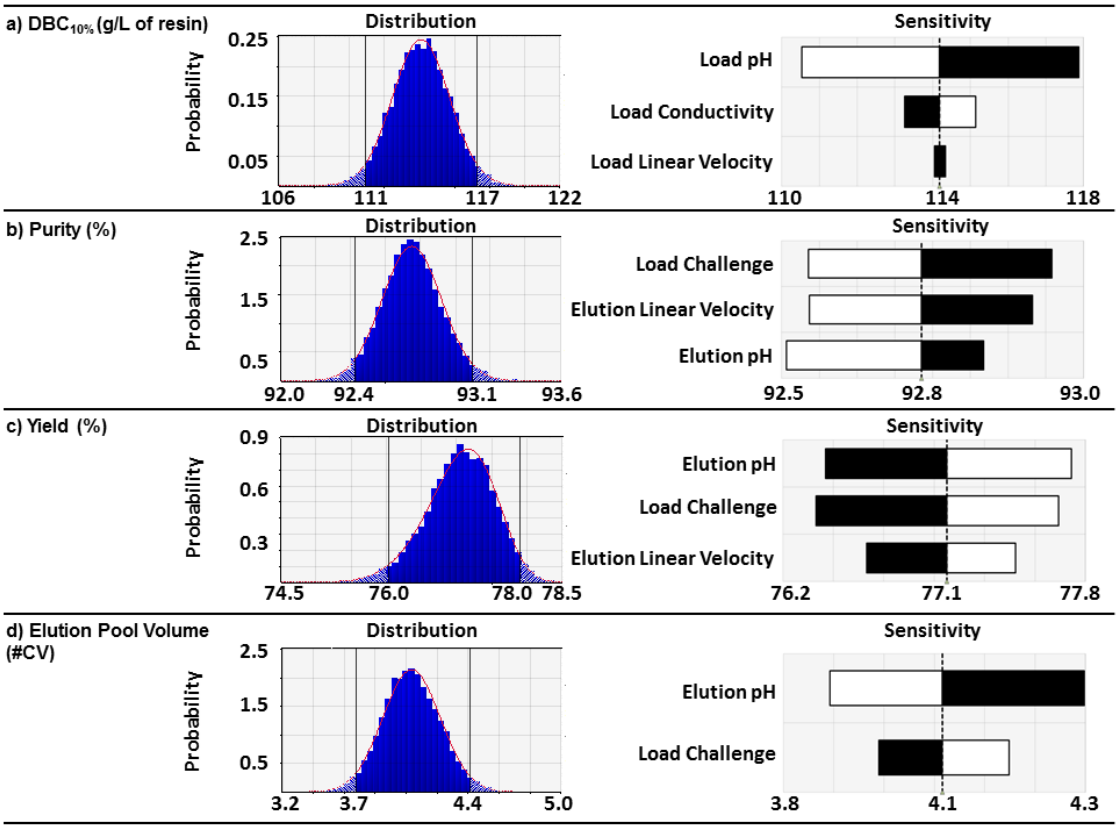


Figure 3.5: Robustness analysis for CEX Resin – 7. Distribution and sensitivity of attributes operating with the identified window of operation under uncertainty. Solid bars in the tornado graphs for the sensitivity analysis indicate lower parameter values than the set-point and hollow bars indicate higher values. Number of Monte Carlo simulations = 10000.

3.3.5. Targeted bench-scale experimentation

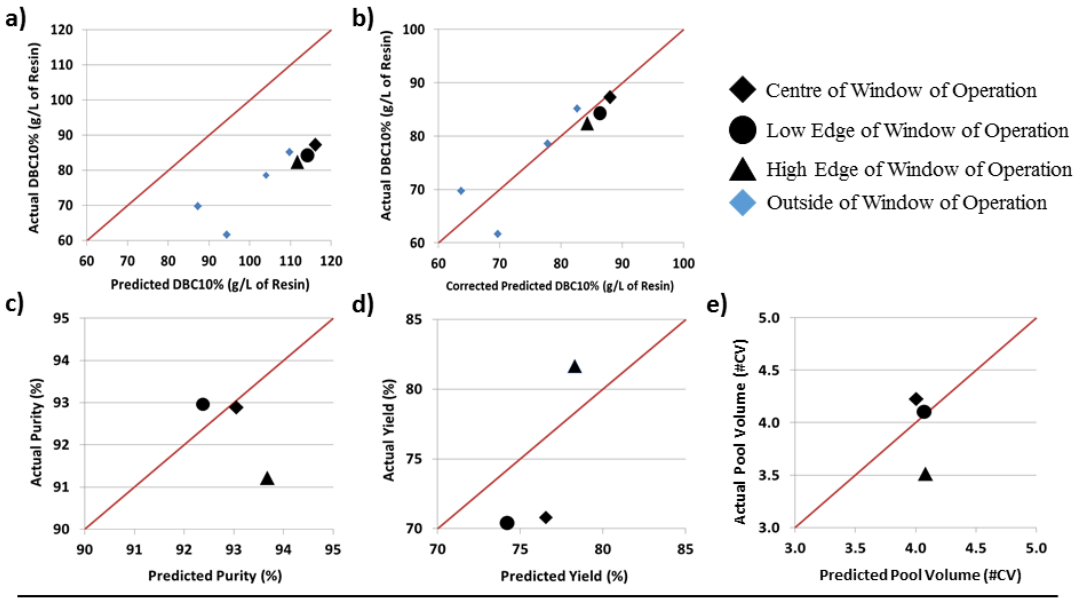
The validity of the predictions made through the implementation the HTPD workflow was assessed by performing targeted experiments at bench-scale with a 20cm bed-height chromatography column packed with Resin-7. Bench-scale experimentation evaluated the operation at the centre of the suggested window of operation (set-point of process parameters) while additional runs were performed deliberately introducing deviations from the set-point within and outside the window of operation.

The $DBC_{10\%}$ was evaluated first in order to determine whether the predicted value of load challenge was feasible. Experimentally achieved (i.e. actual) $DBC_{10\%}$ values were plotted against predicted to visualise their accuracy. It can be noted that $DBC_{10\%}$ was consistently overestimated leading to a shift of the data-points in Figure 8a from the diagonal line to the right. In order to improve the accuracy of the predictions, the

predicted $DBC_{10\%}$ was corrected to account for the collection of a column volume of equilibration buffer in the flow-through pool at the beginning of the loading phase and the hold-up mass from the application of the last column volume (Eq.3.4). Additional breakthrough curves were generated to address the validity of the correction made (Figure 3.6b).

$$\mathrm{DBC}_{10\%}^{\mathrm{Corrected}} = \mathrm{DBC}_{10\%}^{\mathrm{Predicted}} - 2 * \mathrm{C}_{\mathrm{Feed}} \qquad (\mathrm{Eq.}\,3.4)$$

Linear gradient elution experiments followed to evaluate the predictive ability of the elution performance at bench-scale. Experimental values were plotted against predicted for each attribute (purity, yield and elution pool volume) as shown in Figure 3.6c – e. Statistical tests were performed to assess the significance of the difference between the means (t-Test) and the variances (F-Test) of the predicted and the actual values. The test results suggested a good agreement between the predicted and the experimental values for purity and yield and confirmed the over-prediction of the $DBC_{10\%}$ without the application of the correction for the hold-up mass. Additionally, there was a statistically significant difference between the variances of the predicted and the actual datasets on elution pool volume, however within the corresponding probabilistic limits defined by its distribution in Figure 3.5d. In summary, implementation of the proposed HTPD workflow enabled the comparison of a number of CEX resins under conditions that favour each candidate. Two CEX resins qualified as the top-ranked candidates for this particular purification challenge. The CEX Resin-7 was selected based on its performance score for further experimentation to identify a window of operation. The results suggested a mean predicted purity and yield level of 93% and 76%, respectively with an elution pool volume of 4.0 CV. Targeted bench-scale experimentation within the predicted window of operation resulted in similar performance (92% purity, 74% yield and 3.9 CV) and hence provided a preliminary justification of the predictive ability of the model.



	a	b	c	d	e
F-test: p-value	0.37	0.47	0.30	0.09	0.01
t-test: p-value	0.0003	0.92	0.39	0.65	0.68

Figure 3.6: Actual vs Predicted plots and Statistical tests to visualise and verify the proposed window of operation for CEX Resin – 7. Actual refers to the experimental values obtained at bench-scale using a 20cm bed height chromatography column operated with an AKTA Avant 25. Predicted values were obtained using regression correlations developed through analysis of the DoE space. DoE was performed using 600µL miniature pre-packed chromatography columns and operated with a Tecan Freedom EVO® 200.

Note: A 95% confidence level was used. The null hypothesis considers the two samples sets are from the same distribution. P-values ≤ 0.05 reject the null hypothesis. Sample size: 7 for a) and b) and 3 for c), d) and e).

3.4. Conclusions

This chapter demonstrated a hybrid approach for the development of a CEX chromatographic separation process to purify a high-aggregated bispecific antibody. The proposed workflow offers significant benefits in terms of materials' consumption (80% reduction in feedstock material and 50% reduction in buffer consumption) and time requirements compared with bench-scale experimentation in order to achieve a similar level of process understanding. A key focus of the HTPD workflow was the establishment of a systematic framework providing the methods and the tools to cope with the large datasets resulting from HTE and automate a significant part of data manipulation and analysis.

A biopharmaceutical company could identify very rapidly limitations that a specific resin type has for a given product and re-orient development activities accordingly. For instance, as demonstrated here through an industrially relevant case study, a number of well-studied commercial CEX chromatography resins managed to remove only a portion of HMW species thus putting an additional burden on downstream unit operations to provide the desired product profile. Through the introduction of a systematic HTPD workflow process bottlenecks can be identified rapidly at an early stage in process development. Therefore, it would be possible to consider alternative options in order to develop a cost-effective purification process that can meet product and process specifications. Finally, the insights that were gained through the analysis to identify a window of operation and assess its robustness can be further leveraged to guide subsequent experimentation at larger scales. Further experimentation could focus on optimising process parameters that were identified as significant thus mitigating the experimental effort.

Chapter 4. A process economics evaluation of pre-packed chromatography columns in antibody purification

4.1. Introduction

Single-use technologies (SUT) have gained a significant momentum in bio-manufacturing offering a lower capital investment and facility footprint when compared to conventional facilities using fixed, stainless steel process equipment. An example of this momentum from the industry is the introduction by GE Healthcare of modular, prefabricated cGMP bio-manufacturing facilities (KUBio) with a flexible production line. Chapter 1 (Section 1.3.2) described available SUT in bio-manufacturing and discussed the main advantages, limitations and challenges related to the introduction of disposable unit operations in the process sequence. Evidently, the decision to adopt SUT in bio-manufacturing relies upon multiple criteria and objectives. For instance, disposable equipment could decrease the fixed capital investment and mitigate validation activities however size limitations could render infeasible their fit into a large scale facility. Therefore, different considerations such as the drug development stage and available resources as well as the desired annual throughput and the performance of each unit operation could potentially have conflicting effects on the final decision.

The traditional industrial approach in manufacturing of therapeutic antibodies has developed a platform process usually involving three distinct and orthogonal chromatographic purifications steps (Kelley et al., 2009). Already published work has discussed the economic impact of disposable bags (Sinclair & Monge, 2002) and compared single-use, stainless steel (Novais et al., 2001; Sinclair & Monge, 2005) and hybrid bio-manufacturing facilities (Farid et al., 2005). However, there is no published work focusing on the economics of pre-packed chromatography columns in bio-manufacturing. Additionally, there is a lack of commercially available computer aided tools flexible enough to facilitate a comprehensive investigation of pre-packed columns. This chapter focuses on the chromatographic purification and attempts to identify

benefits and limitations of pre-packed columns from a process economics point of view. The overall objective here is the evaluation of pre-packed chromatography columns as an alternative to self-packed columns in the purification of therapeutic antibodies using the cost of goods per gram of product (COG/g) as the key performance metric. A process economics model was developed emphasising on the design of the purification train to capture all chromatography related activities and required resources to allow for the detailed cost comparison of pre-packed and self-packed columns. A series of hypothetical scenarios were designed based on evidence from the industry and academia to simulate a wide decision space and address the impact of multiple parameters on the cost-effectiveness of pre-packed columns. The key focus of the scenario analysis was to determine whether the benefits and limitations of pre-packed columns have a significant impact on the COG/g and identify the scale of manufacturing where pre-packed columns are no longer a feasible or an economically attractive option.

4.2. Materials and methods

To facilitate the investigation of pre-packed chromatography columns a process economics model was developed in MS Excel as described in Chapter 2 (Section 2.3). A benefit of the model is offered through its structure which allows the investigation of individual unit operations and the whole manufacturing train. Throughout this chapter a generic platform manufacturing process for mAbs was used as shown in Figure 4.1. Three different chromatography steps were assumed in the purification train using a Protein A resin for the capture step, a cation exchange resin for the intermediate step and an anion exchange resin for the polishing step.

Before assessing the economics of the complete manufacturing process, the first part of this chapter focuses on an individual chromatography unit operation in order to perform an in-depth comparison of the costs related to self-packed and pre-packed columns. Key operational differences between self-packed and pre-packed columns are the time and cost related for packing and unpacking activities. The number of times that a chromatography column needs to be packed can be estimated based on the effective

chromatography resin lifespan, the number of chromatography cycles per batch and the number of batches performed annually. Increasing packing activity would lead to a surge in the cost of labour and the related chemical reagents and consumables. On the other hand it is assumed that the corresponding packing system and the stainless steel chromatography column can handle the increased utilisation through proper operation and maintenance. Moreover, from a process economics point of view a key model parameter is the ratio of the price of a chromatography resin over the price of an empty pre-packed column. Changing from a self-packed to a pre-packed chromatography column of the same size would cause an increase in the cost of consumables due to the addition of the cost of the empty pre-packed column.

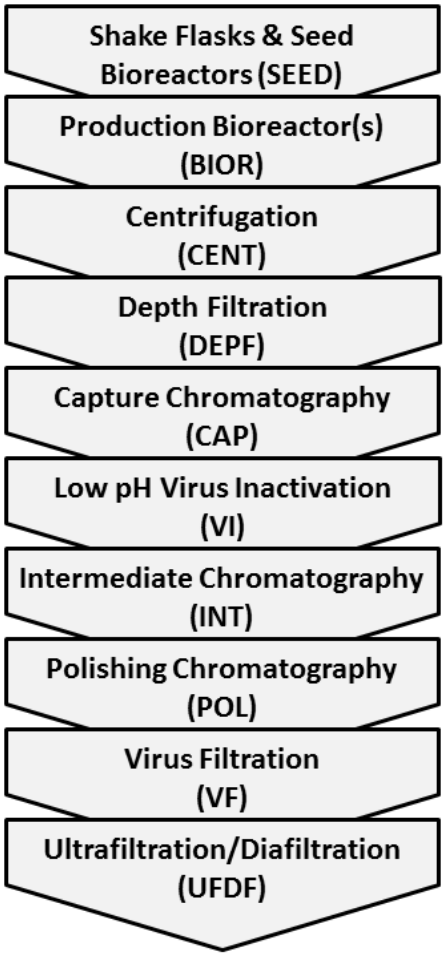


Figure 4.1: A generic manufacturing process flowsheet for mAbs

To investigate these differences a representative range of chromatography column diameters was selected for evaluation under different levels of facility utilisation and a range of resin prices (Table 4.1). Facility utilisation was estimated assuming a maximum

of 20 batches per year using a single production bioreactor. To automate and accelerate the simulation of different scenarios a procedure was programmed in VBA to assign the desired values to model parameters and assumptions and create a record of the cost breakdown for a chromatography step. Following the deterministic comparison of self-packed and pre-packed columns using the base assumption values (Table 4.1), a worst/best case sensitivity analysis was performed to determine model parameters with the greatest impact on the cost of goods for a single chromatography column (Table 4.2).

Table 4.1: Range of parameters used to compare the manufacturing costs between pre-packed and self-packed chromatography columns of the same size and packed with the same resin

Parameter	Range
Diameter (cm)	[5, 20, 45, 60]
Resin Price ($/L)	[500, 2000, 8000]
Batches	[1, 2, 4, 8, 12, 16, 20]
Column type	[Self-packed, Pre-packed]
Empty Pre-packed Column Price ($/cm)	2000
Resin Lifespan (#cycles)	25
DBC (g/L)	50
Number of Cycles per Batch	5

Table 4.2: Model parameters included in the sensitivity analysis to determine their significance in the manufacturing costs of a single chromatography step utilising either a self-packed or a pre-packed column

Parameter	Worst	Base	Best
DBC (g/L)	20	60	100
Resin Price ($/L)	15000	5000	1000
Empty Pre-packed Column Price ($/cm of diameter)	3500	2000	500
Buffer Price ($/L)	7	5	2
Resin Lifespan (#cycles)	20	50	100
Elution Pool Volume (#CV)	7	3	1.5
Linear Velocity (cm/hr)	100	300	500

Note: Using a 30cm in diameter column operated for 5 cycles per batch over 20 batches

Evaluation of a single chromatography step isolated from the rest of the process allows the direct comparison of manufacturing costs related to self-packed and pre-packed columns. Nevertheless, a unit operation is always part of a process flowsheet thus development and optimisation should consider the complete manufacturing process. Hence, to put into perspective the impact of pre-packed columns on the whole process a series of scenarios was created to simulate pre-clinical, clinical and commercial manufacturing of mAbs. Model parameters such as the annual product demand and cell culture titre for each scenario are defined in Table 4.3. In order to achieve a realistic approximation of the different manufacturing stages a set of assumptions was made based on previous published work by (Simaria et al., 2012) regarding a hypothetical mAb and the population to be treated at each stage. In order to estimate the annual product demand, average body weight for each patient of 100kg was assumed. Using Eq.4.1 and the assumptions in Table 4.4 the total annual demand can be approximated. For each manufacturing stage an overproduction factor was applied to account for additional material required for QCQA and process development activities. Finally, at the pre-clinical stage a total annual demand of 0.5kg was assumed regardless of the size of the study in order to have enough material to be used in process development.

$$\text{Demand} = \text{Overproduction factor} * \#\text{Patients} * \frac{\#\text{Doses}}{\text{patient}} * \text{Dosage} * \text{Body weight} \quad (\text{Eq. } 4.1)$$

Table 4.3: Key model assumptions used to evaluate the cost-effectiveness of pre-packed chromatography columns considering the complete manufacturing process

Stage	Total Demand (kg/year)	Cell Culture Titre (g/L)	No. of Batches	Pipeline
Pre-Clinical	0.5	2.5	1	12
Clinical Phase I & II	5	2.5	1	9
Clinical Phase III	20	5	2	2
Commercial	200	5	20	1

Table 4.4: Annual product demand approximation for each phase in the drug development pathway

	Pre-Clinical	Clinical Phase I	Clinical Phase II	Clinical Phase III	Commercial
Success Rate	0.69	0.54	0.34	0.70	0.91
Patients Treated	-	40	150	1300	15000
Doses/patient	-	1	18	18	18
Dosage (mg/kg)	-	15	7	7	7
Overproduction Factor	-	2.5	2.5	1.25	1
Total Demand (kg/year)	0.5	0.2	4.8	20.0	200.0

Note: Average body weight = 100kg

Considering the flowsheet in Figure 4.1 with three chromatography steps and given the two options for each step (self-packed or pre-packed column), there are eight possible configurations for the purification train. A procedure was written in VBA to automate the simulation of every scenario in Table 4.3 and for each purification train configuration. Additionally, using transition probabilities for each development stage as reported by Paul et al. (2010) the required number of drug candidates in the pipeline at each stage can be estimated based on the desired number of successful product launches per year. Therefore the influence of the biopharmaceutical pipeline size on the decision to introduce pre-packed chromatography columns can be evaluated. A stochastic analysis using the Monte Carlo approach was performed to address the robustness of the decision to introduce pre-packed columns at each manufacturing stage in Table 4.3. For each chromatography step in the purification sequence probabilistic values were drawn using triangular distributions with minimum, mode and maximum values as specified in Table 4.5.

Table 4.5: Key model parameters and their distributions that were included in the robustness analysis on the decision to introduce pre-packed columns at different manufacturing stages

Step	Parameter	Distribution (Min., Mode, Max.)
Capture	DBC (g/L)	Tr(35, 40, 45)
	Resin Price ($/L)	Tr(9000, 12000, 15000)
	Resin Lifespan (#cycles)	Tr(40, 50, 60)
	Empty Pre-packed Column Price ($/cm of diameter)	Tr(1800, 2000, 2200)
Intermediate	DBC (g/L)	Tr(55, 60, 65)
	Resin Price ($/L)	Tr(3000, 4000, 5000)
	Resin Lifespan (#cycles)	Tr(75, 100, 125)
	Empty Pre-packed Column Price ($/cm of diameter)	Tr(1800, 2000, 2200)
Polishing	DBC (g/L)	Tr(85, 100, 115)
	Resin Price ($/L)	Tr(2000, 3000, 4000)
	Resin Lifespan (#cycles)	Tr(75, 100, 125)
	Empty Pre-packed Column Price ($/cm of diameter)	Tr(1800, 2000, 2200)

Note: Tr(a,b,c) refers to the triangular probability distribution where a, b, c are the minimum, most likely, and maximum values, respectively.

4.3. Results and discussion

Evaluation of pre-packed chromatography columns as an alternative to self-packed columns was performed through a series of scenarios designed to capture key process economics drivers. Assuming the same size of a chromatography column for both technologies packed with the same resin and operated under the same conditions a relativistic impact of pre-packed columns on manufacturing costs is demonstrated in Table 4.6.

Table 4.6: A qualitative comparison of the manufacturing costs associated with self-packed and pre-packed columns of the same size, packed with the same chromatography resin and operated under identical conditions

Manufacturing Costs		Self-packed	Pre-packed
Direct Costs	Labour	1	1
	Chemical Reagents	1	1
	Packing Labour	1	0
	Packing Chemical Reagents	1	0
	Consumables	1	1(+)
Indirect Costs	Self-packed Column	1	0
	Chromatography Skid	1	1
	Buffer & Product Hold-tanks	1	1(-)
	Column Packing System	1	0

The manufacturing costs in Table 4.6 refer only to the costs associated with a chromatography unit operation. Labour and chemical reagents account for the cost of operating a chromatography column including personnel, buffers for operation and cleaning of hold-tanks. Since the size and operating conditions are the same for both column technologies it is expected that the required time and volume of buffers for operation and therefore the volume of the hold-tanks would remain constant. However, any hold-tanks associated with packing and unpacking buffers would be eliminated in case of pre-packed columns. Additionally, the necessary chromatography skid would remain the same regardless of the technology of the column. On the other hand, any cost related to column packing would be eliminated for a pre-packed column. These costs include the associated personnel and chemical reagents for packing and

unpacking activities along with the cost for the necessary column packing system. Finally, in case of self-packed columns consumables would involve the cost of the chromatography resin and the guard filters for the hold-tanks. On the other hand, in case of pre-packed columns the cost of consumables would be the sum of the cost of the pre-packed column and the guard filters for associated hold-tanks.

The cost of consumables for a self-packed chromatography unit operation as estimated in the process economics model is a function of the volume of the chromatography column and thus a function of its diameter and bed-height. Additionally, the cost of a pre-packed column is estimated as the sum of the cost of the chromatography resin and the cost of the disposable empty column. Here, the cost of the empty pre-packed column is calculated based on the diameter of the column using a unit price per centimetre. Due to the same size and operating conditions between the two column technologies the cost of the disposable guard filters for the hold-tanks would be constant. Hence, guard filters could be excluded in order to evaluate the relationship between the cost of the resin and the disposable empty pre-packed column.

A graphical representation of the relationship between the cost of a chromatography resin and the cost of a pre-packed column is shown in Figure 4.2. As shown in Figure 4.2 increasing resin price and diameter values lead to the dominance of the resin cost. At small diameters (up to 10cm) the cost of the resin varies between approximately 5% and 50%. For instance, a Protein A resin with a price of 12500$/L would account for 50% of the total cost of a pre-packed column packed with the same resin. It should be noted that the curves in Figure 4.2 were generated assuming a price for the empty pre-packed column of 2000$/cm of diameter.

The cost breakdown in Table 4.7 might give the impression that pre-packed columns offer a significant reduction in manufacturing costs however, the end result would also be subject to the ratio between direct and indirect costs. It is expected that indirect costs would decrease in the case of a pre-packed column of the same size compared to a self-

packed column. On the other hand, the answer is not straightforward for the direct costs since the intensity of packing activities could change the balance among different cost components.

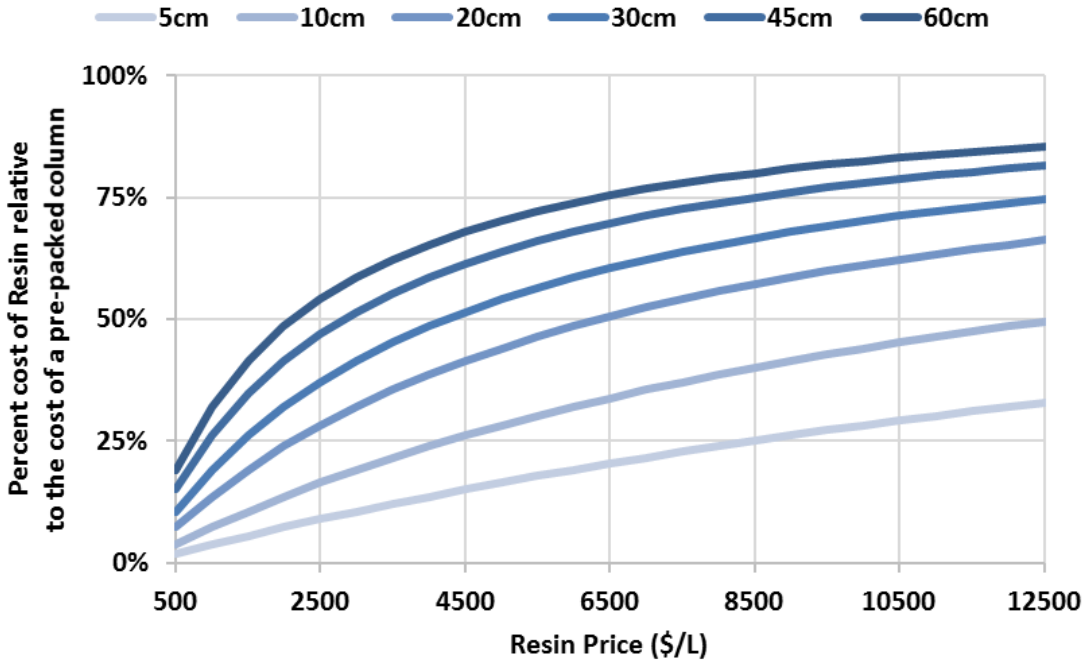


Figure 4.2: Cost of a chromatography resin relative to the total cost of a pre-packed chromatography column against the price of the resin at different column diameters

Figure 4.3 shows different cost ratios for pre-packed columns over self-packed. The top-half of Figure 4.3 plots the consumables cost ratio (i.e. consumables for pre-packed over self-packed column) with increasing facility utilisation and increasing column diameter. As shown in Figure 4.2 increasing resin price and column diameter lead to the dominance of the cost of the chromatography resin over the empty pre-packed column. Figure 4.3 confirms that relationship and demonstrates that the consumables cost ratio of pre-packed over self-packed decreases with increasing resin price and/or diameter. On the other hand, increasing facility utilisation has a moderate effect on the consumables cost ratio as direct costs would increase for both column technologies.

The bottom-half of Figure 4.3 plots the cost ratios of labour, chemical reagents and indirect costs. These cost categories demonstrate a clear benefit from the introduction of pre-packed columns. Their cost ratios are throughout different levels of facility

utilisation and column diameters below 1, indicating that these costs are reduced for the pre-packed option. Furthermore, increasing diameter and/or facility utilisation above 20% have no further cost benefits as labour, reagents and indirect cost ratios reach a plateau value.

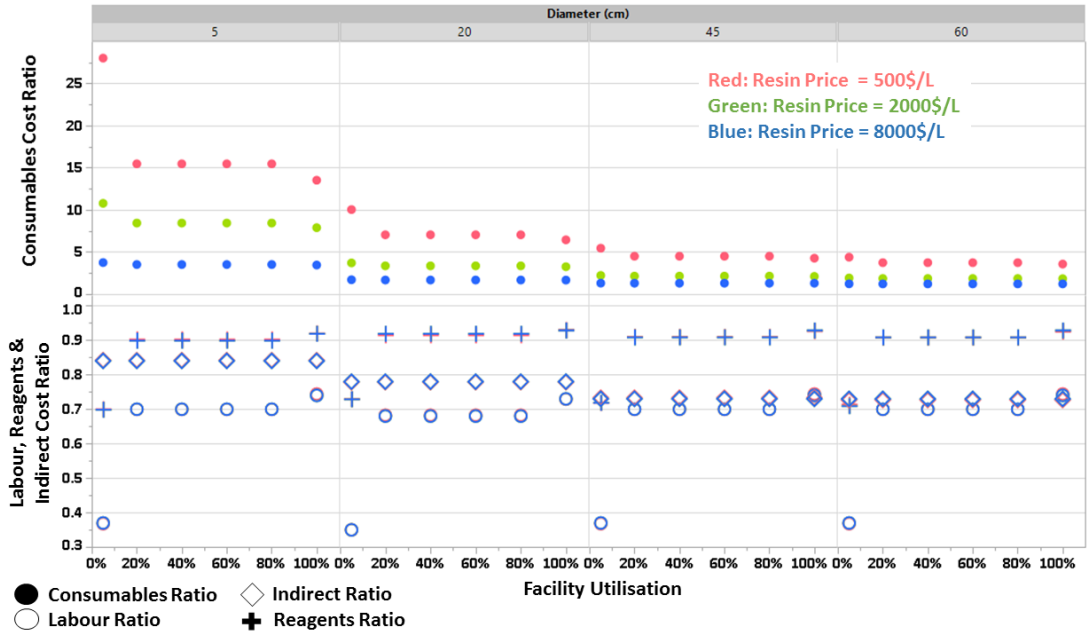


Figure 4.3: Cost ratios of pre-packed over self-packed columns

Overall, Figure 4.3 demonstrates the conflicting effect that pre-packed columns have on the manufacturing costs for a single chromatography step. While labour, reagents and indirect costs benefit from the introduction of pre-packed columns the same cannot be claimed for the cost of the consumables especially with small column diameters.

To better understand how different costs vary between self-packed and pre-packed columns a COG/batch breakdown was plotted at different diameters and resin prices. Assuming full facility utilisation (20 batches), 5 chromatography cycles per batch and a resin lifespan of 25 cycles a self-packed chromatography column would have to be packed four times. Alternatively, four pre-packed columns would be needed. It should be noted that the process economics model generates all cost estimates on an annual basis. Thus, any cost per batch can be estimated by dividing the annual cost with the number of batches in a manufacturing campaign. The bar charts in Figure 4.4 demonstrate the contribution of different costs in the COG/batch.

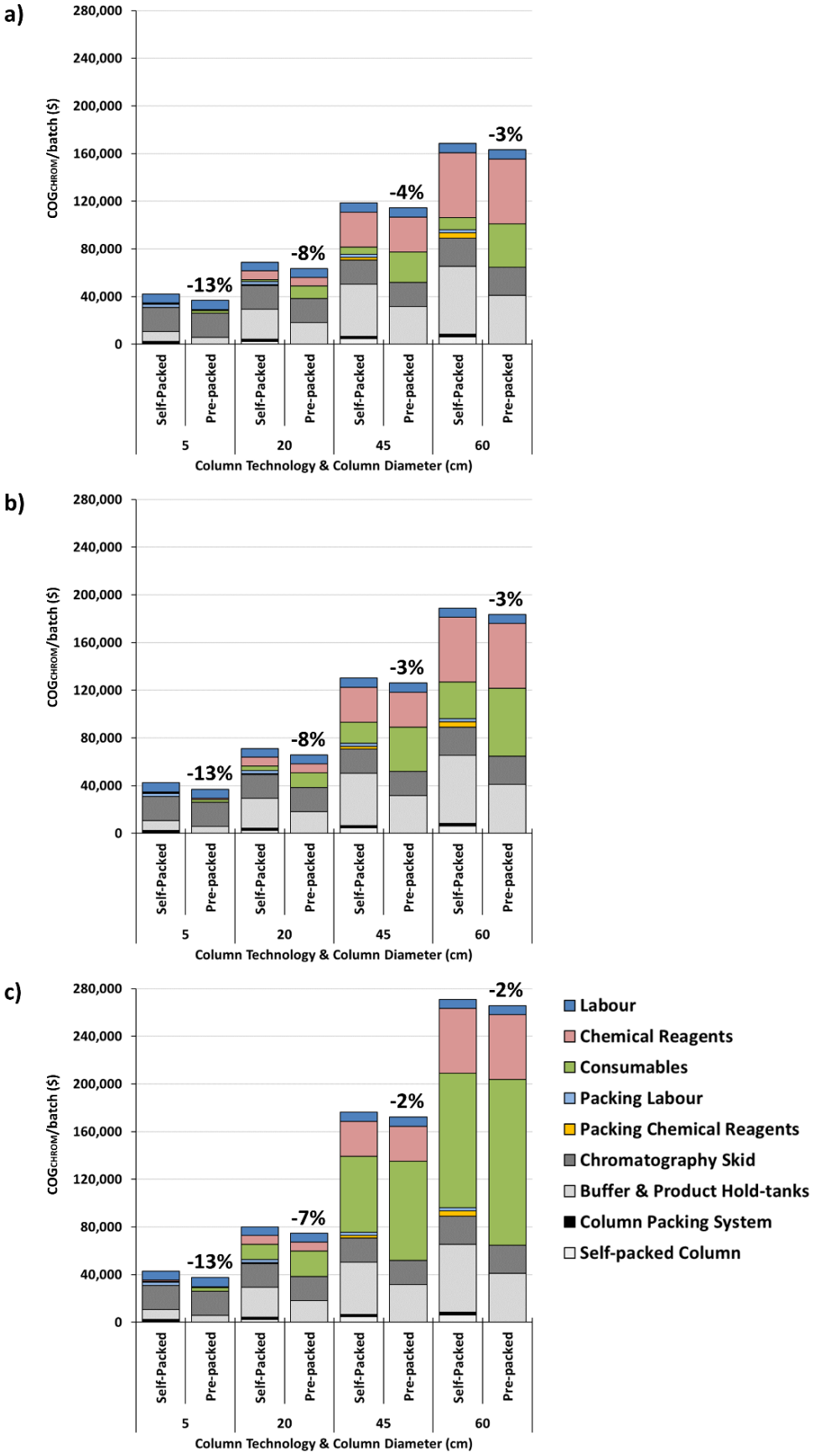


Figure 4.4: Chromatography cost of goods per batch breakdown for a self-packed and a pre-packed chromatography column at different diameters and a) resin price = 500$/L, b) resin price = 2000$/L, c) resin price = 8000$/L

As shown in Figure 4.4 the costs associated with packing and unpacking activities account for only a small percentage (2.5 – 6.5%) of the cost of goods. At small diameters indirect costs dominate the COG. At this size the introduction of pre-packed columns causes the greatest decrease in the COG/batch of approximately 13%. Moreover, the drop in the COG is consistent throughout different prices of a chromatography resin. On the other hand, pre-packed columns offer a 2% drop in the COG/batch using a 60cm diameter column. As column diameter increases the cost savings of pre-packed columns decrease. Nevertheless, pre-packed columns offer a reduction in the COG throughout different sizes and resin prices.

A sensitivity analysis followed to determine key model parameters and assumptions that cause the greater deviations in the cost of goods. Figure 4.5 presents a tornado graph for each column type demonstrating the parameters with the greatest impact on the COG/batch for a single chromatography column (COG_{CHROM}/batch) of 30cm in diameter operated for 5 cycles per batch over 20 batches. Three parameters show more significant impact: the dynamic binding capacity (DBC), the lifespan of the chromatography resin and its price. Additionally, these three parameters have a stronger influence on the pre-packed option, with low DBC and resin lifespan values resulting in greater increase in COG_{CHROM}/batch compared to the self-packed option. For both chromatography column technologies a low DBC value shows the greatest impact on the COG_{CHROM}/batch.

Evaluating a single chromatography step in isolation from the rest of the manufacturing process provided insights regarding the re-distribution of manufacturing costs caused by the introduction of pre-packed columns. To address the impact of the decision to introduce pre-packed columns throughout the purification train a generic process flowsheet was created to simulate the manufacture of a hypothetical mAb at pre-clinical, clinical and commercial stage.

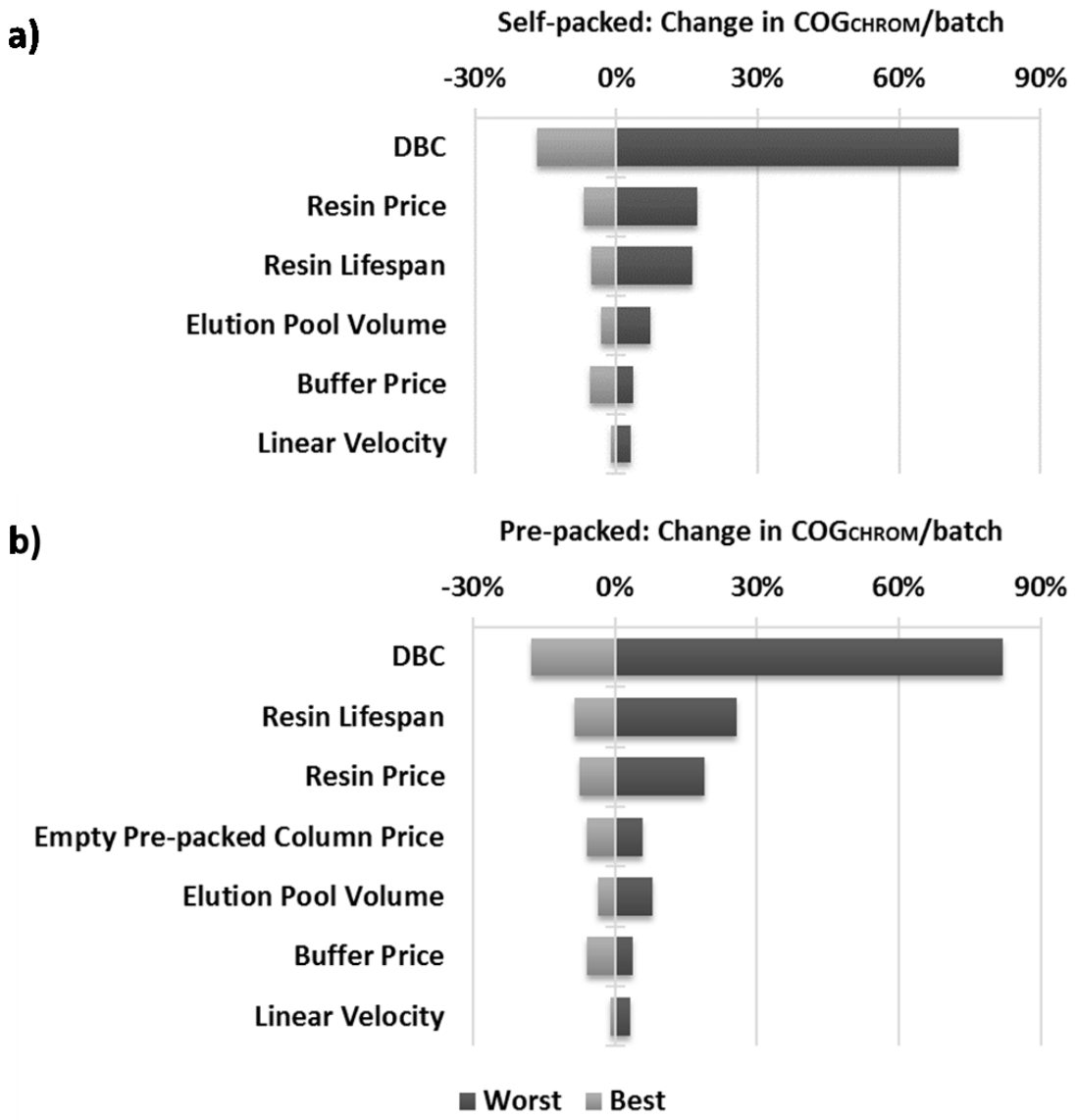


Figure 4.5: Tornado graphs visualising key model parameters related to chromatography and their impact on the COG_{CHROM}/batch for a) self-packed and b) pre-packed column of the same size and packed with the same resin

As discussed in Section 4.2, given three chromatography steps and two column technologies there are eight possible configurations for the purification train. Figure 4.6 shows the cost differences of all purification train configurations that involve at least one pre-packed column against the base case of a full self-packed train. The conflicting effect that pre-packed columns have on the manufacturing costs can also be observed here. Direct costs throughout different manufacturing stages and purification train configurations demonstrate an increase from the base case of a full self-packed train. The incorporation of a single pre-packed column for the last polishing step (train

configuration: SP-SP-PP) demonstrates the minimum impact on direct costs while the greatest impact can be observed for a purification train using only pre-packed columns (PP-PP-PP). The same trend is also noticeable for indirect costs, however, with the opposite effect. Indirect costs benefit throughout the manufacturing stages from the introduction of pre-packed columns with the most significant savings achieved through the use of a full pre-packed purification train.

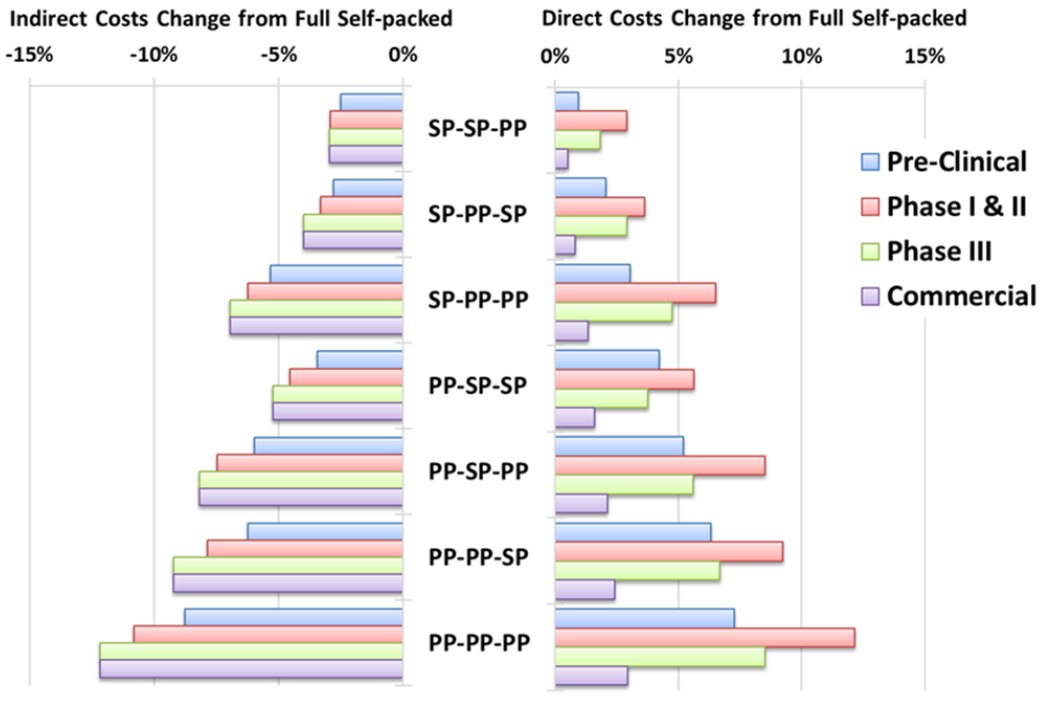
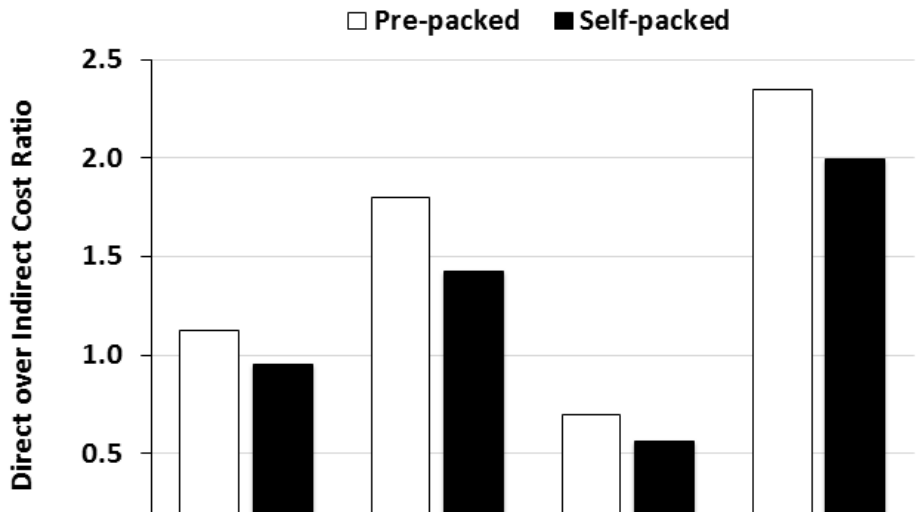


Figure 4.6: Changes in direct and indirect costs of a complete manufacturing process using different configuration of the purification train relative to a full self-packed purification train at different manufacturing stages

The conflict stems from the fact that pre-packed columns cause approximately the same level of increase in direct costs as the level of decrease in indirect costs. Therefore, the final offset in the COG would only be subject to the ratio between direct and indirect costs. Figure 4.7 plots the direct to indirect cost ratios for a full self-packed and a full pre-packed purification train at different manufacturing stages. The full pre-packed option demonstrates a consistent increase of the ratio between direct and indirect costs. However, that increase is capable of changing the cost balance only at the pre-clinical stage. At this stage a full self-packed purification train would result in almost a perfect

balance between direct and indirect cost reaching a ratio close to 1. With the introduction of a full pre-packed train at this stage that ratio exceeds the unit slightly. In contrast, the impact on the direct/indirect ratio although present it does not change the balance at clinical and commercial manufacturing. It should be highlighted that different manufacturing stages involve different pipeline sizes and thus a different level of indirect costs dilution among projects. In this case study the size of the pipelines (Table 4.4) was estimated targeting the successful commercialisation of at least one new therapeutic mAb given the transition probabilities in Table 4.5.



	Pre-Clinical	Clinical Phase I & II	Clinical Phase III	Commercial
Pipeline	12	9	2	1
Demand (kg/year)	0.5	5	20	200
No. of Batches	1	1	2	20
Titre (g/L)	2.5	2.5	5	5
Bioreactor Capacity (L)	400	4000	4000	4000
Campaign Time (Days)	25	28	45	338

Figure 4.7: Direct over indirect cost ratios for full pre-packed and full self-packed purification trains across different manufacturing stages

The changes in the total COG/g for the whole manufacturing process for different configurations of the purification train and across all manufacturing stages are

summarised in Figure 4.8. The cost-effectiveness of the process is not demonstrating a significant improvement from the conventional configuration of the purification train using only self-packed columns. The greatest cost reduction can be observed with the introduction of a full pre-packed purification train at clinical phase III reaching approximately 6%. Pre-clinical, clinical phase III and commercial manufacturing appear to benefit from pre-packed columns with a maximum cumulative cost reduction reaching just above 10%. This can be justified though the combination of Figure 4.6 and Figure 4.7. For instance, at commercial scale direct costs are two times higher than indirect costs. The introduction of pre-packed columns causes approximately a 10% decrease in indirect costs and less than 3% increase in direct costs. Hence, an overall 3% reduction in the COG/g. On the other hand, at clinical phase I & II the increase in direct costs caused by pre-packed columns offsets a similar decrease in indirect costs leading to a slight increase in the COG/g by less than 2%.

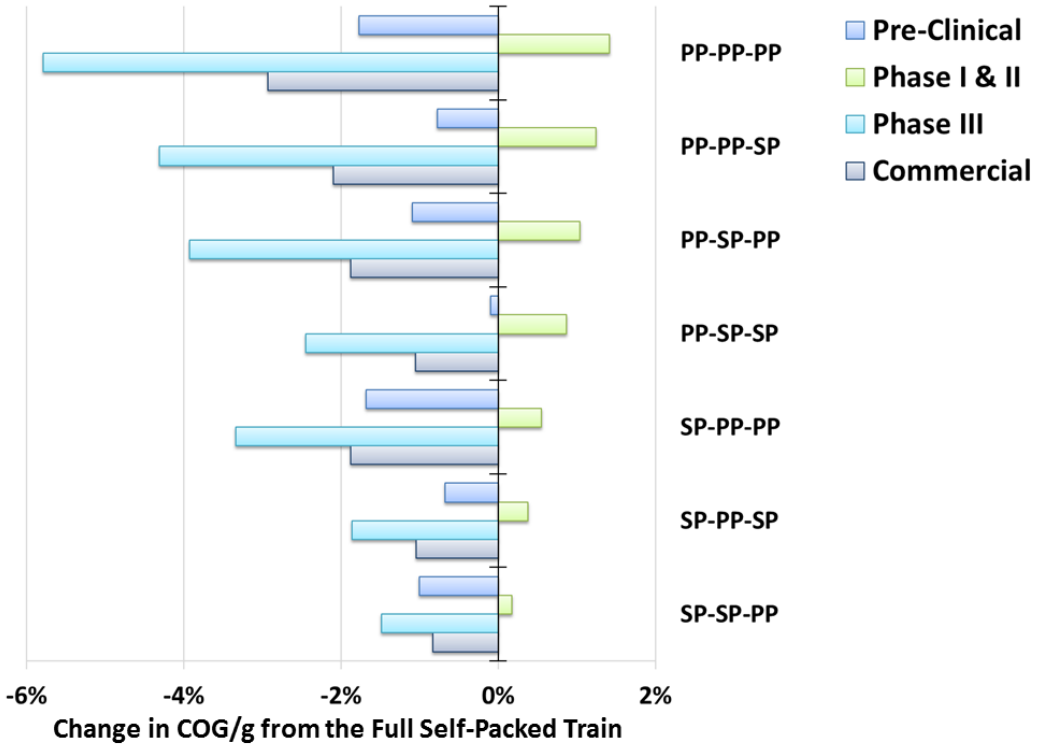


Figure 4.8: Changes in COG/g of a complete manufacturing process at different configuration of the purification train relative to a full self-packed purification train at different manufacturing stages

As discussed, considering Figure 4.8 the most cost-effective configuration of the purification train would be the utilisation of pre-packed columns for all chromatography steps at pre-clinical, clinical phase III and commercial manufacturing. In contrast, at clinical phase I & II the full self-packed configuration offers the lowest COG/g. A direct comparison between full self-packed and full pre-packed configurations across all manufacturing stages is shown in Figure 4.9. The COG breakdown offers an alternative visualisation of the re-distribution of the manufacturing costs caused by the introduction of pre-packed columns. Additionally, the effect of the manufacturing scale on the COG/g is illustrated starting with approximately 1750$/g at the pre-clinical stage and reaching 150$/g (a 90% decrease) at commercial scale.

To further understand the drivers behind the moderate change in the total COG/g caused by pre-packed columns it is necessary to determine the gravity that the chromatographic purification train has on the manufacturing costs. Figure 4.10 demonstrates a COG breakdown per unit operation across different manufacturing stages highlighting the contribution of each chromatography step in the total cost for a full self-packed train and a full pre-packed train. As expected, mainly due to its resin price, the capture step dominates the cost of the whole purification train and covers a significant percentage of the cost of the downstream processing train. In most cases the combined cost of the other two chromatography steps match the cost of the capture chromatography step. By comparing the percentage contribution of corresponding individual chromatography steps the impact of pre-packed columns can be quantified. For instance, at pre-clinical stage a small reduction in the cost contribution occurs for the intermediate and polishing chromatography steps with the introduction of pre-packed columns. On the other hand, a small increase for the capture step can be detected. This observation agrees also with Figure 4.8 where the option of having a self-packed capture step followed by two pre-packed steps (SP-PP-PP) comes very close to the full pre-packed configuration.

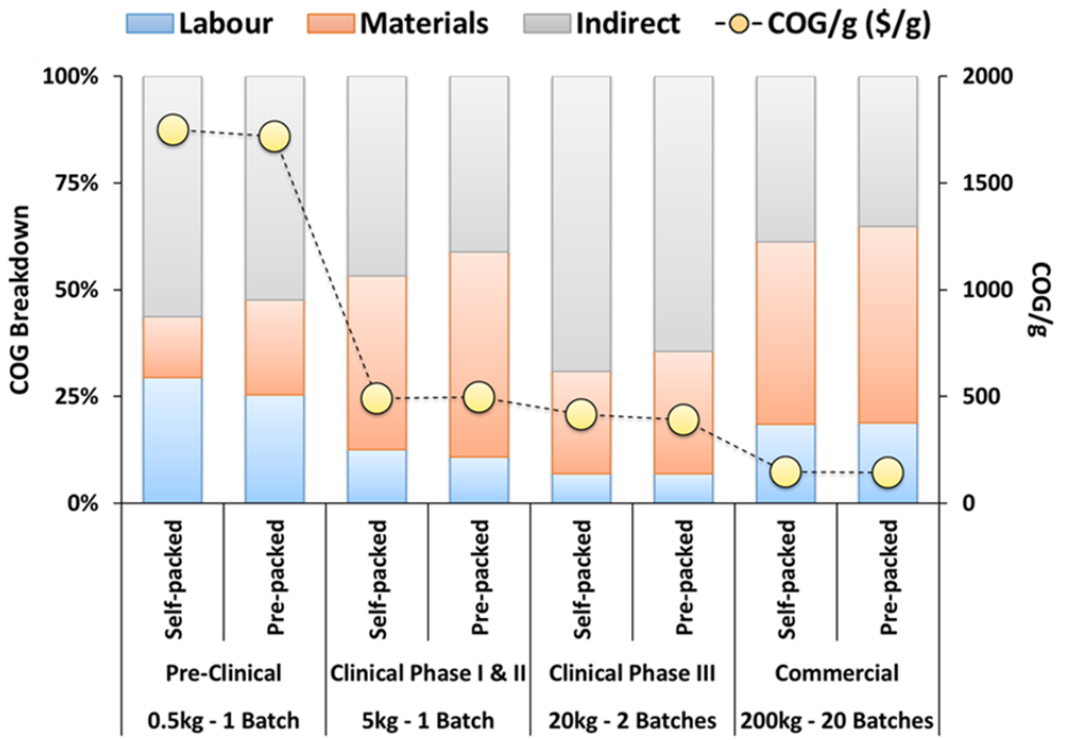


Figure 4.9: Direct comparison and cost of goods breakdown of a full self-packed and a full pre-packed purification train across different manufacturing stages

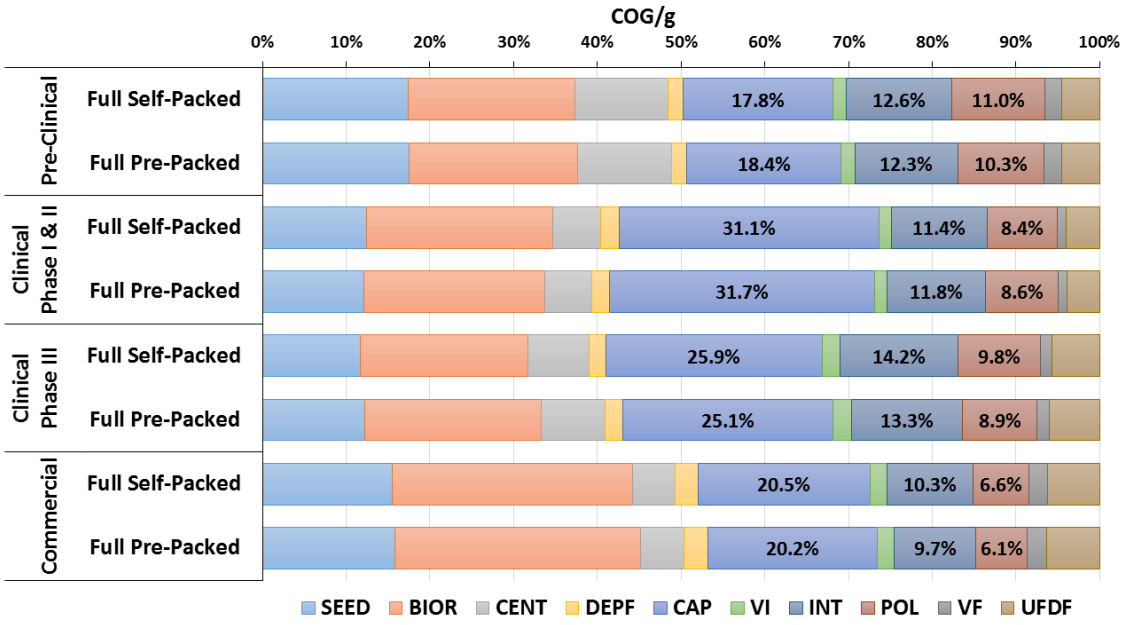


Figure 4.10: Cost of goods breakdown per unit operation across different manufacturing stages for a full self-packed and a full pre-packed purification train
Note: SEED: Flasks & Seed bioreactors, BIOR: Production bioreactor, CENT: Disk-stack centrifuge, DEPF: Depth filtration, CAP: Capture chromatography, VI: Virus inactivation, INT: Intermediate chromatography, POL: Polishing chromatography, VF: Virus filtration and UFDF: Ultrafiltration/Diafiltration

In order to assess the robustness of the decision regarding the selection of the most cost-effective configuration for the purification train at each manufacturing stage a series

of Monte Carlo simulations were performed. The stochastic inputs that were challenged in the analysis are presented in Table 4.6. The DBC, the resin lifespan and the resin price for each chromatography step were selected based on the observations made through the tornado graphs in Figure 4.4. Additionally, the unit price of an empty pre-packed column was included in the analysis in order to leverage the effect of any deviations in the price ratio between the chromatography resin and the empty pre-packed column. The resulted distributions of the COG/g are presented in Figure 4.11 for a full self-packed (black coloured histograms) and a full pre-packed (grey coloured histograms) purification train configuration at different manufacturing stages.

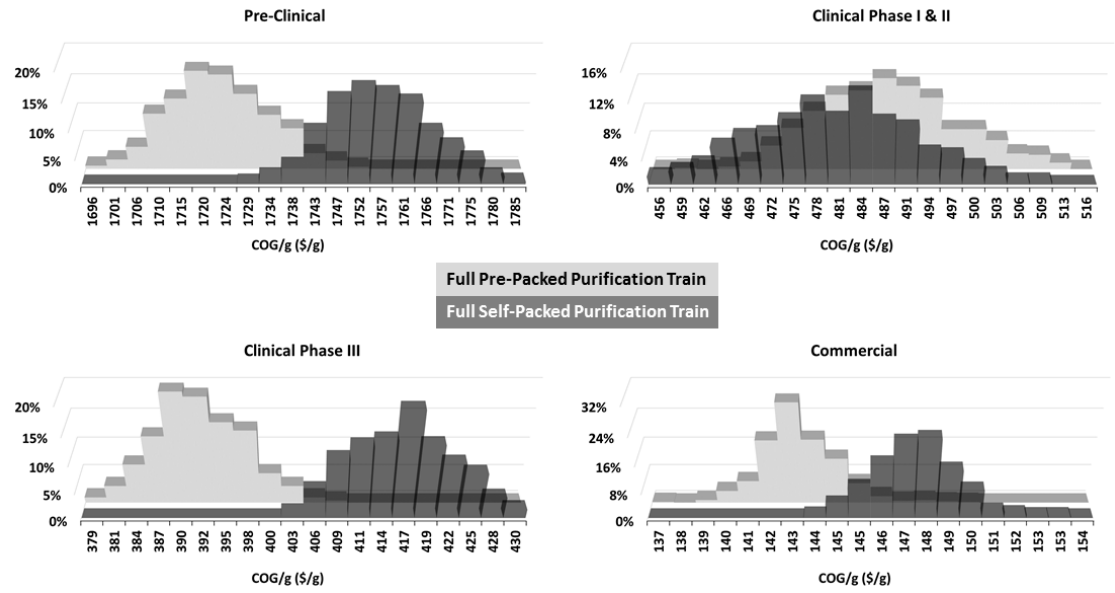


Figure 4.11: COG/g distributions generated through Monte Carlo simulations for a full self-packed (Full SP: black coloured histograms) and a full pre-packed (Full PP: green coloured histograms) purification train across different manufacturing stages

The COG/g distributions confirm the decision made through the initial deterministic analysis. At pre-clinical, clinical phase III and commercial manufacturing stages evaluated in this case study the pre-packed option demonstrates greater cost-effectiveness than the conventional full self-packed option, even under uncertainty on key parameters. Moreover, at clinical phase I & II there is a small shift to the right of the COG/g distribution for the case of a full pre-packed purification train relative to a full self-packed train.

The deterministic and stochastic analysis performed so far demonstrate a moderate benefit from introducing pre-packed columns into the manufacturing process for mAbs from a cost of goods perspective. However, the annual product demand for a commercial mAb could be above the 200kg limit that was challenged here. Therefore, the cost-effectiveness of pre-packed columns should be further evaluated at larger scales of manufacturing. Figure 4.12 shows a plot of the COG/g with increasing annual product demand up to a tone of mAb. On the horizontal axis of the plot there are tabulated five model parameters: the technology of the chromatography columns, the capacity of the production bioreactor, the number of upstream processing (USP) trains operating in staggered mode, the annual product demand and the number of batches performed annually.

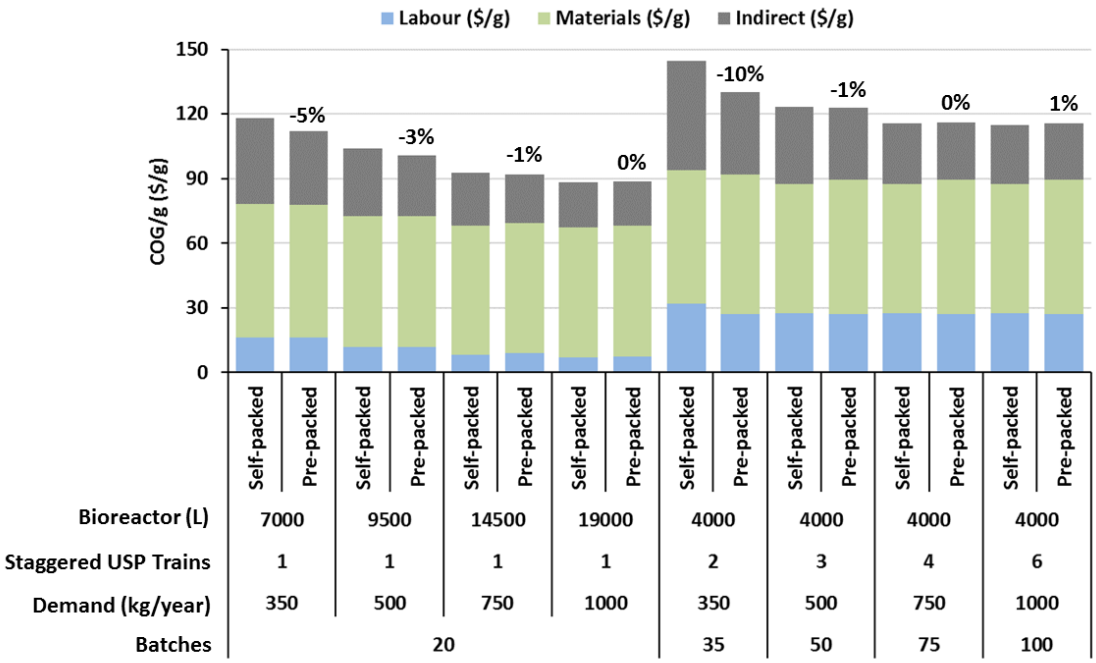


Figure 4.12: Cost of goods breakdown of a full self-packed and a full pre-packed purification train across different commercial annual product demands with increasing and fixed the capacity of the production bioreactor

With a single USP train the facility can run up to 20 batches within a year, thus in order to keep the capacity of the production bioreactor constant throughout late clinical and commercial manufacturing more batches have to be performed. Hence additional USP trains have to be included to maintain the same batch throughput. On the other hand,

there is the option to increase the capacity of the production bioreactor and maintain a single USP train. A comparison between the two options is visualised in Figure 4.12. The option of multiple staggered USP trains to maintain the bioreactor capacity constant demonstrates higher COG/g than the alternative of increasing bioreactor capacity.

The option of adding multiple USP trains and keep the batch throughput constant ensures that the size and the operation of each process step is maintained. In contrast, increasing the batch throughput could require bigger size of process equipment. Although there is a wide range of self-packed column diameters (up to 200cm) available in the market, the largest pre-packed column diameter that was considered in this research was 60cm. Recently, Repligen (Waltham, Massachusetts) commercialised the largest yet pre-packed column of 80cm in diameter. In case of high batch throughput where the required chromatography column diameter is bigger than 60cm, multiple pre-packed columns would have to be operated in parallel. Table 4.7 summarises the size and the processing time for each chromatography step in the purification train using self-packed and pre-packed columns. The size of the purification train changes with increasing batch throughput. For instance, at 1000kg/year and 20 batches annually the capture chromatography step requires 4 pre-packed columns of 60cm in diameter operated over 7 cycles per batch. In contrast only a single self-packed column of 140cm in diameter operated over 6 cycles per batch can be used to achieve the same batch throughput. The increased number of pre-packed columns operated in parallel requires the addition of multiple chromatography skids. Therefore, indirect costs would not necessarily decrease as it is observed with lower batch throughputs (Figure 4.6) where a single chromatography column of no more than 60cm can be used for each chromatography step.

Overall, a full pre-packed purification train shows comparable COG/g with a full self-packed train. The greatest cost reduction from the introduction of pre-packed columns, of approximately 10%, can be seen at 350kg/year with a manufacturing capacity of 2 x 4000L operating 35 batches annually. As annual product demand increases the drop in

the COG/g starts to disappear. At 1000kg/year with 20 batches and 750kg/year with 75 batches the pre-packed option breaks even with the self-packed option. Finally, the intensive utilisation of the DSP train that's associated with the increased number of batches to maintain constant the batch throughput causes a significant increase in the cost of labour. That increase in the cost of labour is mainly responsible for the higher COG/g compared with the operation of a single USP train. At 1000kg/year with 100 batches there is no significant difference between the pre-packed and the self-packed options. It should be highlighted, that in case of multiple USP trains the indirect costs related with the chromatographic purification train account for a small percentage of the total indirect costs for the whole process. Additionally, considering that direct costs are approximately 3 times higher than indirect costs the pre-packed option shows very similar COG/g with the self-packed option.

Table 4.7: Purification train size across increasing annual product demand with increasing bioreactor capacity using self-packed and pre-packed (SP / PP) columns

Chromatography Step	Parameter	Annual Product Demand (kg/year)				
		200	350	500	750	1000
Capture	No. of Columns	1 / 1	1 / 1	1 / 2	1 / 3	1 / 4
	No. of Cycles	6 / 6	6 / 10	6 / 7	6 / 7	6 / 7
	Diameter (cm)	60 / 60	80 / 60	100 / 60	120 / 60	140 / 60
	Processing (hr)	8/ 8	8 / 14	7 / 10	8 / 10	7 / 10
Intermediate	No. of Columns	1 / 1	1 / 1	1 / 1	1 / 2	1 / 3
	No. of Cycles	7 / 7	7 / 7	5 / 9	5 / 7	6 / 6
	Diameter (cm)	45 / 45	60 / 60	80 / 60	100 / 60	110 / 60
	Processing (hr)	9 / 9	9 / 9	7 / 12	7 / 9	7 / 8
Polishing	No. of Columns	1 / 1	1 / 1	1 / 1	1 / 1	1 / 1
	No. of Cycles	8 / 8	6 / 6	5 / 5	5 / 8	6 / 10
	Diameter (cm)	30 / 30	45 / 45	60 / 60	80 / 60	80 / 60
	Processing (hr)	9 / 9	7 / 7	5 / 5	5 / 8	6 / 11

4.4. Conclusions

The main objective of this chapter was to address the cost-effectiveness of pre-packed chromatography columns when compared to conventional self-packed columns in antibody purification. A series of scenarios were performed investigating

chromatography operations as individual units in order to provide an in depth understanding of the costs associated with self-packed and pre-packed columns. Furthermore, pre-packed chromatography columns were evaluated considering their impact on the complete manufacturing process. Through a scenario analysis the utilisation of pre-packed columns at a range of manufacturing capacities for mAbs using a typical platform process sequence was evaluated. The re-distribution of direct and indirect costs caused by pre-packed columns demonstrated a definitive influence on the decision regarding the selection of the most cost-effective chromatography column type. On the other hand, the size limitations of pre-packed chromatography columns did not appear to hinder their use at large scales of manufacturing. Moreover, in cases where both column technologies offer insignificantly different COG values the use of pre-packed columns could be considered due to the increased flexibility they offer especially in multiproduct facilities. However, the potential loss of in-house column packing know-how should also be weighed against the savings in time and cost due to reduced validation effort for packing and unpacking activities.

Chapter 5. Integration of high-throughput purification data with process economics modelling and decision-support tools

5.1. Introduction

The challenges associated with the development of a chromatographic separation unit operation were discussed in Chapter 1. Additionally, the significance of rational and systematic chromatography resin selection was highlighted in Chapter 3. Previous publications have demonstrated the integration of high-throughput screening (HTS) purification data with optimisation algorithms to identify the optimal resin candidates for a single chromatography step (Nfor et al., 2011) and a two-step purification train (Liu et al., 2017). Other work has focused on developing combinatorial optimisation algorithms for 2-step and 3-step column purification trains with the goal of determining the most cost-effective chromatography resin sequence and column sizing strategy, using advanced evolutionary algorithms (Simaria et al., 2012; Allmendinger et al., 2014) and mathematical programming approaches (Liu et al., 2014). This chapter presents a framework for integrating HTS chromatography data with a process economics model and a decision-support tool for the purification train to optimise all the chromatography steps in a purification train in terms of resin sequence and column type (pre-packed or self-packed columns) for both clinical and commercial manufacture.

This chapter builds on the HTPD workflow illustrated in Chapter 3 and the process economics model illustrated in Chapter 4. A decision-support tool was built as a “bridge” linking the HTPD workflow (Chapter 3) with the process economics model (Chapter 4) in order to promote the evaluation of different chromatography resin candidates from a facility perspective and investigate the impact of different purification trains on the total manufacturing costs. The decision-support tool for the chromatographic purification train was developed and linked with the process economics model. The structure and functionality of the tool were described in Chapter 2, Section 2.4. The main features of the tools are: a brute force algorithm to screen all possible purification trains for their

purification capabilities and a multi-attribute decision-making technique to rank alternatives based on multiple objectives. This tool was necessary to explore in an automated fashion the large decision space when considering multiple resins for multiple chromatography steps.

The rest of Chapter 5 presents two case studies demonstrating the functionality of the proposed decision-support tool for the purification train and its integration with an HTPD workflow and a process economics model. The first case study focuses on the synergy between the process economics engine and the decision-support tool for the purification train. Following the scenario analysis presented in Chapter 4 for a hypothetical mAb multiple chromatographic purification trains are evaluated against the platform considering both self-packed and pre-packed columns. A second case study was formulated in collaboration with MedImmune to screen multiple CEX chromatography resins for a series of mAbs and leverage the high-throughput data through an integrated framework. To accommodate the screening of multiple resins for multiple mAbs certain modifications and improvements were implemented on the central component of the HTPD workflow (Design & Execution cycle, Figure 3.1). Finally, a linear mathematical formulation was applied in an option evaluation method to identify the optimum resin sequence and column type across different manufacturing stages. The integrated framework enabled quality and performance targets as well as productivity and financial objectives to be considered when weighing up different chromatography options.

5.2. Materials and methods

The structure of the proposed decision-support tool for the purification train was discussed in Chapter 2 and illustrated in Figure 2.5. Additionally Chapter 2 discussed the information flow of the process economics model (Figure 2.3) and the main components of the HTPD workflow are presented in Chapter 3 (Figure 3.1). A key objective of this chapter is the consolidation of the work presented and discussed in previous chapters of the thesis. A schematic representation of this consolidation is shown

in Figure 5.1. Following the steps involved in the design and execution cycle of the HTPD workflow a resin database can be generated containing properties for each resin candidate and their respective performance for a particular mAb. The resin database can be imported into the chromatography resin library of the decision-support tool to evaluate the performance of different purification trains.

Successful purification trains that meet user specifications and quality attributes were ranked based on their performance using the weighted sum method (Eq.2.5). For the purposes of resin ranking two attributes were selected. The first attribute is the productivity of the chromatographic purification train (Eq.5.1). The second attribute is a metric that attempts to capture simultaneously the price, the binding capacity and the lifespan of a chromatography resin. This metric is referenced here as the resin cost per gram and is expressed in monetary units per gram of product that can be processed (Eq.5.2). The objective is to maximise productivity and minimise the resin cost per gram of product.

$$\text{Train Productivity (g/L/hr)} = \frac{\sum_{i=1}^{3}(\text{DBC} * \text{Yield})_i}{\sum_{i=1}^{3} t_{CYC,i}} \quad \text{(Eq. 5.1)}$$

$$\text{Resin Cost (£/g)} = \sum_{i=1}^{3}\left(\frac{\text{Resin Price}}{\text{DBC} * \text{Resin Lifespan}}\right)_i \quad \text{(Eq. 5.2)}$$

where:

i: index of chromatography resin in a purification train with 3 steps

t_{CYC}: processing time for a single chromatography step (Eq.3.3)

Properties and performance attributes for each chromatography resin of the top-ranked purification trains were imported into the process economics model in order to adjust assumptions related to each chromatography step, update the cost database with the new resin prices and re-synthesise the purification train to meet the operating

specifications for each successful train. To demonstrate the functionality of the decision-support tool in combination with the process economics model a case study was designed evaluating a number of different purification trains across different manufacturing stages. The annual product demand, the cell culture titre, the number of batches performed annually and the size of the pipeline are summarised in Table 4.5.

One of the most challenging elements of the decision-support tool for the purification train is the chromatography resin library. The great difficulty in creating properly a resin library stems from the large number of factors that could potentially offset the level of confidence in the correct approximation of the performance of a purification train. Although the biotech industry has gained significant experience in antibody purification that has led to the development of platform processes (Shukla et al., 2007) not all mAbs respond equally well to the same purification train. Moreover, chromatography steps and resin types might have been standardised, nevertheless there are several different resin choices from a number of vendors that could fit into the purification train. Additionally, the performance of a chromatography resin in a given position in the purification train is subject to the profile of the impurities delivered by the previous unit operation. For instance, assuming the last chromatographic polishing step has a tolerance for HMW species of 5% that would require the previous steps in downstream processing to deliver an antibody solution with less than 5% of HMW species. Such heuristics have been presented in the literature in order to demonstrate the implementation of different approaches in optimisation of antibody purification processes (Simaria et al., 2012; Liu et al., 2014).

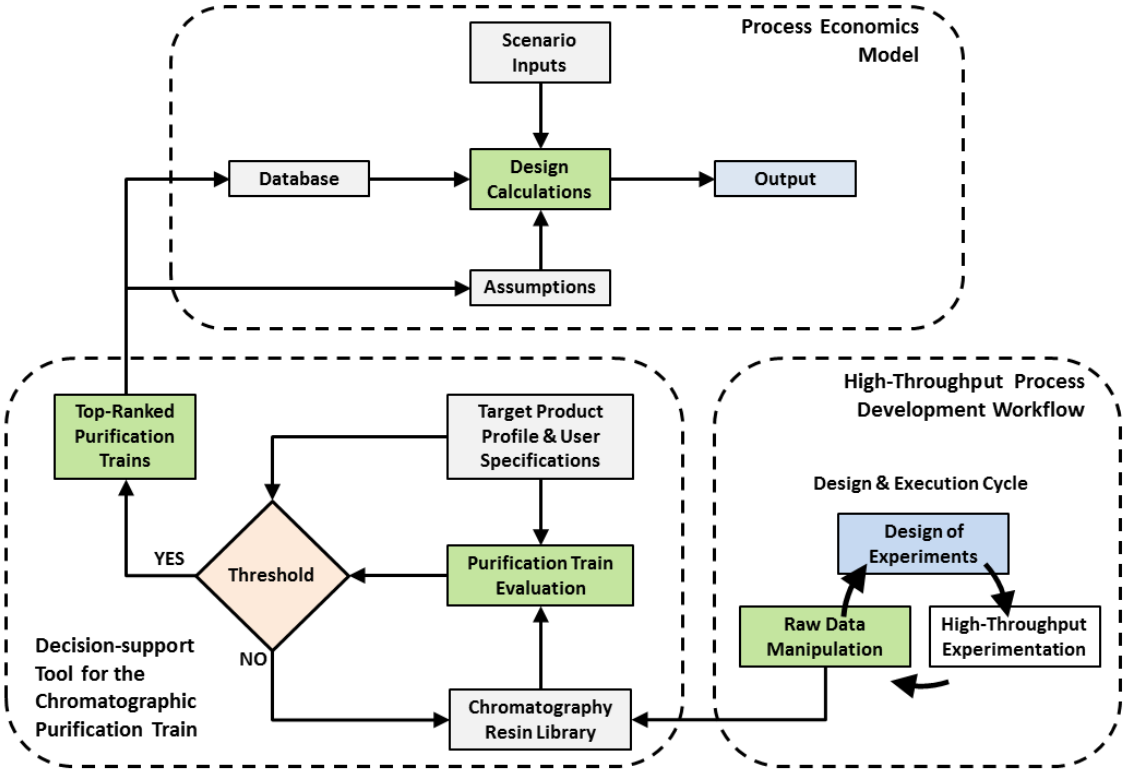


Figure 5.1: Integrated framework in chromatography process development. Schematic illustration of the consolidation of a process economics model, a decision-support tool for the chromatographic purification train and a high-throughput process development workflow

A similar approach was followed here to demonstrate the synergistic functionality of the decision-support tool for the purification train and the process economics model (Figure 5.1). For the purposes of the first case study a conceptual chromatography resin library was created assuming resin properties and performance inspired by the literature, application notes from different chromatography resin vendors and the industrial sponsor (MedImmune). The conceptual resin library for a hypothetical mAb is presented in Table 5.1. A number of different performance characteristics were assumed for each resin along with its position in the purification train. A level of uncertainty was introduced regarding the impurities profile delivered by the primary recovery and clarification process. The overall aim was to identify the optimum resin sequence and column type for each profile of impurities across pre-clinical, clinical and commercial manufacturing. A critical assumption made in the process economics model is the ability of the proposed platform purification train to deliver the desired target product profile. The first case study challenges this assumption by introducing different impurities loads for the same mAb

and evaluating the economics of multiple purification trains that could meet the target product profile. The profiles of different impurities along with quality and performance targets for a purification train are presented in Table 5.2.

Both attributes (productivity and resin cost) that were selected to compare the performance of different purification train using the weighted sum method have an impact on the COG. Parameters such as yield, dynamic binding capacity, processing time and resin price dictate the size of unit operations and the manufacturing costs. Therefore, for the purpose of the first case study each attribute in the score function of the decision-support tool was considered equally important ($w = 0.5$).

The first case study had as the main objective to present the integration of a decision-support tool with a process economics model. Nevertheless, the true purpose of the decision-support tool is to act as a link between the HTPD workflow and the process economics model. Hence, a second case study was formulated to demonstrate this consolidation. Impurities profiles post primary recovery and clarification are shown in Table 5.2. Four different mAbs partially purified using Protein A chromatography following the process as discussed in Chapter 2 were provided by MedImmune. High-throughput experimentation was performed as described in Chapter 2 for the intermediate chromatography step to screen different CEX resins. Finally, a mixed-mode (hydrophobic interaction and anion-exchange) resin was considered for the last chromatography step in the purification train. Assumptions regarding the properties and the performance of the capture and polishing steps for each mAb are presented in Table 5.4. It should be highlighted that high-throughput experimentation was performed only for the intermediate chromatography step (CEX) while the values summarised in Table 5.4 for the capture and polishing steps were derived after discussions with the industrial collaborator, MedImmune.

Table 5.1: Conceptual chromatography resin library for a hypothetical mAb used for the purpose of demonstrating the synergistic functionality of a decision-support tool for the purification train and a process economics model (Case study 1)

Resin Name	Position	Mode of Operation	DBC (g/L)	Yield	Linear Velocity (cm/hr)	Elution CVs	Wash CVs	Resin Lifespan (#cycles)	HMW Reduction (%)	LMW Reduction (%)	HCPs LRV	Price ($/L)
Platform Protein A	1	Bind and Elute	40	97%	350	2.5	2.0	50	15	15	3.5	12000
Platform CEX	2	Bind and Elute	60	93%	350	4.0	2.0	100	85	90	2.5	4000
Platform AEX	3	Flow-Through	100	97%	350	0.0	0.0	100	75	50	2.0	3000
Protein A - 1	1	Bind and Elute	50	90%	250	3.5	2.0	50	15	15	3.5	13000
Protein A - 2	1	Bind and Elute	40	95%	350	3.0	2.0	100	45	35	2.5	16250
Protein A - 3	1	Bind and Elute	30	95%	350	2.5	2.0	50	25	20	2.0	11050
CEX - 1	1	Bind and Elute	65	85%	400	4.0	2.0	100	65	55	1.1	4550
CEX - 3	1	Bind and Elute	75	90%	500	3.5	2.0	80	50	75	1.6	2730
MMCEX - 1	1	Bind and Elute	65	90%	300	5.0	2.0	50	20	60	2.5	5200
MMCEX - 2	1	Bind and Elute	40	95%	450	3.5	2.0	50	80	90	2.0	6500
CEX - 1	2	Bind and Elute	90	85%	500	3.0	2.0	100	80	75	0.5	4550
CEX - 5	2	Bind and Elute	100	90%	350	4.0	2.0	80	75	50	2.4	4940
CEX - 6	2	Bind and Elute	55	95%	300	3.0	2.0	100	85	80	1.2	3900
MMAEX - 2	2	Bind and Elute	60	95%	350	4.0	2.0	60	60	50	1.7	3640
MMAEX - 1	2	Flow-Through	80	95%	300	0.0	0.0	100	45	60	1.4	3900
AEX - 1	2	Flow-Through	75	95%	300	0.0	0.0	150	30	90	0.2	1300
AEX - 2	3	Flow-Through	100	95%	450	0.0	0.0	100	75	75	1.1	5200
MMAEX - 3	3	Flow-Through	80	90%	350	0.0	0.0	100	65	80	2.3	4940
MMAEX - 4	3	Flow-Through	100	95%	400	0.0	0.0	100	85	85	1.5	5850
CEX - 2	3	Bind and Elute	65	90%	250	2.5	2.0	100	60	75	1.0	3770
CEX - 4	3	Bind and Elute	70	95%	350	5.0	2.0	100	80	35	1.5	2340

Table 5.2: Profile of impurities delivered by the primary recovery and clarification process to the first chromatography step (capture) and quality and performance targets as received by the decision-support tool for the purification train

	Case study 1				Case study 2			
	Light	Lower Medium	Upper Medium	Heavy	mAb 1	mAb 2	mAb 3	mAb 4
HMW_{IN} (%)	3.0	6.0	12.0	20.0	6.1	6.3	7.1	20
LMW_{IN} (%)	1.0	2.0	5.0	7.0	0.0	0.1	0.0	0.0
$HCPs_{IN}$ (10^6 ng/mg)	1.0	1.5	3.5	5.0	4.0	1.1	3.6	NA
$Yield_{Target}$ (%)	75.0				Unconstrained			
HMW_{Target} (%)	0.4				0.4	0.4	0.4	1.5
LMW_{Target} (%)	0.1				0.1	0.1	0.1	0.1
$HCPs_{Target}$ (ng/mg)	100.0				100.0	100.0	100.0	NA
$w_{productivity}$	0.5				0.0 – 1.0			

Note: $Yield_{Target}$ refers to the yield of the chromatographic purification train

Case study 1 considered alternative resins for all three chromatography steps. In contrast for Case study 2 it was assumed a certain resin for the capture step and the polishing step. This difference could have a significant impact on the ability of the certain decision attributes to compare efficiently different purification trains. For instance, the high price of a Protein A resin and its relatively low binding capacity could dominate the resin cost per gram (Eq.5.2). Therefore, any small deviations among different CEX resin prices could become undetected or even misleading in predicting the most cost-effective train. In order to determine if this indeed was the case in Case study 2 the weight coefficients were varied for 0 to 1.

The link between the decision-support tool for the purification train and the HTPD workflow was achieved through the modification of the fractionation diagram approach. The fractionation diagram approach as described in Chapter 2 and used in Chapter 3 considered only HMW species as the only impurity in the antibody solution neglecting other product and process-related impurities. In contrast, this chapter requires the evaluation of host cell proteins (HCPs) and LMW species along with HMW species. Hence, the fractionation diagram approach had to be extended in order to account also for HCPs. Samples collected through high-throughput experimentation were sent for an in-house analysis by Medimmune to measure the concentration of HCPs using ELISA on a disc. HCPs concentration values were received in units of ng/ml thus for a given fraction volume the mass of HCPs in each fraction (f) can be calculated. Additionally, following the same methodology in the fractionation diagram approach, the cumulative mass faction of HCPs (Z) can be estimated using Eq.5.3. Finally, each pair of purity-yield corresponds to a concentration of HCPs in the elution pool which can be determined as described in chapter 2 and initially proposed by Ngiam et al. (2001). By determining the elution pool volume, the required fractions for collection are identified thus enabling the estimation of the total mass of HCPs collected in the elution pool.

$$Z = \frac{\sum_{f}^{f+n} M_{HCPs,f}}{\sum_{1}^{F} M_{HCPs,f}} \qquad \forall\, n \in \mathbb{N}, \qquad n \leq F - 1 \qquad (Eq.\,5.3)$$

The design and execution cycle illustrated in Figure 3.1 was followed for mAb-1, mAb-2 and mAb-3 shown in Table 5.2 (Case study 2) to screen eight CEX resins: Poros™ XS and Poros™ HS50 (Life Technologies, California U.S.A), Toyopearl® GigaCap S-650(S) and Toyopearl® GigaCap CM-650(M) (Tosoh Biosciences, Redditch UK), Capto™ S Impact (GE Healthcare Life Sciences, Buckinghamshire UK), Fractogel® EMD COO^- (M) and Eshmuno® CPX (EMD Millipore, Darmstadt Germany) and Nuvia™-HRS (Bio-Rad Laboratories, California U.S.A). On the other hand, mAb-4 is the same molecule as the one used to demonstrate the HTPD workflow in Chapter 3 and due to low availability of additional feedstock material the performance of each CEX resin was obtained from the

results of the screening part of the HTPD workflow in Chapter 3. As a consequence data on HCPs for mAb-4 are not available since HCPs were not considered in the initial configuration of the fractionation diagram approach. For the rest of the molecules in Case study 2 (mAb-1 to mAb-3) raw data manipulation was performed applying the modifications implemented in the fractionation diagram approach to include HCPs.

In order to simplify and accelerate experimentation only one set of operating conditions was evaluated for each mAb, thus eliminating the need for the development of a DoE space. This approach can rapidly provide information regarding the fit of a CEX resin into a platform purification train, although operating under the same conditions does not guarantee the optimum performance for every CEX resin for a given mAb. Nevertheless, the implementation of the fractionation diagram approach offers the ability to identify several purity-yield pairs thus promoting a comprehensive evaluation of a given set of operating conditions (i.e. pH, linear velocity etc.). Furthermore, due to the structure of the chromatography resin library the same resin can be evaluated multiple times simply by assigning a different index value to it every time it is imported into the library under different conditions.

Results from the implementation of the design and execution cycle led to the construction of a chromatography resin library for each mAb. The resin library that was generated for each mAb can be found in the Appendix (Table A.4 – A.7). Subsequently, the decision-support tool evaluated all possible CEX resins that can be used in the intermediate step of the purification train and can achieve quality and performance specifications given in Table 5.2. Then each successful purification train was assigned a score and ranked against each other. Finally, properties related to each top-ranked purification train were imported to the process economics model to evaluate its cost-effectiveness considering the complete manufacturing process.

For the purposes of the Case study 2, the 4 mAbs included in the evaluation are considered to have successfully passed the pre-clinical stage of the drug development

pathway and preparing to enter clinical trials. Therefore, the manufacturing costs were estimated for the clinical phase I & II, clinical phase III and commercial stage. The scenario inputs used for Case study 2 are summarised in Table 5.3, assuming only these four mAbs in the pipeline of the facility at early stage in the clinical trials and the commercialisation of a single mAb.

Table 5.3: Scenario inputs in the process economics model used in Case study 2 to demonstrate the implementation of the integrated framework

Stage	Total Demand (kg/year)	Cell Culture Titre (g/L)	No. of Batches	Pipeline
Clinical Phase I & II	5	2.5	1	4
Clinical Phase III	20	5	2	1
Commercial	200	5	20	1

The overall objective here was to identify the optimum CEX resin candidate for each mAb to be included in the purification train across clinical and commercial manufacture and minimise the total COG. The flow of information in the integrated framework is presented in Figure 5.2. Purification data can be generated rapidly through the design and execution cycle of the HTPD workflow and create a database with a purification profile for each resin candidate. That resin database is then imported into the chromatography resin library of the decision-support tool for the purification train using a brute force algorithm to screen all possible resin sequences and creates a record with the purification train that have successfully met the quality and performance threshold. The target values for quality and performance attributes that were considered in both case studies and for each mAb are shown in Table 5.2 along with the impurities loads.

All successful purification trains are ranked based on the cost of the resin sequence and the productivity of the train using the weighted sum method. Then the user can specify the number of these successful purification trains for further evaluation using the process economics model. For instance, it might be desirable to evaluate the top-5 or the top-10

from the pool of successful purification trains. The decision-support tool allows the user to specify the size of pool containing the top-ranked purification trains and import them into the process economics model. Once a purification train has been imported into the process economics model relevant assumptions are adjusted to simulate the performance of the new train and generate a COG value.

In order to identify the optimum purification train for each mAb it is important to recognise the possibility that a certain train might not be the most cost-effective throughout different manufacturing stages. Moreover, the decision regarding the selection of the appropriate resin sequence should be consistent throughout all manufacturing stages in order to avoid introducing a different chromatography resin in the purification train at later development stages. Furthermore, pre-packed columns were evaluated in both case studies at each manufacturing stage and for every successful purification train as determined by the decision-support tool. Hence, in this chapter the investigation of different purification strategies was performed considering three main factors: the manufacturing stage “m”, the purification train (or resin sequence) “p” and the chromatography column type “c”. Additionally, the final decision was based on the cumulative COG achieved for each combination of manufacturing stage, resin sequence and chromatography column type ($COG_{m,p,c}$).

The mathematical formulation for the option evaluation method used to identify the optimum purification strategy for each mAb in both case studies is as follows. A binary variable ($b_{m,p,c}$) was assigned to each $COG_{m,p,c}$ value since the decision to choose any purification strategy (m,p,c) would be either true or false. To achieve the unique selection of a resin sequence across all manufacturing stages an additional set of binary variables (I_p) was introduced along with a set of constraints to ensure the consistency of the selected resin sequence throughout all manufacturing stages (Eq.5.4 to Eq.5.6). Finally optimisation was performed in MS Excel by employing the Simplex LP engine in SOLVER optimisation tool add-in with the objective to minimise the sum of COG across all the manufacturing stages (Eq.5.7). The abbreviations MM, MP and MC indicate the

maximum number of manufacturing stages, alternative purification trains (resin sequences) and column type configurations, respectively.

Binary Variables: $\mathrm{b},\ \mathrm{I} \in [0,1]$

Constraints:

$$\sum_{p=1}^{MP}\sum_{c=1}^{MC} b_{m,p,c} = 1 \quad \forall\, m \in [1, MM] \qquad (Eq.\ 5.4)$$

$$\sum_{m=1}^{MM}\sum_{c=1}^{MC} b_{m,p,c} = MP * I_p \quad \forall\, p \in [1, MP] \qquad (Eq.\ 5.5)$$

$$\sum_{p=1}^{MP} I_p = 1 \qquad (Eq.\ 5.6)$$

Objective function:

$$\min\left[\sum_{m=1}^{MM}\sum_{p=1}^{MP}\sum_{c=1}^{MC}\left(b_{m,p,c} * COG_{m,p,c}\right)\right] \qquad (Eq.\ 5.7)$$

Table 5.4: Key assumptions regarding the properties and the performance of platform resins for the capture and polishing chromatography steps for each mAb involved in Case study 2

mAb	Resin Name	Mode of Operation	Position	DBC (g/L)	Yield (%)	Linear Velocity (cm/hr)	Elution CVs	Wash CVs	Resin Lifespan (#cycles)	HMW Reduction (%)	HCPs LRV	Price ($/L)
1	Protein A	Bind & Elute	1	40	95	300	3.5	2.0	50	10.0	3.5	10000
	MM-AEX	Flow-Through	3	80	97	300	0.0	0.0	100	90.0	1.0	3200
2	Protein A	Bind & Elute	1	40	95	300	3.2	2.0	50	10.0	3.5	10000
	MM-AEX	Flow-Through	3	80	99	300	0.0	0.0	100	90.0	1.0	3200
3	Protein A	Bind & Elute	1	40	97	300	2.5	2.0	50	10.0	3.5	10000
	MM-AEX	Flow-Through	3	90	99	300	0.0	0.0	100	90.0	1.0	3200
4	Protein A	Bind & Elute	1	30	93	300	3.0	2.0	50	10.0	NA	10000
	MM-AEX	Flow-Through	3	65	95	300	0.0	0.0	100	90.0	NA	3200

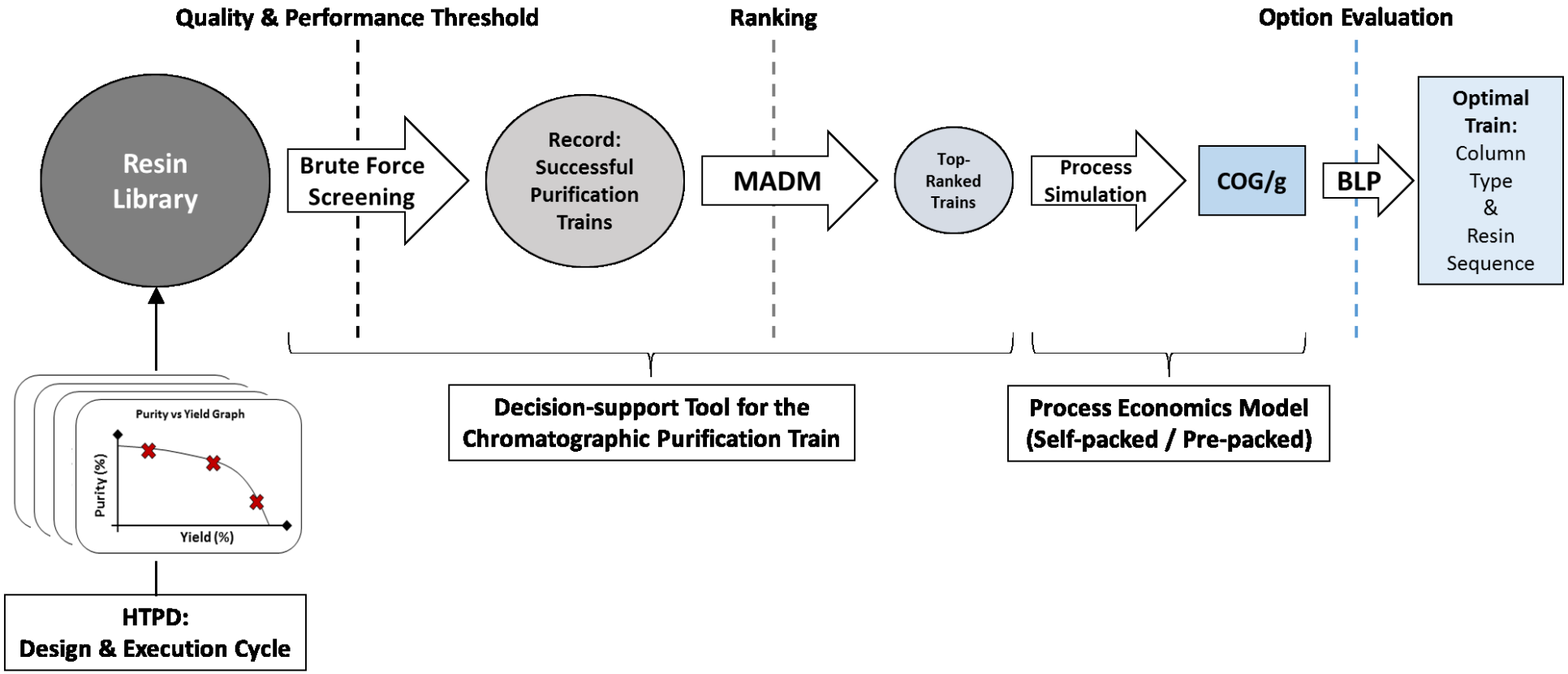


Figure 5.2: Schematic illustration of the information flow within the integrated framework. HTPD: High-Throughput Process Development, MADM: Multi-Attribute Decision-Making, COG/g: Cost of goods per gram of product, BLP: Binary Linear Programming

5.3. Results and discussion

The overall objective of this chapter was to further leverage the work presented in previous chapters. An integrated framework in chromatography process development is demonstrated combining high-throughput experimentation with process economics and decision-making analysis. A decision-support tool for the purification train was developed as described in Chapter 2 to link a HTPD workflow for the chromatographic purification of therapeutic antibodies with a process economics model for bio-manufacturing facilities. Initially, a case study was formulated to present the synergy between the process economics model and the decision-support tool. A second case study followed to demonstrate the implementation of the integrated framework using four mAbs provided by MedImmune.

5.3.1. Case study 1: Decision-support tool and process economics model synergy

For the purpose of Case study 1, according to the number of chromatography resins available for each position in the purification train (Table 5.1) there are more than 300 possible purification trains. Figure 5.3 presents the number of successful purification trains across different impurities profiles. As impurities become more challenging the number of successful trains decreases. The fewest purification train alternatives that were identified by the decision-support tool correspond to the heavy load of impurities. In order to proceed with the process economics evaluation and optimisation the size of the top-ranked train pool was set to the minimum number of successful purification trains across all impurities loads.

The top-six purification trains for each impurities load were further evaluated considering the whole manufacturing process. Additionally, for each top-ranked purification train the process economics model simulated its operation using either a full self-packed or a full pre-packed column train. Insights that were gained through the evaluation of pre-packed chromatography columns (Chapter 4) concluded that the greatest cost benefits can be observed when all three chromatography steps operate using pre-packed columns.

Hence, in this chapter only the full self-packed and the full pre-packed configurations of the purification train were evaluated in order to simplify and accelerate the scenario analysis. The comparison between self-packed and pre-packed purification trains is consistent through different impurities loads with relatively small deviations. The changes caused in the COG/g from the introduction of a full pre-packed column train against a full self-packed train with the same resin sequence are presented in Figure 5.4. Pre-packed columns demonstrate a reduction in the COG/g at pre-clinical, clinical phase III and commercial scale with the greatest benefits observed at phase III of approximately 6%. On the other hand, a small increase in the COG/g using pre-packed columns appears at clinical phase I & II. The same trend was also noticed in Chapter 4 and it was expected here since the same scenario inputs were used. The error bars in Figure 5.4 represent the standard deviation of the COG/g among the top-ranked trains.

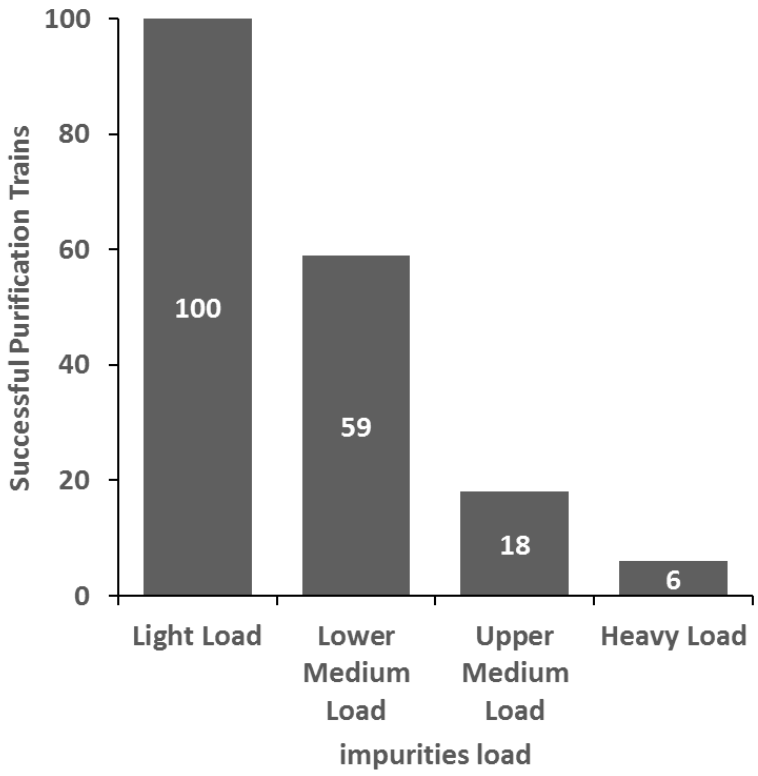


Figure 5.3: Number of successful purification trains for different impurities loads as identified using the decision-support tool for the chromatographic purification train

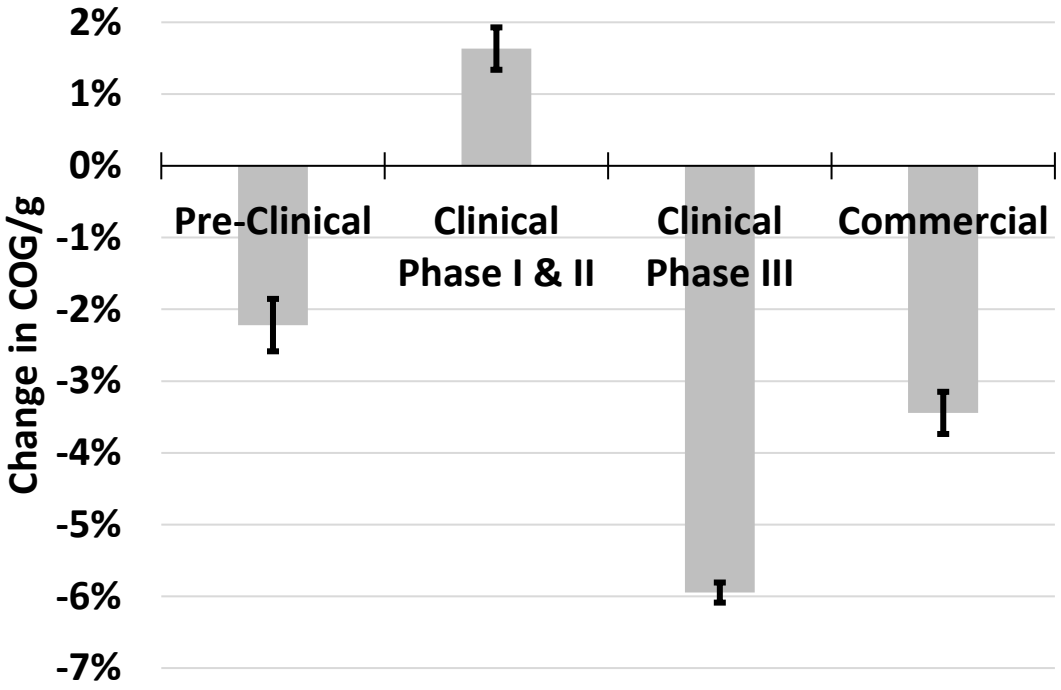


Figure 5.4: Average change in the COG/g among different loads of impurities for a full pre-packed train relative to a full self-packed train across all manufacturing stages. Error bars show two standard deviations

According to Figure 5.4 the decision to introduce pre-packed chromatography columns in the purification train is consistent throughout different impurities loads and across all top-ranked purification trains. The most cost-effective configuration would be a full self-packed purification train for the manufacture of material for clinical phase I & II and a full pre-packed purification train for pre-clinical, clinical phase III and commercial manufacture.

Using the weighted sum method each successful purification train was assigned a score expressing its performance it terms of productivity and the resin cost per gram of product. Figure 5.5 plots the top-six purification trains for each impurities load based on their respective resin cost and productivity values. According to Figure 5.5 as the load of impurities increases the purification trains that manage to meet the quality and performance threshold achieve a high resin cost and a low productivity. At a light load of impurities the vast majority of the top-ranked purification trains are located on the bottom-right quadrant of the scatter plot in Figure 5.5 with high productivity and low resin cost. On the other hand, at a heavy load of impurities top-ranked purification trains are mostly

located on the top-left quadrant of the scatter plot with low productivity and high resin cost. It is worth highlighting that the top-ranked purification trains for the lower and upper impurities loads demonstrate almost parallel profiles with average productivity and high resin cost.

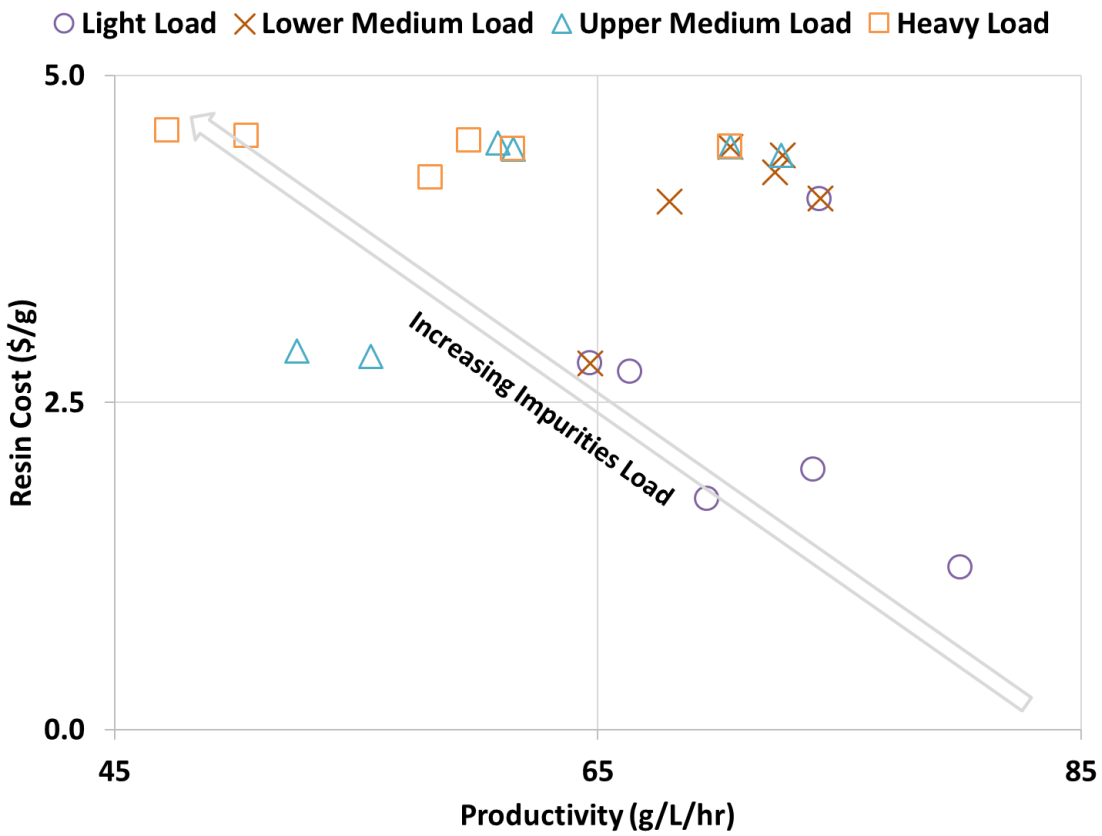


Figure 5.5: Resin cost per gram of product against productivity of the purification train considering the top-six purification trains for different loads of impurities

Due to the fact that both the resin cost and productivity have a direct impact on the COG, the top-six purification trains for each impurities load were evaluated considering the whole manufacturing process using the process economics model. A summary of key results for each purification train is presented in Table 5.5. For each impurities load, Table 5.5 provides the top-six resin sequences with descending score order along with the train yield and the delivered impurities profile. The last column in Table 5.5 shows the cumulative COG across pre-clinical, clinical and first year of commercial manufacturing. Highlighted purification trains in Table 5.5 indicate the train with the minimum cumulative COG for each load of impurities.

Table 5.5: Top-six purification trains and their respective quality and performance profile for Case study 1

Impurities Load	Capture	Intermediate	Polishing	Score	Train Yield (%)	HMW Out (%)	LMW Out (%)	HCPs Out (ng/mg)	Total Clinical & first Year Commercial COG ($M)
Light	CEX-3	MMAEX-1	Plat. AEX	100.0%	82.9	0.26	0.06	10.0	35.0
	CEX-3	MMAEX-2	AEX-2	88.5%	81.2	0.19	0.04	39.8	36.9
	CEX-3	MMAEX-2	Plat. AEX	85.3%	82.9	0.19	0.08	5.0	35.9
	MMCEX-1	CEX-5	AEX-2	75.4%	77.0	0.20	0.07	1.0	38.3
	MMCEX-2	CEX-1	Plat. AEX	74.9%	78.3	0.04	0.02	31.6	39.1
	MMCEX-1	CEX-5	MMAEX-4	73.2%	77.0	0.12	0.04	0.4	38.3
Lower Medium	MMCEX-2	CEX-1	Plat. AEX	87.9%	78.3	0.08	0.03	47.4	39.1
	MMCEX-1	CEX-5	MMAEX-4	85.0%	77.0	0.25	0.08	0.6	38.3
	MMCEX-2	MMAEX-1	AEX-2	83.7%	85.7	0.21	0.03	47.4	38.9
	MMCEX-2	CEX-5	AEX-2	83.0%	81.2	0.10	0.03	4.7	39.0
	MMCEX-2	CEX-5	MMAEX-4	79.5%	81.2	0.06	0.02	1.9	39.0
	MMCEX-2	MMAEX-1	Plat. AEX	79.4%	87.5	0.20	0.05	6.0	38.3
Upper Medium	MMCEX-2	CEX-5	AEX-2	86.7%	81.2	0.22	0.09	11.1	39.0
	MMCEX-2	CEX-5	MMAEX-4	83.0%	81.2	0.13	0.06	4.4	39.0
	MMCEX-1	Plat. CEX	MMAEX-4	75.4%	79.5	0.33	0.05	1.1	38.2
	MMCEX-1	CEX-6	MMAEX-4	70.6%	81.2	0.32	0.09	22.1	37.7
	MMCEX-2	Plat. CEX	AEX-2	70.2%	83.9	0.13	0.02	8.8	39.4
	MMCEX-2	CEX-5	MMAEX-3	68.8%	77.0	0.33	0.08	0.7	40.1
Heavy	MMCEX-2	Plat. CEX	Plat. AEX	73.4%	85.7	0.24	0.06	1.6	38.8
	MMCEX-2	CEX-5	MMAEX-4	67.2%	81.2	0.25	0.09	6.3	39.0
	MMCEX-2	Plat. CEX	AEX-2	50.2%	83.9	0.24	0.03	12.6	39.4
	MMCEX-2	Plat. CEX	MMAEX-4	37.2%	83.9	0.15	0.02	5.0	39.5
	MMCEX-2	Plat. CEX	MMAEX-3	12.9%	79.5	0.36	0.02	0.8	40.6
	MMCEX-2	CEX-6	MMAEX-3	0.0%	81.2	0.35	0.05	15.8	40.3

According to Table 5.5, in most cases the lowest cumutive COG can be observed with purification trains that have managed to achieve a high place in the ranking list. An exception is for the upper medium load of impurities where the fourth ranking purification train demonstrated the lowest cumulative COG however with a small difference from its alternatives. The most cost-effective (first option) purification train for each impurities load is plotted in Figure 5.6. Although at each load of impurites a different resin sequence demonstrated the lowest cumulative COG, there is a common resin sequence among the heavy and the two medium impurity profiles. This common resin sequence is also part of the list with the successful purification trains for the light impurities load. However, its avarage productiviry and high resin cost prevented its progression to the top-ranked list. Nevertheless, it is of high value to identify resin sequences that could handle a variaty of impurity profiles especially at early stage in process development. The value stems from the uncertanty at early stage that is assoiated with the profile of the impurities that could be delivered to the chromatographc purification train.

Additionally, Figure 5.6 illustrates the resin sequence and the cumuative COG for each purification train and plots the platform puritication train to visualise how it compares with the proposed alternatives. It should be highlighted that all the alternative purification trains suggest the replacement of the Protein A resin for the capture chromatography step.

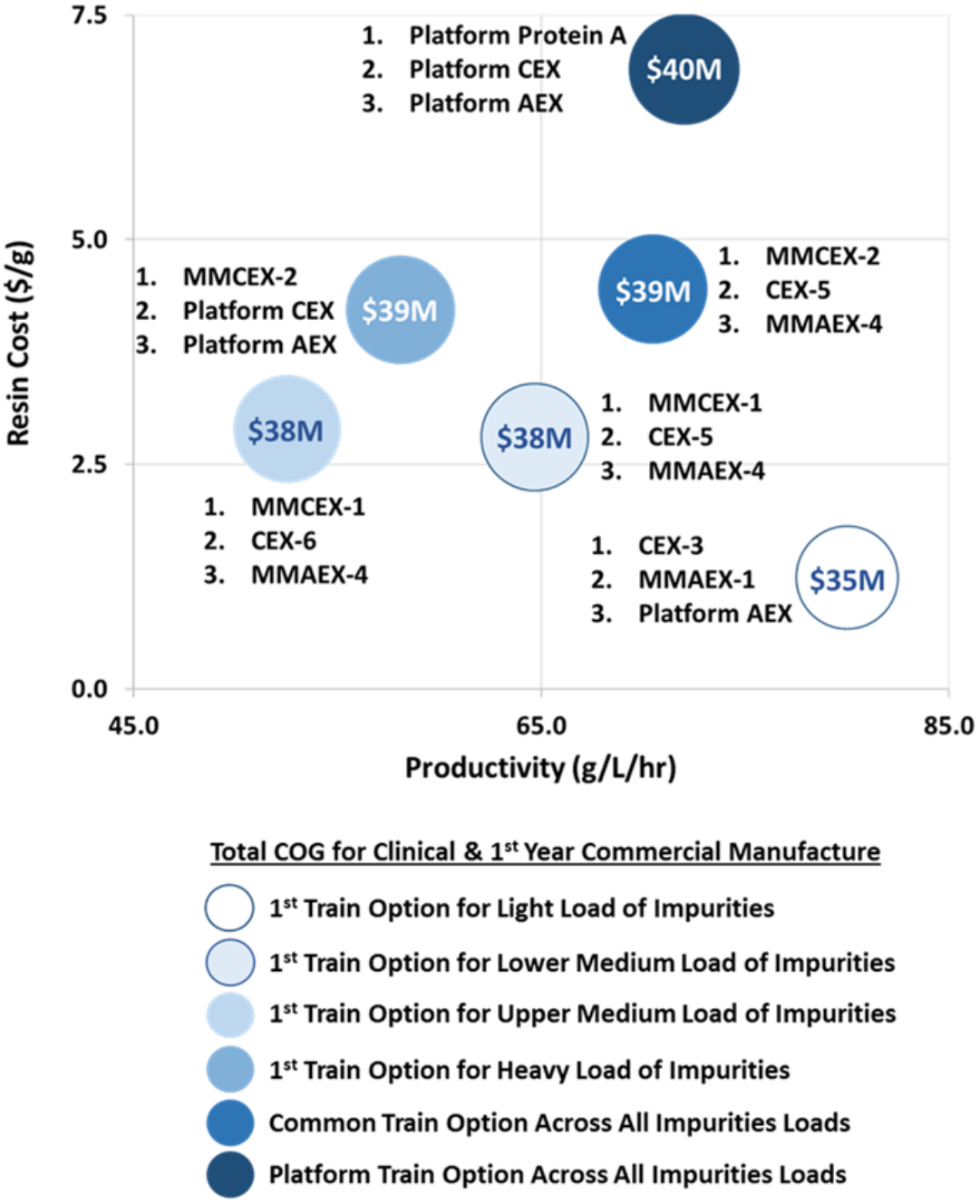


Figure 5.6: Resin cost per gram of product against productivity of the purification train graph illustrating the most cost-effective (first option) purification train at different loads of impurities. A common purification train option and the platform option are also plotted. The resin sequence for each purification train is indicated along with the cumulative COG for pre-clinical, clinical and first year of commercial manufacture

5.3.2. Case study 2: Integrated framework in chromatography process development

An industrially-relevant case study was formulated to demonstrate the implemenation of the consolidation of the HTPD workflow (Chapter 3) with the process economics model

(Chapter 4) and the decision-support tool for the purification train. Due to the lack of experimental data for the capture and polishing chromatography steps for the four mAbs provided by MedImmune the evaluation here refers only to the intermediate chromatography step. Hence, resin screening and analysis considered certain resins for the capture and the polishing steps (Table 5.4). A consequence of this formulation is the low responsiveness of the resin cost metric to different CEX resins introduced in the purification train. Figure 5.7 presents the average and the standard deviation for the productivity and the resin cost across all four mAbs. The first three mAbs are represented with a single bar due to their similar performance. For the fourth mAb a separate bar was needed due to its signifiant differnent performance. It should be highlighted that for the fourth mAb the decsion-support tool could not identify a CEX resin among those tested that could fit into the given purification train and deliver the desired product profile. Instead, for the fourth mAb, the tolerance for HMW species had to be increased to 1.5% in order to identify any purification trains for further evaluation for Case study 2.

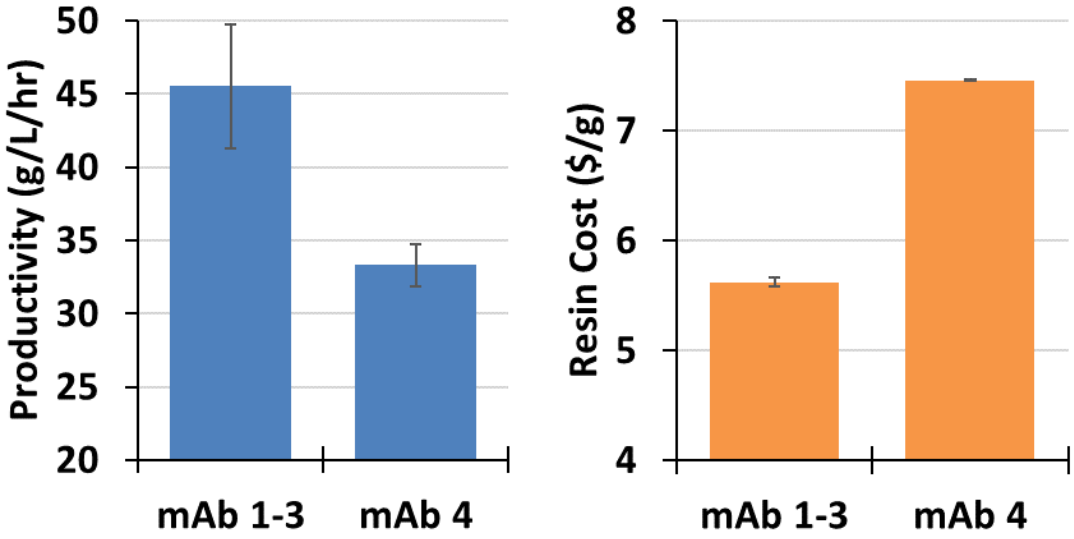


Figure 5.7: Average productivity and resin cost across all successful purification trains that have met their respective quality and performance threshold. Error bars indicate two standard deviations

As expected the resin cost across all successful purification trains demonstrates almost a constant value of 5.7$/g for mAbs 1-3 and 7.4$/g for mAb 4. Hence, the resin cost metric is unable in this case to provide a good comparator for different purification trains.

In order to neglect the resin cost per gram of product and rank the successful purification train only based on their productivity the weight coefficient for productivity was set to 1.0.

Similarly to Case study 1, the top-six purification trains were selected for each mAb to be imported into the process economics model and simulate their respective COG values. For each top-ranked purification train and each mAb the process economics model simulated the COG for a full self-packed and a full pre-packed purification train. A comparison between full self-packed and full pre-packed purification trains across all 4 mAbs is presented in Figure 5.8. Throughout different manufacturing stages pre-packed columns demonstrate a reduction in the COG compared to self-packed columns, regardless of the resin sequence and the mAb. In contrast to Chapter 4 and Case study 1 of this chapter, here pre-packed columns demonstrate cost benefits throughout clinical manufacture. The difference can be observed for clinical phase I & II and the root cause of it can be explained considering the different size of the pipelines. For instance, Figure 5.4 considers 9 products in the pipeline (Case study 1) while Figure 5.8 assumes only 4 products at clinical phase I & II (Chapter 2). That difference translates into a change in the direct to indirect costs ratio which has a definitive impact of the cost-effectiveness of pre-packed columns as discussed in Chapter 4.

A summary of the top-ranked CEX resins for each mAb is presented in Table 5.6. The resin indices refer to the resin lists in the Appendix (Table A.4 – A.7) for each mAb respectively. Different CEX resin options are presented in a descending order from high to low productivity. Additionally, only the concentration of HMW species and HCPs in the final product are indicated since the initial concentration of LMW species was very low and below the specified targets in Table 5.2.

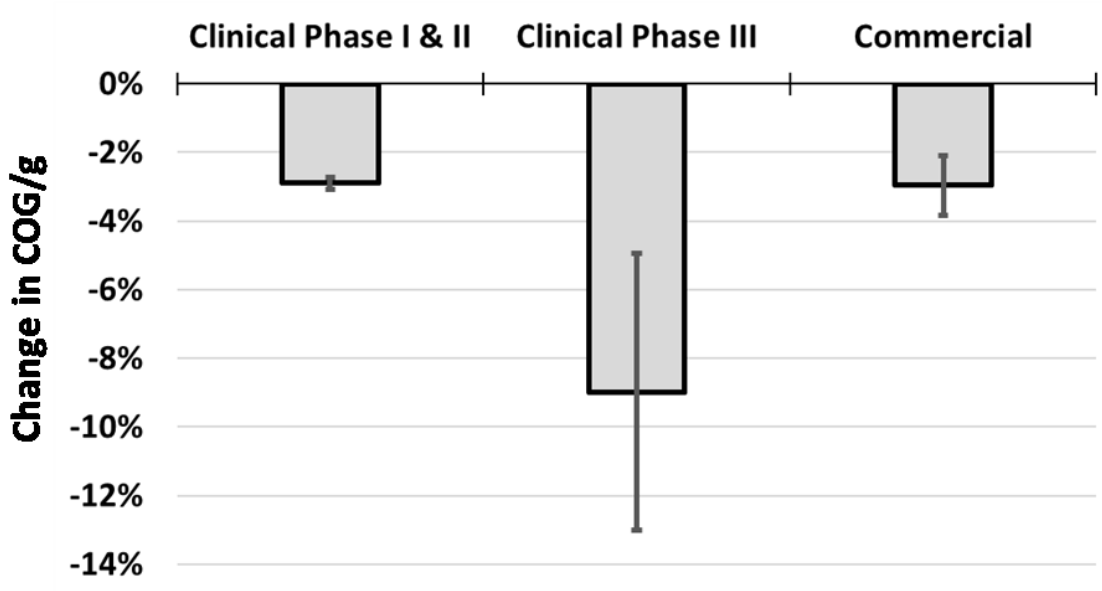


Figure 5.8: Average percentage change in COG/g for pre-packed purification trains relative to self-packed at different manufacturing stages across the top-ranked purification trains and across all 4 mAbs

The last column in Table 5.6 shows the cumulative COG for clinical (phase I, II & III) and first year commercial manufacture and highlighted rows indicate the CEX resin that demonstrated the lowest cumulative COG. The cumulative COG values in Table 5.6 correspond to purification trains that use pre-packed columns throughout all chromatography steps. For mAb-1 and mAb-2 the first option with the highest productivity train resulted also in the most cost-effective option. In contrast, for mAb-3 and mAb-4 the CEX resins that resulted in the lowest cumulative COG achieved a lower position in the top-ranked list. Nevertheless, the differences in productivity among the top-six CEX resins for each mAb were relatively low. On the other hand, greater deviations can be observed among the train yields for different CEX resins. Hence, the cumulative COG was plotted against the purification train yield to determine their correlation. Evidently, Figure 5.9 shows a negative correlation between COG and yield. Increasing train yield results in a reduction in the cumulative COG throughout different mAbs. The yield of the purification train was defined as a constraint in the decision-support tool for the purposes of the Case study 1. In contrast, for Case study 2 that constraint was removed in order to focus the brute force screening on determining CEX resins capable of removing impurities.

Table 5.6: Top-six CEX resins and their respective quality and performance profile for Case study 2

mAb	CEX Resin (index)	Train Productivity (g/L/hr)	Train Yield (%)	HMW Out (%)	HCPs Out (ng/mg)	Total Clinical & first Year Commercial COG ($Million)
1	R5 (32)	44.8	77.9%	0.39	69.8	46.5
	R7 (20)	44.4	69.8%	0.35	61.2	48.5
	R5 (24)	44.0	72.9%	0.31	61.1	47.3
	R7 (12)	43.6	65.2%	0.28	57.8	50.1
	R3 (6)	43.3	62.8%	0.39	75.1	50.6
	R5 (16)	43.2	68.2%	0.25	55.0	48.9
2	R7 (20)	46.5	70.7%	0.40	6.3	47.3
	R7 (12)	45.3	66.3%	0.35	6.4	48.7
	R5 (24)	44.4	69.9%	0.38	5.2	48.1
	R7 (4)	44.3	61.9%	0.31	6.4	50.6
	R5 (16)	43.4	65.5%	0.33	5.1	49.2
	R5 (8)	42.5	61.1%	0.29	4.9	50.5
3	R7 (20)	53.2	77.7%	0.33	49.3	45.6
	R7 (12)	52.1	72.5%	0.25	46.8	46.9
	R5 (24)	51.1	82.1%	0.33	48.3	45.0
	R7 (4)	50.9	67.6%	0.19	44.2	48.4
	R5 (16)	50.3	76.8%	0.27	43.9	46.0
	R5 (8)	49.5	71.6%	0.21	35.8	46.8
4	R1 (17)	35.4	57.5%	1.39	0.0	54.4
	R7 (20)	35.1	60.1%	1.36	0.0	53.8
	R1 (9)	34.4	53.2%	1.25	0.0	56.6
	R7 (12)	34.2	56.0%	1.26	0.0	54.9
	R7 (4)	33.4	51.9%	1.14	0.0	58.0
	R2 (26)	31.9	64.8%	1.41	0.0	53.3

The negative correlation between COG and yield can be justified considering a specific product demand. For instance, assuming a throughput of 10kg/batch and a process yield of 50% it would require the production of 20kg/batch during the cell culture. As the process yield decreases the batch throughput increases to meet the product demand.

Hence, decreasing process yield would require the use of larger process equipment to maintain the batch throughput, thus increasing the cost.

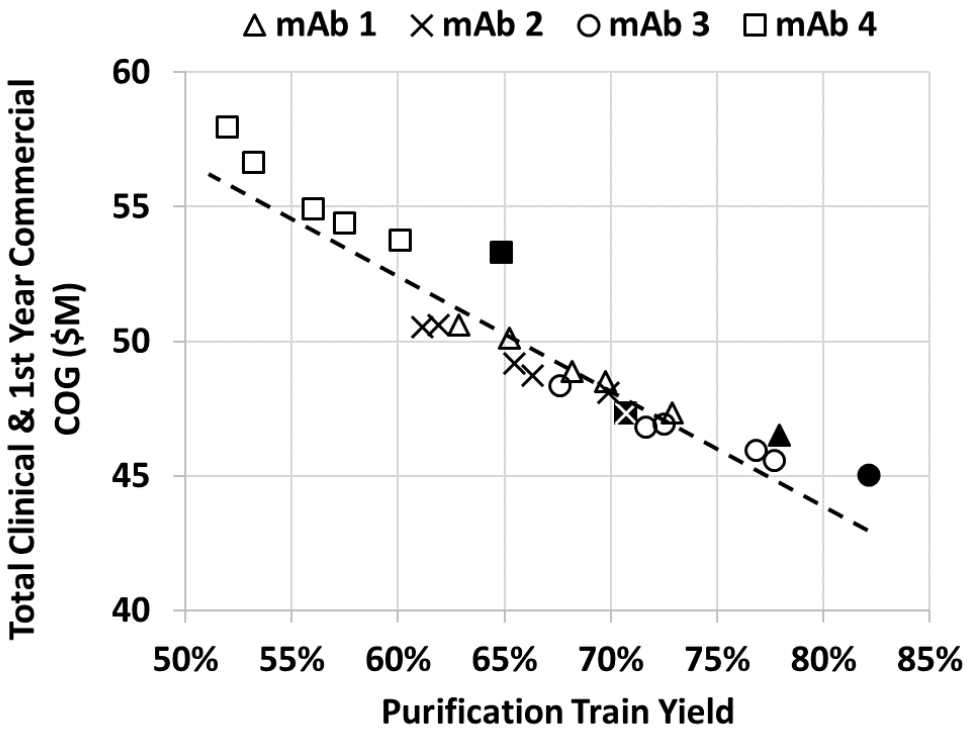


Figure 5.9: Total COG for clinical (phase I, II & III) and first year commercial manufacture against product yield of the purification train across different mAbs. Solid shapes indicate the CEX resin that demonstrated the lowest cumulative COG for each mAb

Cross-referencing the CEX resin indices in Table 5.6 and the corresponding resin list for each mAb in the Appendix (Table A.4 – A.7) it can be seen that certain CEX resins appear throughout the top-ranked lists in Table 5.6 for different mAbs. Figure 5.10 shows on the left vertical axis the frequency that a CEX resin appears in Table 5.6 for all mAbs. Two CEX resins show the greater frequency of approximately 38% and 45% for Resin-5 and Resin-7, respectively. These two CEX resins combined dominate the top-ranked lists in Table 5.6 due to their use under different sets of yield and impurities removal capability.

Through the use of the fractionation diagram approach incorporated into the HTPD workflow a series of Pareto diagrams can be generated to describe the profile of each impurity against product yield. The decision-support tool for the purification train can

import a large set of impurities-yield data-points and assign to each of them a different index number. The appearance of the same resin multiple times in the top-ranked list indicates its superior performance against other resin candidates. Additionally, it could also demonstrate a wider operating window considering that every time the same CEX resin appears in the top-ranked list is under different conditions. Furthermore, Figure 5.10 plots on the right vertical axis the number of mAbs that each top-ranked CEX resin managed to purify. The most frequent CEX resin (Resin-7) managed to reach the top-ranked list for every mAb that was included in this study. In contrast Resin-5 managed to reach the top-ranked list of the first three mAbs only to be excluded for mAb-4. It should be highlighted that mAb-4 had a challenging concentration of HMW species and the decision-support tool could not identify a CEX resin among those tested that could fit into the purification train and deliver the desired target product profile. Hence, in order to allow for the comparison among different CEX resins the target for HMW species was incrementally increased to 1.5%.

Overall, Resin-5 and Resin-7 demonstrated superior performance against other CEX resin candidates. It should be mentioned that Resin-7 was also determined the best CEX resin candidate using the resin selection tool of the HTPD workflow in Chapter 3 (Figure 3.2). Similarly to Case study 1 the synergy between the decision-support tools and the process economics model has revealed the optimum purification train and has identified a purification train that could handle multiple mAbs. A COG/g breakdown is shown in Figure 5.11 comparing the costs for the most cost-effective purification train using self-packed and full pre-packed columns for each mAb and across clinical and commercial manufacture. The most cost-effective options are highlighted in Table 5.6. The use of pre-packed columns offers a reduction in the COG/g at clinical and commercial manufacture and across different mAbs with the greatest cost savings achieved at clinical phase III.

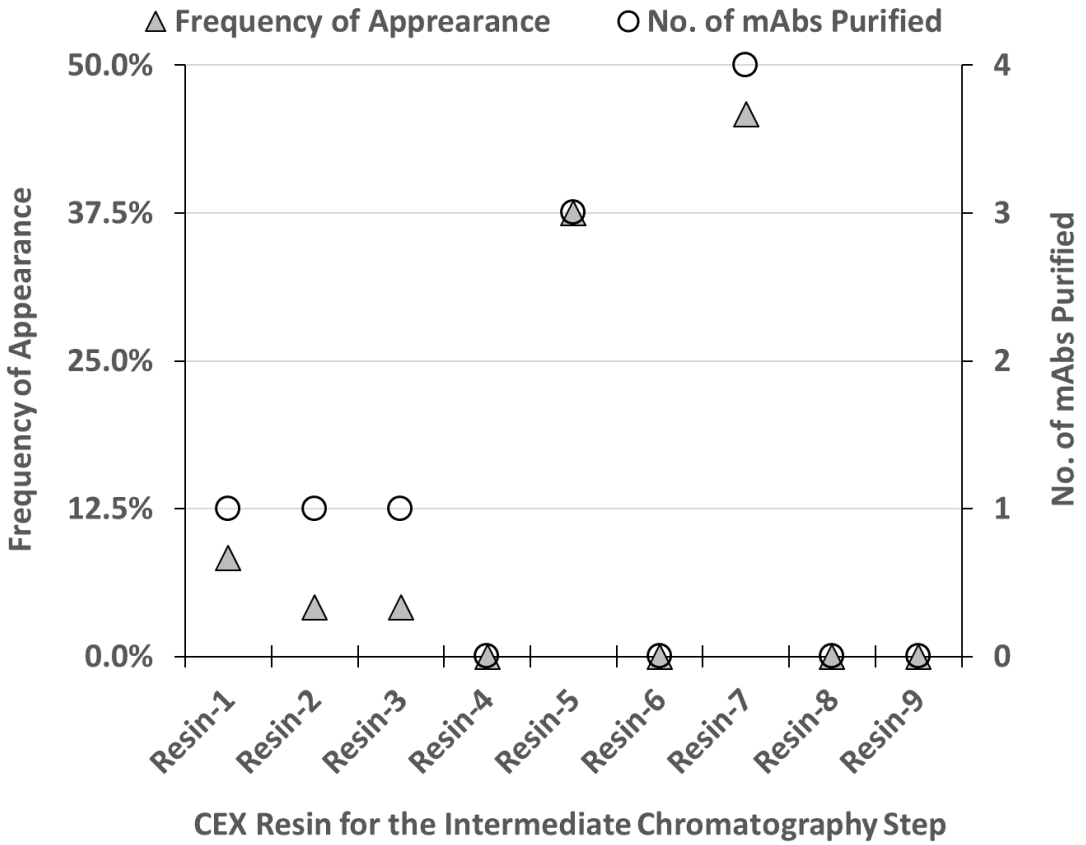


Figure 5.10: Frequency of appearance and number of purified mAbs for each CEX resin included in Case study 2. Resin-8 was used only for mAb-4 and it was replaced with Resin-9 for mAb 1-3

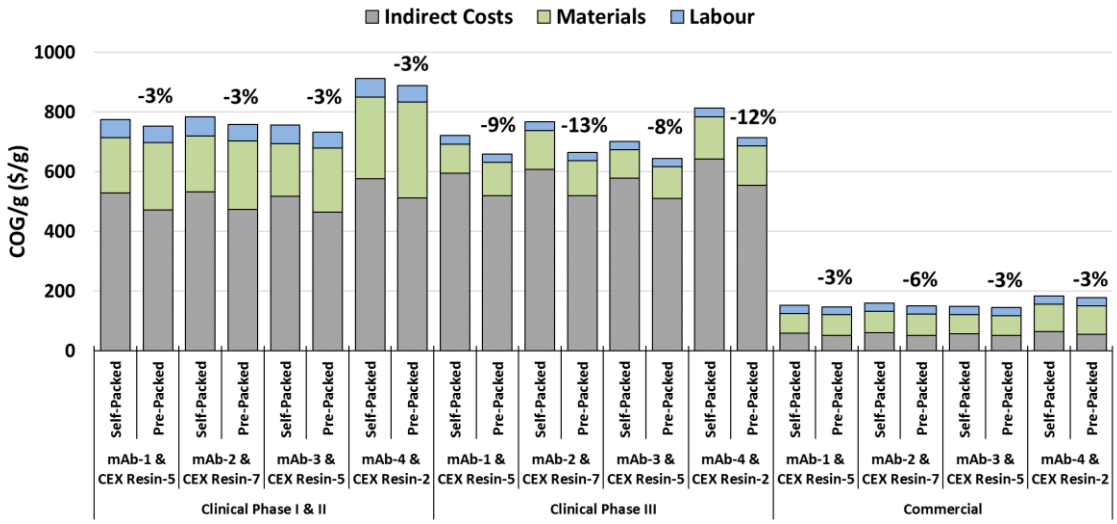


Figure 5.11: COG/g breakdown for the first option (i.e. most cost-effective) for the purification train using self-packed and pre-packed chromatography columns across clinical and commercial manufacture and for each mAb included in Case study 2

Finally, Figure 5.12 demonstrates the total COG across clinical and first year commercial manufacture for the first option (most cost-effective) and the second option regarding the

CEX resin for the intermediate purification step. Both options are plotted considering the use of pre-packed columns for all chromatography steps and throughout different manufacturing stages. Moving towards the selection of a platform CEX resin that could fit into the purification train for different mAbs, Figure 5.12 demonstrates that CEX Resin-7 could be a suitable candidate. CEX Resin-7 ranks consistently in the top-two options with a difference in the cumulative COG of 1 – 4%. Having identified CEX resins with superior performance against other candidates it is important to attempt and determine the resin properties associated with their performance.

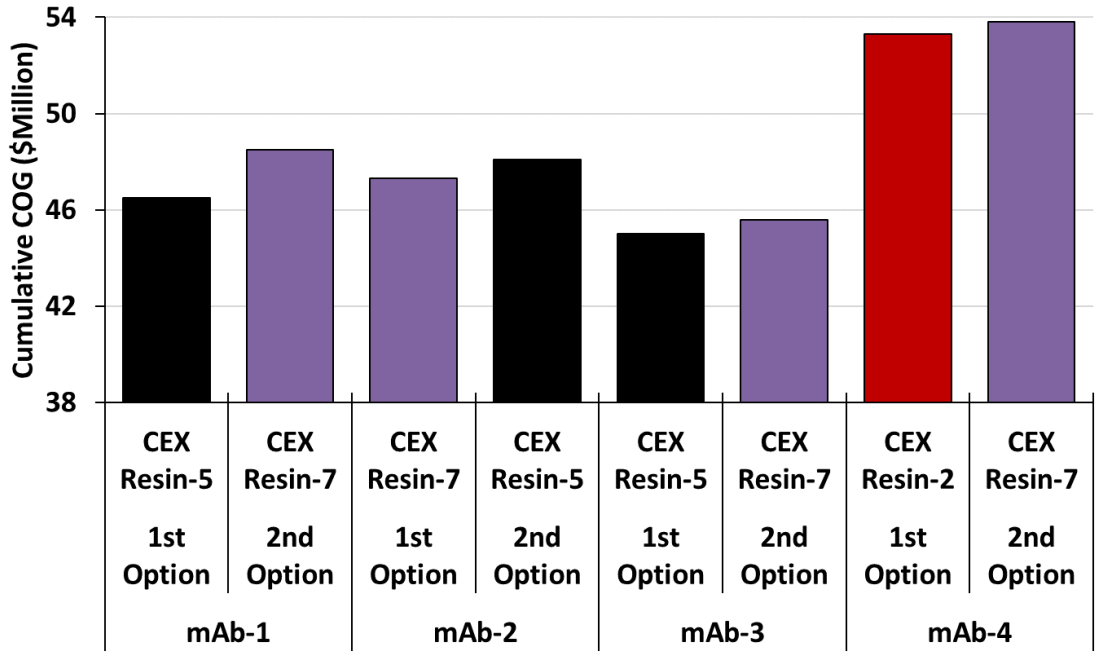


Figure 5.12: Total COG for clinical (phase I, II & III) and first year of commercial manufacture of the first and the second option for the CEX resin for the intermediate chromatography step in the purification train across all mAbs included in Case study 2. Pre-packed columns were assumed throughout.

As discussed in Chapter 4 the dynamic binding capacity of a chromatography resin has a strong impact on the economics of the unit operation with low DBC values leading to high cost (Figure 4.X). According to Figure 5.13, CEX Resin-5 and CEX-Resin-7 report consistently high DBC values throughout different mAbs. However, other CEX resins demonstrate comparable DBC without necessarily leading them to a high ranking place. For instance, CEX Resin-3 shows a high DBC, however it manages to purify only one mAb out of four (Figure 5.10).

Therefore, suitability and cost-effectiveness are not guaranteed simply with high DBC. Additionally, yield demonstrates a strong correlation with the COG (Figure 5.9). Nevertheless, neither the throughput or product recovery can be considered without being able to deliver the desired target product profile. Thus resins that demonstrate high yield and high DBC might be excluded from the evaluation due to their insufficient purification profile. In order to determine whether the purification profile of a chromatography resin could potentially provide early evidence of superior cost-effectiveness, Figure 5.14 plots HMW reduction, HCPs logarithmic reduction values (LRV) and elution pool volume against yield for each CEX resin and across all mAbs included in Case study 2. A visual inspection of the data plotted in Figure 5.14 reveals the trade-offs among different attributes (e.g. HMW reduction, yield, etc.).

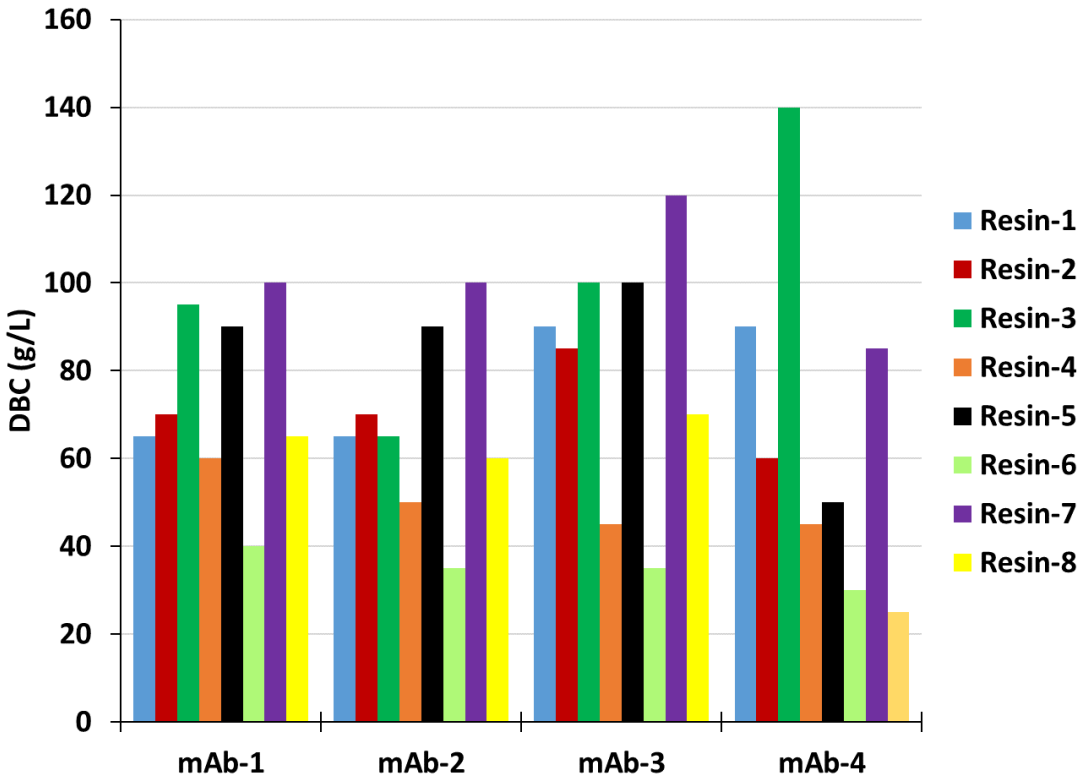


Figure 5.13: Dynamic binding capacity for each CEX chromatography resin across different mAbs included in Case study 2.

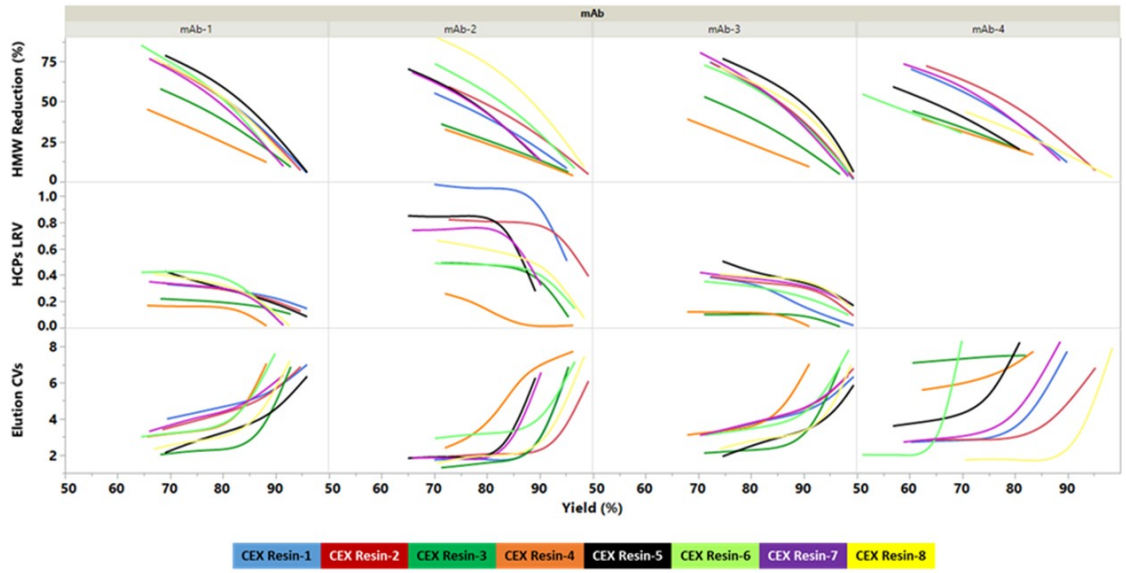


Figure 5.14: Impurities removal and elution pool volume against yield for each CEX resin and each mAb included in Case study 2. Profiles generated through the implementation of the HTPD workflow using the resin databases shown in the Appendix (Table A.4 – A.7)

For mAb-2 the greatest HMW reduction profile is provided by CEX Resin-8 followed by CEX Resin-6. On the other hand, the greatest reduction of HCPs was observed for CEX Resin-1. Nevertheless, none of these resins managed to reach the top-ranked list. In contrast, CEX Resin-5 and Resin-7 showed average HMW and HCPs reduction capabilities and managed to dominate the top-ranked list for mAb-2 (Table 5.6). It should be highlighted that CEX Resin-5 and Resin-7 demonstrate the two highest DBCs for mAb-2 with average yield. Similar trends can be observed for the other mAbs in Figure 5.14. Hence, an overall trend emerges for these four mAbs and eight CEX resins. Figure 5.15 illustrates a simplistic decision-tree for the selection of CEX resins for the intermediate chromatographic purification step. The first decision level is the impurities reduction capabilities followed by yield and concluding with DBC. As demonstrated in Case study 2, CEX resins can be considered only when they can meet product quality targets. The next major influence is the product recovery that can be achieved for a given impurities reduction level. Step yield affects directly the whole manufacturing process influencing the size and the operation of all process steps. Finally, having secured a good

yield and impurities reduction level the cost-effectiveness of the process can benefit from high DBC values.

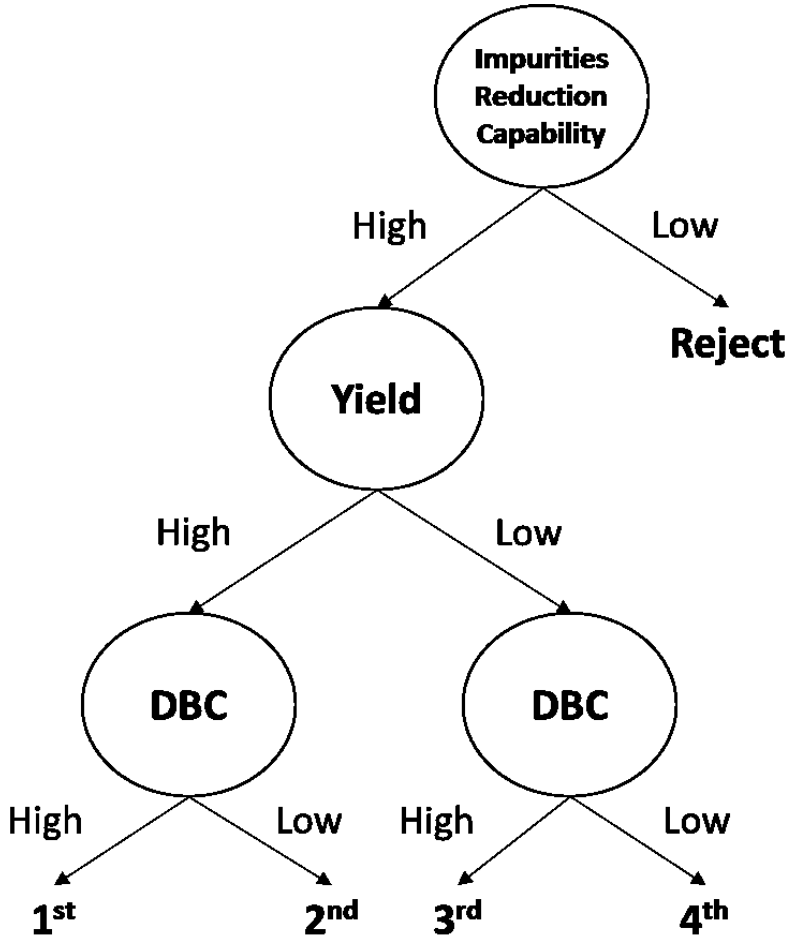


Figure 5.15: A simple decision-tree illustrating the main decision levels and their order for the selection of CEX resins for the intermediate chromatography step in the purification train for the mAbs included in Case study 2

5.4. Conclusions

This chapter focused on demonstrating an integrated framework in chromatography process development leveraging high-throughput purification data using advanced decision-support tools. Through the implementation of the integrated framework a great number of different purification trains can be evaluated considering the economics of the whole manufacturing process. The progressive flow of information in the framework allowed for a systematic data manipulation and storage providing the flexibility to the user to trace any piece of information at any point in the analysis. For instance, it would not be possible to determine a common purification train in Case study 1 without being

able to access the lists of successful purification trains in retrospect. The value of such a level of flexibility was demonstrated in both case studies by determining at least one purification train that could be used for different mAbs or could handle greater deviations in the profile of the product. Hence, through the integrated framework, a greater process understanding and the establishment of a platform process can be achieved.

Furthermore, process bottlenecks can be identified and alternative solutions can be considered very rapidly at early-stage in process development. This capability of the framework was demonstrated through Case study 2. For the case of mAb-4 it was determined that none of the CEX resins that were tested could fit into the given purification train and deliver the desired product profile. Hence, further development efforts could be focused on different resin types for the intermediate chromatography step in the purification train to replace the CEX resin. Additionally, further research could be initiated to investigate the root cause of the inadequate performance of multiple well-established CEX resins.

Chapter 6. Process validation

Process validation is a major concern in the pharmaceutical industry and its main objective is to provide guarantees that the final product, which will be administrated to the patients, consistently meets specific pre-determined quality characteristics (FDA, 2011).

6.1. Initial approach in process validation

Initially, the industry was very sceptical when it came to the introduction of new technologies into their manufacturing process, due to the uncertainty on how the regulators might respond. Additionally, the manufacturing processes were usually designed insufficiently and operated under sub-optimal conditions, which had tremendous implications in the profitability of the industry (Pujar et al., 2009; Rathore & Winkle, 2009). The ‘’traditional’’ process validation approach was based mainly, if not entirely, on empirical information. Additionally, there was a lack in predictive tools to evaluate the effects of scale-up on product quality and process performance. Moreover, there was little understanding on manufacturing failures and how they were caused (Rathore & Winkle, 2009).

6.2. Quality by design approach in process validation

Quality by design (QbD) is an initiative to help both the industry and the regulators to communicate more efficiently and accelerate process development, following a more systematic approach throughout the lifecycle of a therapeutic drug (ICH Q8(R2), 2009; Rathore & Winkle, 2009). QbD incorporates sound scientific knowledge and risk-based evaluation with management of the performance of the manufacturing process, in order to develop a flexible and robust process, capable of delivering consistently the desired product quality (Rathore & Winkle, 2009; Francis, 2012). QbD is based upon the fundamental principal that product quality cannot be tested and hence it has to be build-in the process.

The guidelines provided by the regulators offer instructions for pharmaceutical development and product lifecycle management. They prompt the adaption of a more systematic approach in process development, using prior knowledge and experience. In such an approach results from design of experiments (DoE) studies and quality risk assessment are combined in order to acquire an in-depth process and product understanding (ICH Q8(R2), 2009; ICH Q9, 2005; ICH Q10, 2008).

Furthermore, the guidelines encourage the incorporation of process analytical technologies (PAT) to help improve process understanding and identify the appropriate tools for analysing and controlling the manufacturing process (FDA, 2004; Rathore et al., 2010). A central objective of the QbD approach is to define the target product profile (TPP), identify critical quality attributes (CQAs) and critical process parameters (CPPs) and develop connective correlations between them (Chhatre et al., 2011). Gaining an in-depth process and product understanding can support the establishment of a design space. The design space is defined as ‘*the multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality*.’’ Operating within the design space permits changes in the manufacturing process without initiating regulatory concerns. On the other hand, any changes that can cause operation outside the design space require revalidation to ensure that the process can still deliver the desired quality (ICH Q8(R2), 2009).

In 2009, a consortium of leading biotech companies gathered to combine their knowledge and experience in QbD focusing on guidelines contained in ICH Q8, Q9 and Q10 (CMC Biotech Working Group, 2009). Through the collective effort of the consortium and its interaction with the regulators a case study (A-Mab) in bioprocess development was formulated to discuss the guidelines and their application to real world scenarios. The final document discusses different elements in QbD from product and process characterisation to determining CQAs and CPPs and establishing a design space and a process control strategy.

More recently, Roche/Genentech published a special Section of Biologicals demonstrating an integrated framework implementing QbD principles for mAbs (Kelley, 2016). The first chapter of the publication described the overall QbD roadmap that was created to enhance and streamline decision-making in development activities. The authors highlighted the benefits of an enhanced approach in process development that links systematically a risk-based control strategy with advanced understanding of CQAs. Additionally, it was emphasised that development activities should begin considering the end goal. The control strategy should be progressively created to ensure the consistent delivery of the target product profile. Thus the starting point should be the definition of the target product profile with the determination of CQAs and CPPs gradually leading to the establishment of a design space (Finkler & Krummen, 2016).

The rest of the chapters in this special Section of Biologicals describe in detail the key components of the proposed integrated framework in QbD. Alt et al. (2016) discussed a systematic approach towards an in-depth understanding of quality attributes and the determination of the impact on product safety and efficacy. Hakemayer et al. (2016) focused on developing the tools to facilitate process characterisation, identify potential CPPs and their acceptable operating range and define a design space. Kepert et al. (2016) discussed the tools created to establish a control system by defining acceptance criteria and a testing approach for impurities. The implementation of the control strategy was demonstrated by Ohage et al. (2016) along with a Post Approval Lifecycle Management (PALM) plan. The PALM plan enables the linkage between the development and the commercial stages of the product and helps to manage risks after commercialisation. The final chapter presented the integration of all the tools developed throughout the proposed QbD roadmap (Kelley et al., 2016).

The work presented in the context of this thesis considered QbD principles and guidelines in the development of a decision-support framework in protein chromatography process development. Prior knowledge and experience from the sponsor company were used to guide the development of a high-throughput process

development workflow. Core objective of the HTPD workflow is to leverage high-throughput purification data using a novel approach linking design of experiments (DoE) with the fractionation diagram method. Multi-variate data analysis of the DoE space enabled the development of predictive correlations to estimate potential CQAs as a function of potential CPPs. Furthermore, predictive correlations were integrated with a multi-attribute decision-making method to perform a fair comparison among different CEX chromatography resins and identify the most suitable candidate to deliver the desired product quality and process performance. Finally, the HTPD workflow described a novel methodology in identifying potential windows of operation under process deviations. Overall, the HTPD workflow offers the ability to gain an enhanced process understanding with limited feedstock material from an early stage in process development. Insights can be further leveraged in subsequent assessments for the establishment of a design space and a control strategy.

The significant effort from the industry, the regulatory agencies and academia has highlighted the importance in adapting a QbD strategy from the beginning of a product's lifecycle. As new therapeutic proteins and advanced therapy medicinal products (ATMPs) are discovered new challenges will emerge. Hence, the early establishment of a QbD driven framework is paramount to ensure consistent product quality.

Chapter 7. Concluding remarks and recommendations for future work

The research project discussed throughout the chapters of the current thesis focused on the establishment of advanced decision-support tools in early-stage protein chromatography process development. The tools that were developed combine a variety of methodologies with the overall objective of gaining an in-depth process understanding and accelerating process development activities. This chapter summarises the main features of the decision-support tools developed throughout this research and the benefits associated with their use. Additionally, current limitations and potential areas of improvement are identified paving the road towards future research.

7.1. High-throughput process development workflow

Chapter 3 demonstrated the implementation of a high-throughput process development (HTPD) workflow for the chromatographic purification of a highly aggregated bispecific monoclonal antibody (mAb). The HTPD workflow links high-throughput experimentation (HTE) at micro-scale with design of experiments (DoE), regression analysis, multi-attribute decision-making and stochastic analysis. HTE often results in a large number of samples which could potentially increase the analytical and data analysis effort (Chhatre et al., 2009). Additionally, a chromatogram offers much more information than a single pair of purity-yield values. Typically, a chromatogram can be transformed into a Pareto frontier demonstrating a relationship between product recovery and purity (Ngiam et al., 2001; Ngiam et al., 2003). Thus considering a single point on the Pareto curve neglects the rest of the purification profile.

A key focus of the HTPD workflow was the establishment of a systematic framework providing the methods and the tools to cope with the large datasets and leverage information regarding the complete purification profile. A novel approach in chromatogram analysis was illustrated that integrates DoE with the fractionation diagram

approach in order evaluate the trade-offs between purity and yield at different operating conditions. Although the fractionation diagram approach can be used very efficiently for the transformation of a chromatogram into a Pareto frontier graph it requires meticulous calculations (Ngiam et al., 2001; Ngiam et al., 2003). Considering also the large number of chromatograms, the data analysis effort could increase substantially becoming a development bottleneck. The HTPD workflow leverages the advantages of the fractionation diagram approach and automates the whole analysis for all the chromatograms through the synergistic use of SOLVER (optimization tool add-in) and VBA in MS Excel. Hence, user intervention is mitigated to importing raw data received from different analytical tools in the laboratory and initiating a VBA script (Macro) to perform raw data manipulation.

The HTPD workflow offers the ability to screen multiple chromatography resins and develop regression correlations between process parameters and quality/performance attributes. Chromatography resin selection can become challenging due to the need to satisfy usually conflicting resin properties. Furthermore, visual inspection of the results from the DoE analysis could prove even more challenging to determine with confidence, resin candidates with superior performance. To address these issues the HTPD workflow utilizes a resin selection tool build in MS Excel. The resin selection tool leverages the regression correlations developed for each resin candidate and uses SOLVER through VBA to optimize the set-point of process parameters and achieve user defined quality and performance targets. The comparison of the resin candidates is performed by combining two multi-attribute decision-making techniques: satisficing and weighted sum. The satisficing method compares prioritized threshold values of user defined targets to the performance of individual resin candidates and reports the values achieved for each attribute. Then, in case all attributes were satisfied for more than one resin, a performance score was estimated for each resin candidate based on the weighted sum of normalized attribute values. Thus the final comparison is based on a relative score among resin candidates rather than threshold values.

Another function of the HTPD workflow is the optimization of process parameters and the definition of windows of operation under uncertainty. Conventionally, windows of operation are identified and represented graphically (Salisbury et al., 2006; Boushaba et al., 2011). However, in cases where the number of parameters is greater than 2 and/or the interaction of different parameters has an impact on the shape of the window of operation, its graphical determination becomes extremely difficult. Additionally, depending on the sensitivity of the attributes on deviations from the set-point of different parameters the shape of the window of operations could change dramatically. To overcome these challenges a robustness assessment tool was built in MS Excel using SOLVER through VBA. A series of Monte Carlo simulations were performed introducing a level of uncertainty on each process parameter and the probability of failing to meet any of the user-specified threshold values of the desired attributes was calculated. Using SOLVER the robustness assessment tool manages to iterate through different set-point values for each parameter and determine a sweet-spot able to tolerate process deviations.

Insights gained throughout the implementation of the HTPD workflow can be further leveraged to guide experimentation at larger scales towards the establishment of a design space and a control strategy. Evidently, the HTPD workflow is greatly influenced by the QbD paradigm towards gaining a thorough understanding of chromatographic separation processes for therapeutic proteins. The establishment of the workflow required iterative discussions with the industrial sponsor (MedImmune) in order to determine the appropriate experimental and analytical protocols and develop the framework to its current state. Nevertheless, there are still known and unknown challenges that would require the modification of certain components of the workflow in order to avoid creating a bottleneck in process development.

Through the implementation of the HTPD workflow for the purposes of Chapter 3 and Chapter 5 a previously unreported source of error was identified. The potential error stems from the operation of the robotic arm in a liquid handling system and more

specifically the capacity of its pipettes. To achieve a target load challenge the required volume depends on the feed concentration. Therefore, at a low feed concentration and a high load challenge the necessary volume might exceed the maximum volume of the pipettes. Thus multiple aspiration/dispersion cycles would be required to achieve the target load challenge. In such cases, the delay of the robotic arm would increase the residence time that could cause a surge in the dynamic binding capacity. Hence, for future studies the delay of the robotic arm should be quantified and considered for the calculation of the flowrate at micro-scale.

Another known challenge that the HTPD workflow currently faces is related to the analytics. High-throughput screening can lead to the generation of a large number of samples, thus potentially increasing manual intervention, materials consumption and analytical time. As an indication, during the implementation of the workflow demonstrated in Chapter 3, more than 4000 samples were generated for binding and elution studies combined. The use of a UPLC system in sample analysis was of great benefit due to its capability to process up to 8x96 samples without any manual intervention. However, using the current protocol it would require a few days of continuous operation for the UPLC system while generating the samples would take only a few hours.

Further improvements on the operating protocol for the UPLC system could accelerate the analysis. Residence times for different impurities and the product can be determined prior to the analysis of the samples in order to determine the cut-points and potentially shorten the analytical timeframe. Such an approach might need fine tuning of the protocol for different molecules. Another suggestion would be to introduce an intermediate analytical step (e.g. Infinite® 200 PRO, Tecan) to selectively pick samples with a protein concentration above a pre-specified threshold. Proper calibration would be necessary to avoid excluding samples with protein or including empty samples. Additionally, a data management system has to be established to generate indices or barcodes for samples to enable sample tracking across different analytical tools in the laboratory.

A significant part of the HTPD workflow both on the experimental and the computational front is fully automated. For instance, both the resin selection tool and the robustness approach to determine windows of operation under uncertainty make use of the SOLVER optimization add-in tool in MS Excel through VBA. Additionally, through the same synergy of tools in MS Excel it was possible to fully automate raw data manipulation and analysis to determine the responses in the DoEs (e.g. DBC, Purity, etc.). On the other hand there are key points of manual intervention. For example in data transfer from different analytics to the tools of the HTPD workflow for further manipulation and analysis. Hence, in order for the HTPD workflow to become part of an automated process development platform additional modifications are required. Critical areas of improvement in automation that could be prioritized include:

- The data transfer between the laboratory and the computational tools of the HTPD workflow
- The data transfer between MS Excel and JMP
- The model fitting process in JMP by developing a script that could follow a procedure as described by Kumar et al. (2013) and followed in the current thesis

7.2. Process economics model

Chapter 4 demonstrated the functionality of a process economics spreadsheet model simulating a typical manufacturing process for mAbs. The spreadsheet model offers a dashboard that allows the user to perform deterministic and stochastic analysis. The purpose of the model was to enable the evaluation of pre-packed chromatography columns as an alternative to traditional self-packed columns in antibody purification. Hence, a built-in function of the model is the sizing of pre-packed columns and the estimation of related direct and indirect costs. Additionally, great attention was given to the design of ancillary equipment (e.g. buffer hold-tanks) and estimating the requirements in materials for operating (e.g. guard filters) and cleaning (CIP buffers, WFI, etc.).

Single-use technologies (SUT) could potential offer significant cost benefits (Lopes, 2015). Although pre-packed chromatography columns have been extensively used in research and process development activities (Scharl et al., 2016) there are limited studies discussing their fit into pilot and commercial scale facilities (Grier & Yakubu, 2016). The utilization of pre-packed columns could eliminate the need for a packing system and a self-packed column. On the other hand, the cost of consumables is expected to increase. Hence, the main objective of Chapter 4 was the detailed investigation of the changes in the manufacturing costs associated with the use of pre-packed columns for a given set of assumptions. Additionally, through a sensitivity analysis it was possible to address the significance of different parameters and assumptions on the cost of goods. Through a series of simulations the cost-effectiveness of pre-packed columns was evaluated for every chromatography step in a purification train. Additionally, it was demonstrated that pre-packed columns could potentially replace large scale self-packed chromatography columns despite the size limitations. The analysis in Chapter 4 demonstrated the cost-effectiveness of pre-packed columns despite the assumption regarding a column packing success rate of 100% that favors the self-packed columns. In practice, such a high column packing success rate would be very difficult to achieve. A lower packing success rate would lead to an increase in personnel and reagents cost for self-packed columns. Additionally, significant disruptions might occur in scheduling and operation of a manufacturing process and its support activities (e.g. media and buffer preparation, column packing, etc.). The consequences of a low column packing success rate in a process economics analysis would highlight even further the benefits of pre-packed columns.

The cost-effectiveness of pre-packed columns depends on several factors related to the product, the process, the facility and the portfolio of a biopharmaceutical company. A cost of goods analysis focuses on capturing the costs related to manufacturing thus neglecting the cost associated with R&D. As discussed in Chapter 1, the introduction of single-use technologies could mitigate the cleaning activities in a bio-manufacturing

facility and reduce the effort in validation studies. Hence, a cash-flow analysis would be necessary to capture both the cost of goods and the cost of development for a drug candidate in order to determine the cost-effectiveness of an alternative technology.

The process economics model developed here provides a framework to rapidly evaluate technical and financial metrics in manufacture related to pre-packed and self-packed chromatography columns and determine the most cost-effective configuration of the purification train for a given scenario. A level of flexibility was achieved through the utilization of VBA which allowed for the development of scripts (Macros) to automate the analysis of multiple scenarios and the creation of a database to store all the model outputs.

A current limitation of the model is the restriction to simulate a certain flowsheet using a Chinese Hamster Ovary (CHO) cell-based manufacturing process. Any intention to simulate an alternative flowsheet would require additional model development effort. The biopharmaceutical industry has a number of different therapeutic proteins expressed intracellularly or extracellularly using different cell lines (e.g. bacterial, yeast or mammalian cell lines). Additionally, the process economics model was developed to simulate batch and fed-batch mode of operation thus, neglecting the option of continuous bio-manufacturing. The design calculations applied to a batch or a fed-batch process require further tuning to simulate accurately continuous and/or hybrid manufacturing strategies. Designing individual flowsheets for each case would require intense development work and could lead into a very large spreadsheet that would be difficult to use and maintain. Hence, in order to acquire a greater level of flexibility in synthesizing alternative flowsheets the use of an object oriented programming language would be preferred. Thus future work could focus on determining a suitable programming language to improve and re-write the design equations.

An additional consideration for future work could be to update the cost database of the process economics model. Currently, the database is populated with equipment and

materials purchase cost and size values based on literature and commercially available computer-aided tools. Moreover, the cost of the vast majority of unit operations was estimated based on the 6/10 rule assuming a base size, a base cost and a scaling factor (Peters & Timmerhaus, 1991). A more systematic approach would be to create a list of alternative process equipment with their sizes and purchase costs for each unit operation in bio-manufacturing. One challenge of this approach could be the maintenance of the database as new equipment and technologies emerge. Moreover, in case an equipment or material is withdrawn from the market changes in the cost database would be needed to reflect the changes in availability.

7.3. Integrated framework

Although the process economics model offers the capability to simulate different chromatography resins through VBA, the requirements in user intervention increase exponentially with the number of alternative resins. Increased manual intervention is more prone to error and decelerates the whole analysis. To overcome this challenge a decision-support tool for the chromatographic purification train was developed in MS Excel as an extension of the process economics model.

The tool for the purification train can be operated independently from the process economics model for the rapid evaluation of multiple purification trains using a brute force search algorithm written in VBA. Chapter 5 demonstrated the linkage of the decision-support tool for the purification train and the process economics model. Additionally, the HTPD workflow was used to generate and manipulate purification data and create the appropriate datasets that can be further leveraged using the integrated framework. Hence, the purification performance of different chromatography resins can be assessed using real data within a process economics engine evaluating the cost-effectiveness of multiple purification trains across different stages in the drug development pathway.

The level of difficulty to interpret a scenario output and determine the optimum purification strategy depends on the complexity of the scenario. In case of a relative

simple scenario set-up the optimum purification train could be identified using a graphical approach to visualize key metrics among a few options. However, in cases of a complex scenario set-up that searches for the optimum within a conditional environment across a large number of alternatives and across different manufacturing stages, simple visualization becomes challenging. In such cases the integrated framework offers a mathematical formulation for the linear transformation of the objective function and the constraints of an option evaluation method. The Simplex LP engine in SOLVER has been used very efficiently to identify the optimum configuration of the purification train in complex scenario configurations (Chapter 5, Case study 1 & 2).

Implementation of the framework can be of great benefit in establishing platform purification processes and determining manufacturing bottlenecks at early-stage in process development. Through an industrially-relevant case study, high-throughput data were used to evaluate eight CEX resins for the intermediate chromatography step in the purification train and for four different mAbs. Two CEX resins demonstrated superior performance considering their technical and financial impact on the whole manufacturing process.

Already published work (Simaria et al., 2012; Liu et al., 2014; Liu et al., 2015) has focused primiraly on the development of the optimisation algoritms to determine the most cost-effective resin sequence. Hence, a simplistic apparoch was followed in the development of the chromatography resin database. Typically, a resin database used in a process economics model simulating a bio-manufacturing facility would consider a chromatography resin operating at a set of conditions and delivering a certain purification and recovery performance. Therefore, potentially valuable information from the complete purification profile (e.g. purity versus yield curves) could be neglected. The integrated framework offers the ability through the use of the HTPD workflow to build a more comprehensive resin database that contains several data-points for each resin candidate. Thus providing a greater and more flexible decision space to the user by offering the ability to identify resin candidates that otherwise could be left unnoticed.

The challenges and limitations that were discussed so far with regards to the HTPD workflow and the process economics model also apply here. Future work could focus on developing experimental strategies that could provide a more comprehensive chromatography resin library. A DoE strategy can be created to perform resin screening considering all chromatography steps in the purification train. For instance, operating under different conditions the capture chromatography step might result in significantly different product profiles in the elution pool. Thus, a different product profile could be delivered to the next chromatography step and have a definitive impact on its performance. Therefore, the load of impurities to each chromatography step could be a factor in the DoE.

Another advantage of the integrated framework is that it allows the use of purification data from any source or scale of experimentation. The user can follow the structure of the chromatography resin library (e.g. Table 5.3) and import relevant information from any data source. However, special care must be taken in cases of incorporating datasets from different sources at the same time. For instance, Chapter 3 showed that the 600μL miniature chromatography columns offer a better approximation of the purification performance of a bench-scale column compared to the 200μL columns. Purification data using the 200μL columns can provide critical insights and offer significant savings in materials consumption especially in screening studies. Nevertheless, when creating a chromatography resin library a mathematical transformation might be required before combining data from different scales.

Furthermore, high-throughput purification data and process economics data could be leveraged along with resin characteristics using multivariate data analysis to develop predictive correlations. These predictive correlations could potentially dictate the resin characteristics and purification performance that are required to design a cost-effective purification train that could deliver the desired product quality. A simplified example was given in Figure 5.15 illustrating a decision-tree for the selection of CEX resins for the intermediate chromatography step in the purification train. Through the implementation

of the integrated framework, it was possible to identify key parameters that had a significant impact on the decision to select a CEX resin candidate. However, additional experimental effort and more advanced data mining techniques such as Classification and Regression Trees (Yang et al., 2014) are required to define threshold values for key decision parameters (e.g. yield and DBC). Moreover, the development of predictive correlations would be subject to other process related factors (e.g. impurities load from previous step and purification performance of downstream steps). A potential challenge of this approach could be the data requirements to develop useful correlations that could screen resin characteristics for multiple resin sequences and identify the most suitable resin candidates for the design of a cost-effective purification process.

To sum up, the process economics model offers a solid base that combined with additional work at the Advanced Centre for Biochemical Engineering at UCL could establish the framework for a multi-purpose tool package. A multi-purpose tool package could attempt to simulate various aspects in bioprocessing including:

- Techno-economic evaluation
- Drug development modelling
- Capacity planning
- Supply-chain modelling
- Portfolio management

Through the use of such a package a more comprehensive evaluation of alternative manufacturing technologies can be performed. For instance, in relation to the evaluation of pre-packed chromatography columns it would be possible to assess the potential cost savings during R&D related to column packing activities. Additionally other parameters could be factored in the analysis (e.g. relationship with vendors, availability of backup options for vendors, potential loss of packing know-how etc.). Furthermore, the integrated framework could become part of a platform approach in chromatography process development both in academia and industry. All individual components are

described throughout the thesis in order to allow other researchers and process developers to follow the integrated framework as it stands or even to dismantle it and use parts of it. Alternatively, the work presented here hopefully can inspire the development of novel and more advanced tools with greater automation and flexibility.

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Appendix

Table A.1: List of process equipment and materials as assumed in the process economics model. Base costs are given in USD ($)

Equipment & Materials	Base Size	Min. Size	Max. Size	Units	Base Cost	Scaling Factor
Bioreactor	200	2	25000	L	240000	0.384
Hold-Tanks	500	25	20000	L	40000	0.384
Chromatography Skid	3	3	100	L/min	160000	0.245
Chromatography Column	60	5	200	cm	66500	0.900
Packing System	50	10	100	L/min	35000	0.363
Centrifuge	600	50	3000	L/hr	430000	0.155
Filter Housing	2	1	5	m^2	3500	0.306
TFF Skid	20	1	85	m^2	245000	0.295
PW Vessel	1000	1000	40000	L	27500	0.384
WFI Vessel	1000	1000	25000	L	36500	0.384
Hold-Tank Guard Filter	1000	20	20000	L	310	0.645
Shake Flask	0.50	0.015	1.5	L	500	0.400
Depth Filter	1	NA	NA	m^2	300	NA
Virus Removal Membrane	1	NA	NA	m^2	6800	NA
Ultrafiltration Membrane	1	NA	NA	m^2	3200	NA
Empty Pre-packed Column	1	NA	NA	cm	2000	NA
Cell Culture Media	1	NA	NA	L	50	NA
Buffers	1	NA	NA	L	5	NA
PW	1	NA	NA	L	0.07	NA
WFI	1	NA	NA	L	0.59	NA
Incubator	NA	NA	NA	NA	5000	NA

Sources: UCL Decisional Tools database, Simaria et al. (2012), Repligen, GE Healthcare, Sigma-Aldrich (Merck) & Biosolve (BioPharm Services). Base costs are given in USD ($).

$$\text{Cost} = \text{Cost}_{\text{Base}} * \left(\frac{\text{Size}}{\text{Size}_{\text{Base}}}\right)^{\text{Scaling Factor}}$$

Table A.2: Key assumptions for each unit operation used in the process economics model as identified through discussions with the industrial sponsor (MedImmune)

Assumption	Shake Flasks	Seed Bioreactors	Production Bioreactor(s)
Preparation Time (hr)	1	5	5
Load Time (hr)	0	3	3
Operation Time (hr)	24	72	336
Turnaround Time (hr)	2	48	48
Inoculation Ratio (%)	10	10	10
Fed-batch Media addition (%)	NA	NA	25
H/D tank ratio	NA	3	3
Space Efficiency (%)	50	75	75
Assumption	**Depth Filtration**	**Virus Filtration**	**Ultrafiltration/Diafiltration**
Preparation Time (hr)	0.5	0.5	1
Turnaround Time (hr)	1	1	1
Duration Limit (hr)	5	6	8
Flush Volume (L/ m^2)	100	100	100
Filter Flush Preparation	0.8	0.8	0.8
Filter Flush Recovery	1.5	1.5	1.5
Hold-up Volume (L/ m^2)	10	2	0.3
Flux (LMH)	200	250	40
Step Yield (%)	95	99	98
Capacity	200 **L/** m^2	10 **kg/** m^2	NA
Acidic Buffer (%)	NA	20	NA
Neutralisation Buffer (%)	NA	20	NA
Diafiltration Cycles	NA	NA	5.5
Diafiltration Time Limit (hr)	NA	NA	3
Assumption	**Chrom. Self-packed**	**Chrom. Pre-packed**	**Disk-Stack Centrifuge**
Preparation Time (hr)	1	1	1
Packing Prep. Time (hr)	8	0	NA
Unpacking Prep. Time (hr)	4	0	NA
Turnaround Time (hr)	1	1	1
Duration Limit (hr)	16	16	4
Resin Overfill (%)	10	10	NA
Pre-packing (CVs)	14	0	NA
Pre-First Operation (CVs)	16	16	NA
Equilibration (CVs)	4	4	NA
Wash (CVs)	2	2	NA
Strip (CVs)	3	3	NA
Regeneration (CVs)	3	3	NA
Packing (CVs)	20	0	NA
Unpacking (CVs)	5	0	NA
Sanitisation (CVs)	4	4	NA
Storage (CVs)	3	3	NA
Step Yield (%)	Resin Specific	Resin Specific	85

Table A.3: Key design calculations to estimate the size of each unit operation used in the process economics model

	Upstream Processing	
Unit Operation Sizing	$V_{BIOR} = \frac{\text{Demand}}{\text{Batches} * \text{Titre} * \text{Yield}_{DSP}}$ $V_{SEED,q} = V_{BIOR} * r^{q}, \text{ for } q = [1,2,3] \text{ if } V_{SEED,q} < \min[BV] \Rightarrow V_{SEED,q} = 0$ $V_{Flask} = r * \min\left[V_{SEED,q}\right]_{q=1}^{3} \quad \text{for } V_{SEED,q} > 0$	
	Chromatography Step	**Centrifugation & Depth Filtration**
Unit Operation Sizing	$D = \sqrt{\frac{4 * \frac{M_{IN}}{DBC * n_{COL} * n_{CYC}}}{\pi * H}}$ find D_A for $\min[(D - D_A)^2]$ Recalculate n_{CYC} for D_A $n_{CYC} \Longrightarrow n_{CYC,new}$ Sizing algorithm available in Figure A.1	$\text{Flowrate}_{CENT} = \frac{V_{IN}}{\text{Duration} * \#\text{Units}}$ $A_{DEPF} = \frac{V_{IN}}{\text{Capacity}}$ $V_{VI} = V_{IN} * (1 + \text{Acid}) * (1 + \text{Neutr.})$ $A_{VF} = \frac{M_{IN}}{\text{Capacity}}$ $A_{UFDF} = \frac{V_{IN}}{\text{Flux}} * \left(\frac{1}{\text{Duration} - t_{Diaf}} + \frac{DFC}{t_{Diaf} * CF}\right)$

$$\text{Yield}_{DSP} = \prod_{DSP} \text{Yield}_{step}$$

q: Seed bioreactor's index

r: Inoculation ratio

V_{IN}: Volume in (L)

M_{IN}: Mass in (g)

D: Calculated chromatography column diameter (cm)

D_A: Available chromatography column diameter (cm)

n_{COL}: Number of chromatography columns in parallel

n_{CYC}: Number of chromatography cycles per batch

$n_{CYC,new}$: Recalculated number of chromatography cycles per batch

A: Filter/Membrane area (sqm)

DFC: Diafiltration cycles

t_{Diaf}: Diafiltration time limit (hr)

CF: Concentration factor

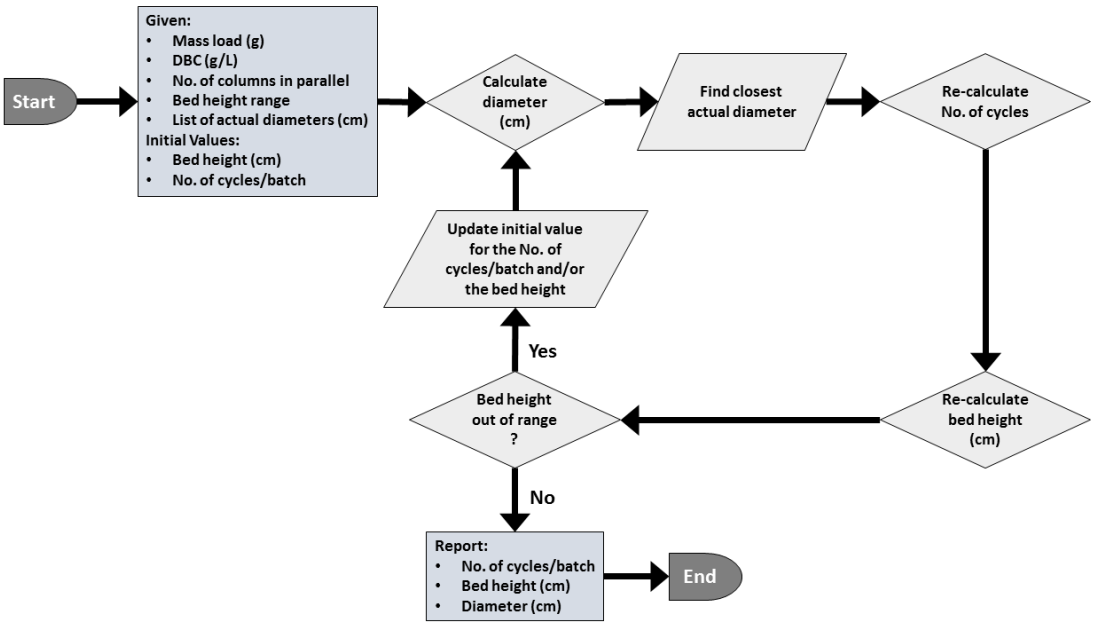

Figure 16: Algorithm for sizing chromatography columns used in the process economics model

Method A.1:

Method for calculating weight coefficient of each decision attribute considered in the resin selection tool based on its factor of significance.

Consider four decision attributes (j = [1, 2, 3, 4]) with factors of significance (f = [a, b, c, d] where a, b, c and d $\in \mathbb{R}^{+}$). The objective is to normalize the factors of significance (f_j) and transform them into weight coefficients (w_j). The ratios among a, b, c, and d should be reflected by the ratios among w_1, w_2, w_3 and w_4. Thus, $w_1/w_2 = a/b$. Given also that $\sum_{j=1}^{4} w_j = 1$, the weight coefficients are calculated as; $w_j = f_j / \sum_{j=1}^{4} f_j$. The benefit of Method A.1 is the flexibility that it offers to the user to define its own scale for the factors of significance instead of attempting to assign values strictly between 0 and 1.

Table A.4: CEX Chromatography resin list for mAb-1 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield (%)	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
3	Resin-6	40	64.5	3.0	85.2	0.42	1000
4	Resin-7	100	66.0	3.3	76.5	0.35	2500
5	Resin-8	65	66.9	2.3	76.8	0.41	2000
6	Resin-3	95	68.1	2.0	57.8	0.22	1900
7	Resin-4	60	65.6	3.0	45.2	0.17	1900
8	Resin-5	90	69.0	2.1	78.5	0.43	2300
9	Resin-1	65	69.4	4.0	72.8	0.33	2800
10	Resin-2	70	68.5	3.4	73.5	0.34	1800
11	Resin-6	40	69.3	3.2	75.3	0.42	1000
12	Resin-7	100	70.7	3.7	68.7	0.33	2500
13	Resin-8	65	71.8	2.7	68.6	0.38	2000
14	Resin-3	95	72.9	2.2	51.0	0.21	1900
15	Resin-4	60	70.1	3.2	38.9	0.16	1900
16	Resin-5	90	74.0	2.7	71.0	0.36	2300
17	Resin-1	65	74.4	4.3	64.3	0.31	2800
18	Resin-2	70	73.4	3.8	65.5	0.32	1800
19	Resin-6	40	74.1	3.4	66.8	0.42	1000
20	Resin-7	100	75.7	4.2	58.5	0.31	2500
21	Resin-8	65	76.7	2.9	60.1	0.35	2000
22	Resin-3	95	77.8	2.4	42.9	0.20	1900
23	Resin-4	60	74.7	3.4	32.5	0.16	1900
24	Resin-5	90	79.1	3.2	61.2	0.31	2300
25	Resin-1	65	79.5	4.7	53.7	0.30	2800
26	Resin-2	70	78.5	4.2	55.4	0.30	1800
27	Resin-6	40	79.0	3.7	54.7	0.39	1000
28	Resin-7	100	80.7	4.4	45.7	0.29	2500
29	Resin-8	65	81.7	3.3	48.5	0.30	2000
30	Resin-3	95	82.7	2.6	33.2	0.18	1900
31	Resin-4	60	79.2	3.7	25.8	0.15	1900
32	Resin-5	90	84.4	3.7	47.8	0.25	2300
33	Resin-1	65	84.8	5.0	40.6	0.26	2800
34	Resin-2	70	83.7	4.7	42.6	0.26	1800
35	Resin-6	40	84.3	4.3	37.8	0.31	1000
36	Resin-7	100	86.0	5.1	29.5	0.24	2500
37	Resin-8	65	87.1	3.9	32.6	0.22	2000
38	Resin-3	95	87.8	3.0	21.7	0.16	1900
39	Resin-4	60	83.8	4.1	18.9	0.14	1900
40	Resin-5	90	90.0	4.3	29.7	0.19	2300
41	Resin-1	65	90.3	5.6	24.6	0.23	2800
42	Resin-2	70	89.2	5.3	26.6	0.22	1800

Table A.4 (cont.): CEX Chromatography resin list for mAb-1 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield (%)	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
43	Resin-6	40	89.9	7.9	14.4	0.07	1000
44	Resin-7	100	91.5	6.5	9.3	0.00	2500
45	Resin-8	65	92.7	7.5	10.9	0.00	2000
46	Resin-3	95	92.9	7.3	9.0	0.10	1900
47	Resin-4	60	88.3	7.4	12.2	0.00	1900
48	Resin-5	90	96.0	6.5	5.4	0.08	2300
49	Resin-1	65	96.0	7.1	5.1	0.14	2800
50	Resin-2	70	94.8	7.0	6.6	0.12	1800

Table A.5: CEX Chromatography resin list for mAb-2 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
3	Resin-6	35	70.1	2.9	73.5	0.49	1000
4	Resin-7	100	65.8	1.8	68.0	0.74	2500
5	Resin-8	60	70.5	1.5	89.6	0.66	2000
6	Resin-3	65	71.3	1.3	36.0	0.49	1900
7	Resin-4	50	72.0	2.5	32.7	0.25	1900
8	Resin-5	90	65.0	1.8	70.1	0.85	2300
9	Resin-1	65	70.0	1.7	55.2	1.09	2800
10	Resin-2	70	72.7	1.9	59.2	0.82	1800
11	Resin-6	35	75.2	3.1	64.7	0.48	1000
12	Resin-7	100	70.4	1.9	61.5	0.74	2500
13	Resin-8	60	75.6	1.9	82.2	0.63	2000
14	Resin-3	65	76.1	1.4	30.5	0.49	1900
15	Resin-4	50	76.9	3.0	27.3	0.22	1900
16	Resin-5	90	69.6	1.9	64.0	0.84	2300
17	Resin-1	65	74.9	1.8	48.2	1.06	2800
18	Resin-2	70	77.8	2.0	51.9	0.81	1800
19	Resin-6	35	80.3	3.3	55.1	0.47	1000
20	Resin-7	100	75.2	1.9	53.2	0.74	2500
21	Resin-8	60	80.9	2.1	71.9	0.58	2000
22	Resin-3	65	81.0	1.8	24.7	0.46	1900
23	Resin-4	50	81.7	3.9	21.8	0.10	1900
24	Resin-5	90	74.2	2.0	56.0	0.83	2300
25	Resin-1	65	79.9	1.9	40.2	1.04	2800
26	Resin-2	70	83.0	2.2	43.0	0.79	1800
27	Resin-6	35	85.6	3.5	42.9	0.45	1000
28	Resin-7	100	80.0	2.0	42.7	0.74	2500
29	Resin-8	60	86.4	2.3	57.2	0.51	2000
30	Resin-3	65	85.8	2.0	19.0	0.44	1900
31	Resin-4	50	86.6	6.8	16.1	0.01	1900
32	Resin-5	90	79.0	2.1	45.6	0.81	2300
33	Resin-1	65	84.9	2.0	30.9	1.02	2800
34	Resin-2	70	88.3	2.3	32.4	0.77	1800

Table A.5 (cont.): CEX Chromatography resin list for mAb-2 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield (%)	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
35	Resin-6	35	91.0	3.8	27.4	0.42	1000
36	Resin-7	100	85.1	2.4	29.4	0.71	2500
37	Resin-8	60	92.2	2.7	36.7	0.46	2000
38	Resin-3	65	90.7	2.4	12.7	0.38	1900
39	Resin-4	50	91.5	7.0	10.0	0.02	1900
40	Resin-5	90	84.0	2.2	32.4	0.81	2300
41	Resin-1	65	90.0	2.0	20.3	1.01	2800
42	Resin-2	70	93.7	2.4	19.6	0.76	1800
43	Resin-6	35	96.7	7.5	7.9	0.12	1000
44	Resin-7	100	90.3	7.0	12.7	0.28	2500
45	Resin-8	60	98.5	7.9	8.5	0.04	2000
46	Resin-3	65	95.5	7.4	6.0	0.05	1900
47	Resin-4	50	96.4	7.7	3.8	0.02	1900
48	Resin-5	90	89.2	6.7	15.6	0.22	2300
49	Resin-1	65	95.2	7.0	8.1	0.45	2800
50	Resin-2	70	99.3	6.5	4.4	0.35	1800

Table A.6: CEX Chromatography resin list for mAb-3 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
3	Resin-6	35	71.1	3.1	72.4	0.35	1000
4	Resin-7	120	70.3	3.1	80.2	0.42	2500
5	Resin-8	70	74.1	2.3	71.3	0.41	2000
6	Resin-3	100	71.1	2.1	52.9	0.10	1900
7	Resin-4	45	67.9	3.1	39.1	0.12	1900
8	Resin-5	100	74.6	1.9	76.6	0.51	2300
9	Resin-1	90	72.2	3.2	74.4	0.38	2800
10	Resin-2	85	72.4	3.2	74.0	0.39	1800
11	Resin-6	35	76.2	3.3	66.0	0.33	1000
12	Resin-7	120	75.5	3.4	71.8	0.39	2500
13	Resin-8	70	79.5	2.8	63.0	0.38	2000
14	Resin-3	100	76.1	2.2	46.1	0.10	1900
15	Resin-4	45	72.5	3.3	33.2	0.12	1900
16	Resin-5	100	80.0	2.5	68.8	0.42	2300
17	Resin-1	90	77.4	3.5	65.8	0.35	2800
18	Resin-2	85	77.6	3.6	65.9	0.36	1800
19	Resin-6	35	81.4	3.6	57.0	0.31	1000
20	Resin-7	120	80.8	3.9	60.7	0.37	2500
21	Resin-8	70	84.9	3.1	54.1	0.38	2000
22	Resin-3	100	81.1	2.4	38.1	0.10	1900
23	Resin-4	45	77.2	3.5	27.2	0.11	1900
24	Resin-5	100	85.5	3.1	58.6	0.38	2300
25	Resin-1	90	82.9	4.0	54.7	0.32	2800
26	Resin-2	85	83.0	4.0	55.2	0.33	1800

Table A.6 (cont.): CEX Chromatography resin list for mAb-3 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
27	Resin-6	35	86.8	4.0	44.7	0.26	1000
28	Resin-7	120	86.4	4.3	46.2	0.34	2500
29	Resin-8	70	90.5	3.5	41.8	0.35	2000
30	Resin-3	100	86.3	2.7	28.6	0.10	1900
31	Resin-4	45	81.8	3.8	21.2	0.11	1900
32	Resin-5	100	91.3	3.5	44.4	0.33	2300
33	Resin-1	90	88.5	4.3	40.7	0.17	2800
34	Resin-2	85	88.6	4.5	41.5	0.31	1800
35	Resin-6	35	92.5	4.5	28.1	0.21	1000
36	Resin-7	120	92.2	4.7	27.4	0.31	2500
37	Resin-8	70	96.4	4.1	25.2	0.28	2000
38	Resin-3	100	91.6	3.0	17.4	0.09	1900
39	Resin-4	45	86.5	4.5	15.2	0.10	1900
40	Resin-5	100	97.5	4.3	24.9	0.25	2300
41	Resin-1	90	94.4	4.8	23.0	0.09	2800
42	Resin-2	85	94.5	5.0	23.8	0.27	1800
43	Resin-6	35	98.6	8.1	5.6	0.09	1000
44	Resin-7	120	98.5	6.5	2.9	0.18	2500
45	Resin-8	70	99.0	7.9	2.7	0.12	2000
46	Resin-3	100	96.9	7.3	4.4	0.00	1900
47	Resin-4	45	91.1	7.3	9.3	0.00	1900
48	Resin-5	100	99.5	6.5	0.0	0.14	2300
49	Resin-1	90	99.5	6.5	0.5	0.02	2800
50	Resin-2	85	99.5	7.0	1.2	0.07	1800

Table A.7: CEX Chromatography resin list for mAb-4 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
3	Resin-6	30	51.0	2.0	54.7	NA	1000
4	Resin-7	85	58.8	2.7	73.3	NA	2500
5	Resin-8	25	70.5	1.7	43.7	NA	2000
6	Resin-3	140	60.5	7.1	44.2	NA	1900
7	Resin-4	45	62.3	5.6	39.3	NA	1900
8	Resin-5	50	56.8	3.6	59.2	NA	2300
9	Resin-1	90	60.2	2.7	70.1	NA	2800
10	Resin-2	60	63.1	2.8	72.0	NA	1800
11	Resin-6	30	54.8	2.0	49.7	NA	1000
12	Resin-7	85	63.3	2.9	68.2	NA	2500
13	Resin-8	25	75.8	1.8	37.2	NA	2000
14	Resin-3	140	64.6	7.2	39.8	NA	1900
15	Resin-4	45	66.5	5.8	35.0	NA	1900
16	Resin-5	50	61.1	3.8	53.8	NA	2300
17	Resin-1	90	65.0	2.8	64.0	NA	2800
18	Resin-2	60	68.0	2.9	66.4	NA	1800

Table A.7 (cont.): CEX Chromatography resin list for mAb-4 used in Chapter 5, Case study 2

Index	Resin Name	DBC (g/L)	Yield	Elution CVs	HMW Reduction (%)	HCPs LRV	Price (USD/L)
19	Resin-6	30	58.5	2.2	45.2	NA	1000
20	Resin-7	85	67.9	3.0	63.1	NA	2500
21	Resin-8	25	81.2	1.9	29.9	NA	2000
22	Resin-3	140	68.9	7.3	35.1	NA	1900
23	Resin-4	45	70.7	6.0	30.7	NA	1900
24	Resin-5	50	65.6	4.0	47.5	NA	2300
25	Resin-1	90	70.2	2.9	56.1	NA	2800
26	Resin-2	60	73.3	2.9	58.9	NA	1800
27	Resin-6	30	62.3	2.4	40.5	NA	1000
28	Resin-7	85	73.8	3.4	51.6	NA	2500
29	Resin-8	25	86.8	2.0	21.8	NA	2000
30	Resin-3	140	73.2	7.4	29.9	NA	1900
31	Resin-4	45	74.9	6.4	26.2	NA	1900
32	Resin-5	50	70.4	4.5	40.0	NA	2300
33	Resin-1	90	75.9	3.1	45.9	NA	2800
34	Resin-2	60	79.3	3.0	48.4	NA	1800
35	Resin-6	30	66.1	2.6	35.5	NA	1000
36	Resin-7	85	80.2	4.0	37.3	NA	2500
37	Resin-8	25	92.5	2.4	12.8	NA	2000
38	Resin-3	140	77.6	7.5	24.4	NA	1900
39	Resin-4	45	79.2	6.8	21.5	NA	1900
40	Resin-5	50	75.4	5.0	31.0	NA	2300
41	Resin-1	90	82.3	3.7	32.1	NA	2800
42	Resin-2	60	86.2	3.5	33.0	NA	1800
43	Resin-6	30	69.9	9.0	30.3	NA	1000
44	Resin-7	85	88.6	8.5	12.9	NA	2500
45	Resin-8	25	98.5	8.5	2.5	NA	2000
46	Resin-3	140	82.1	7.5	18.4	NA	1900
47	Resin-4	45	83.5	7.8	16.9	NA	1900
48	Resin-5	50	80.9	8.5	20.0	NA	2300
49	Resin-1	90	89.9	8.0	11.8	NA	2800
50	Resin-2	60	95.3	7.0	6.7	NA	1800

Abbreviations

Abbreviation	Description	Abbreviation	Description
AEX	Anion Exchange	**HTE**	High-Throughput Experimentation
ATF	Alternative Tangential Flow	**HTPD**	High-Throughput Process Development
BE	Bind-and-Elute	**HTS**	High-Throughput Screening
BLA	Biologics Licence Application	**ICH**	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
BLP	Binary Linear Programming	**IEX**	Ion Exchange
CEX	Cation Exchange	**IND**	Investigational New Drug
cGCP	current Good Clinical Practice	**LMW**	Low Molecular Weight
		mAb	Monoclonal Antibodies
cGLP	current Good Laboratory Practice	**MADM**	Multi-Attribute Decision-Making
CHO	Chinese Hamster Ovary	**MILFP**	Mixed-Integer Linear Fractional Programming
CIP	Cleaning-in-place	**MINLP**	Mixed-Integer Non-Linear Programming
COG	Cost of Goods	**MLR**	Multiple Linear Regression
COG/g	COG per gram of product	**NDA**	New Drug Application
CPPs	Critical Process Parameters	**NPV**	Net Present Value
CQAs	Critical Quality Attributes	**OFAT**	One-factor-at-a-time
DBC	Dynamic Binding Capacity	**PMDA**	Pharmaceutical and Medical Devices Agency, Japan
$DBC_{10\%}$	DBC at 10% breakthrough	**PW**	Process Water
DoE	Design of Experiments	**QbD**	Quality by Design
DSP	Downstream Processing	**QCQA**	Qaulity Control and Quality Assurance
EMA	European Medicines Agency	**R&D**	Research and Development
Fab	Antibody Fragment	**SEC**	Size Exclusion Chromatography
FCI	Fixed Capital Investment	**SIP**	Sterilisation-in-place
FDA	Food and Drug Administration	**SUT**	Single-Use Technologies
FT	Flow-Through	**UPLC**	Ultra-High Performance Liquid Chromatography
GMP	Good Manufacturing Practice	**USP**	Upstream Processing
HA	Hydroxyapatite Chromatography	**VBA**	Visual Basic for Applications
HCPs	Host Cell Proteins	**WFI**	Water For Injection
HIC	Hydrophobic Interaction Chromatography	**WPC**	Weak Partitioning Chromatography
HMW	High Molecular Weight		