# A Dynamic Decision Support Tool For Use in The Design of Bio-manufacturing Facilities and Processes

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I, Adam Stonier, 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.

# Abstract

The effect of uncertainty in biopharmaceutical manufacturing can be a barrier to robust, scalable process design. The ideal is for a process in development to complete technology transfer to full scale manufacturing with no redevelopment costs or surprises. Essential to achieving this is a systematic method for analysing large complex datasets and extracting critical combinations of fluctuations that lead to product loss and scheduling delays.

This thesis describes a dynamic database-driven decision-support tool to facilitate such efforts and identify robust optimal purification strategies to match the high productivity cell cultures whilst coping with uncertainties. The benefits of a database-driven approach using MySQL (MySQL AB, Uppsala, Sweden) are harnessed to capture the process, business and risk features of multiple biopharmaceutical purification sequences in a multi-product facility and better manage the large datasets required for multiple processes, uncertainty analysis and optimisation.

Principal component analysis combined with clustering algorithms are used to analyse the complex datasets from complete batch processes for biopharmaceuticals. The challenge of visualising the multidimensional nature of the dataset was addressed using hierarchical and k-means clustering as well as parallel co-ordinate plots to help identify process fingerprints and characteristics of clusters leading to facility fit issues. Industrially-relevant case studies are presented that focus on tech transfer challenges for therapeutic antibodies moving from early phase to late phase clinical trials.

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# Chapter 1

# Introduction

## 1.1 Drug Development

In biopharmaceutical manufacturing the aim is to deliver a product to the market in the shortest available time with a robust, economically viable process. In order to reach the market there are several stages of development, each with specific challenges. These stages are summarised in figure 1.1. Figure 1.1 also shows the typical scale of operation highlighting one of the key process development challenges of producing a scalable process at 10L which will perform at 10,000L scale.

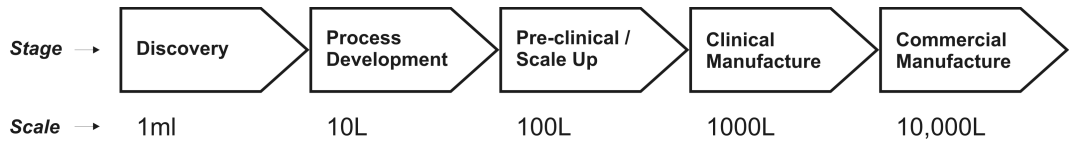



Figure 1.1: Stages of drug development and associated scale of operation. Scale of operation refers to the typical volume prior to purification.

Up to the point of late phase clinical manufacture, it is common for minor process changes which may also be referred to as process intensification, to be made to improve robustness and scalability. As the process moves through the development pathway, the regulatory burden increases and the justification for changes as having no impact on the product become more stringent. By the time of commercial manufacture the process is in essence locked and any changes must be fully justified to the regulators and supported with costly experimental studies.

Development of a biopharmaceutical is currently expected to take approximately 6-7 years. (Rosenberg, 2000) Over this time there is significant risk associated with

each stage in the process. (Zabriskie and Sofer, 2000) Figures for the average percentages of biopharmaceuticals which pass the stages of clinical trials validation are reported as; 15% of products pass phase 1, 40% for phase 2 and 80% for phase 3. This results in less than 5% of the products entering clinical trials making it to market.

Over the past 15-20 years, the cost to develop biopharmaceuticals has seen a significant rise. Figures quoted by Tufts in a report of Nov 2005 (Tufts, 2005) placed the average cost of developing a biopharmaceutical in 1987 at US$231m, this figure rose to US$802m in 2000. Generally, this rise is attributed to more rigorous regulatory requirements, specifically longer clinical trials.

The emphasis on clinical trials may suggest that process development timelines do not lie on the critical path to market. Broadly speaking this is the case, however one key trend in the industry is the rise of small companies which outsource development and manufacture of their portfolio to contract manufacturers as opposed to cash rich large pharmaceutical companies that maintain the capacity in-house. For these small companies the aim is to demonstrate efficacy such that the process can be sold and taken to market by larger pharmaceutical companies. For these companies the end of the process is not commercialisation, it is early phase clinical trials and the timelines and cost for development are therefore often compressed.

Each stage in the drug development pathway presents a particular tech transfer challenge. The development of platform technologies and processes can provide a solid basis for process development efforts. An appropriately scoped platform can ensure that the process developed within its bounds does, at a high level, fit to the target facilities. Even with a solid platform process however, there are still process specific fit challenges that will arise.

This thesis is aimed at the development of a simulation framework to address some of the challenges that occur upon scale up from the process development scale through to commercial manufacture. With the knowledge gained at each stage of development, the aim is to simulate the performance at latter stages to target process intensification efforts.

## 1.2 Platforms Processes and Technologies

The development of platform processes reduces the potential for major fit issues and act to target development efforts inline with the capability of commercial facilities. This section coverers some of the major platform technologies in used in the purification of mAbs from mammalian cell lines. (Fahrner et al., 2001; Shukla et al., 2007) Any simulation engine developed to investigate mAb production processes must be capable of simulating the process types and unit operations outlined in this section.

### 1.2.1 Platform Process Paradigms

Elements of a typical platform process can be summarised as follows:

- **Product capture** is typically carried out using affinity-based chromatographic separation.
- **Virus inactivation** is generally achieved through either solvent/detergent treatment or more commonly low pH inactivation.
- **Ultrafiltration and diafiltration (UFDF)** is used to to condition the product stream by changing the concentration through ultrafiltration and the buffer composition through diafiltration to be optimal for subsequent polishing steps.
- **Polishing** or further purification or is then achieved through a further sequence of chromatography operations with intermediate ultrafiltration and diafiltration (UF/DF) steps for buffer exchange and concentration.
- **Nano-filtration** is employed for the reduction of viruses and along with virus inactivation forms the two orthogonal virus reduction steps as required by regulatory authorities. (ICH, 1999)
- **Final bulk product formulation** is achieved by ultrafiltration / diafiltration, to adjust to the final concentration and transition the product into the final formulation buffer.

More detail on each of these steps is outlined below.

#### 1.2.1.1 Product Capture

The promise of >90% purity and high yields in a single step has led to protein A becoming the dominant capture technology (Hober et al., 2007; Fahrner et al., 2001; Shukla et al., 2007). This power does however come at a price and protein A affinity resins may account for up to 25% of the batch cost. (Gülich et al., 2000)

Protein A affinity chromatography is operated in bind and elute mode with the protein A ligand binding the Fragment Crystallisable (Fc) region which is common to all mAbs. As such this one technology can be used with a generally high degrees of success for most mAb processes. (Vunnum et al., 2008) Once bound to the column, the column is washed using a number of wash buffers to remove non-specifically bound components such as DNA, host cell proteins and cell culture media components. After washing, the product is eluted by using a low pH buffer which causes the product to dissociate from the resin and flow off the column. Once the product is eluted the column is stripped of more tightly bound proteins at an even lower pH before being regenerated and cleaned using chaotropic agents such as guanidine or urea, (Girot et al., 1990) or for alkali stable resins, sodium hydroxide is used. (Hahn et al., 2006)

Once regenerated the resin may be reused to form part of multi-cycle runs. Depending on the cleaning conditions resins may be used for in excess of 100 cycles (Hale et al., 1994) meaning that for commercial manufacture the potential exists for the high cost of the resin to be amortised across a large number of batches. For clinical trails manufacture where the campaigns are generally smaller the cost advantage of multi-cycling is smaller as the resin life is often not reached.

**Scalability and Facility Fit Considerations:** Chromatography steps are scaled by maintaining constant residence time of the product and buffers as they flow through the column as well as a constant column resin loading capacity. Loading capacity is defined as grams of product per litre of resin ($g_{product}$/$L_{resin}$). The most common and simplest strategy to maintain residence time is to use constant bed height and linear flow rate across all scales. The column step is then scaled by changing the diameter of the column and the number of cycles run. This leads to a trade off between running large numbers of cycles in a small column or few cycles in a larger column. This

equates to a cost vs. time optimisation challenge. Facility fit considerations for chromatography are focused on the maximum achievable flow rates and column diameters at scale as well as schedule constraints. The relative complexity of the step compared to other chromatography operations with respect to wash buffers means that the buffer demands for protein A operations can be high and may approach the limits of buffer storage.

#### 1.2.1.2 Ultrafiltration / Diafiltration and Final Formulation

UFDF steps are designed to concentrate the product stream and for buffer exchange. The majority of platform processes will have a UFDF step after the product capture step and possibly between polishing steps depending on the order and selection of chromatography steps used. It is possible to optimise the buffering systems of the polishing steps such that only conductivity or pH adjustments by titration are required to condition the process steam for loading. The majority of mAb processes will have a UFDF step at the end of the process to formulate the product into its final formulation buffer and concentration. (Teeters et al., 2011)

The primary technology of UFDF steps for the purification of mAbs is tangential flow filtration (TFF). In TFF the product is re-circulated around one side of a membrane with a molecular weight cut off smaller than the antibody (usually 30 - 50kDa), a transmembrane pressure is applied across the membrane which forces the buffer to permeate through the membrane whist the product is retained. In ultrafiltration mode the buffer permeates through the membrane and the volume of the retained product stream drops, thus increasing the product concentration. In diafiltration mode, the buffer into which the product is to be exchanged is continually pumped into the retained product stream such that the product concentration is maintained. The result is that over time the concentration of buffer components will be exchanged. The volume of the process stream prior to the start of diafiltration is referred to as the diafiltration volume (DV). In order to achieve near total buffer exchange, 8-10 DVs of diafiltration buffer must be used. (Teeters et al., 2011) Total buffer exchange is required for final formulation operations however for product conditioning it is normally acceptable for a lower number of DVs to be used as long as pH and conductivity specifications are met.

**Scalability and Facility Fit Considerations:** There are various methods to scale UFDF operations. Principle amongst these is to use constant trans-membrane pressure across all scales, the area of the filter can then be determined based on data for the permeate flux decay rate and scheduling constraints. Facility fit considerations for scale up are the maximum installable membrane area which will determine the minimum processing time.

#### 1.2.1.3 Virus Inactivation and Nano-filtration

Two orthogonal virus reduction and removal steps must be built into any mAb process in order to satisfy regulatory requirements. (ICH, 1999) The term orthogonal means that these two steps must use different mechanisms for virus reduction and inactivation. In most cases this takes the form of a chemical inactivation step and a separate normal flow nano-filtration step.

Chemical inactivation works by disrupting the proteins and or genome of viruses. Numerous methods exist, such a treatment with solvents, detergents, heat or UV. As mAbs are generally tolerant to low pH environments the most common method for virus inactivation is titration with acid to a low pH. This also fits well with the use of protein A affinity capture as the product often elutes at a low pH and requires only minor adjustment to be within the inactivation range of between pH3.0 and 4.0 and is depended on product stability. (Vunnum et al., 2008) Once at the inactivation pH the product is held for between 30min and 1h depending on the pH used (Brorson et al., 2003) prior to adjusting to a neutral pH to improve product stability prior to forward processing.

Nano-filtration is almost exclusively used as the second orthogonal virus removal step in mAb production. (Zhou et al., 2008) Key to its success is it's reliability and relative simplicity as a step. Nano-filtration steps are normally operated at constant pressure and experience a flux decay as the filter fouls. A number of nano-filtration products are available and due to the high cost of the membranes the focus for suppliers is on increased capacity (i.e reduced flux decay). Nano-filtration steps are generally placed towards the end of the process, often immediately prior to final formulation, where the volumes are smaller and consequently the filter area is minimised.

Other technologies such as depth filtration and charged membrane chromatography have been documented for use as dedicated virus removal steps however their adoption within platform processes for this use is still not widespread. (Zhou et al., 2008)

**Scalability and Facility Fit Considerations:** Virus inactivation only requires a mixing vessel which would typically be the protein A eluate pool tank and as such there are no step specific scale up issues. Nano-filtration is scaled by maintaining consistent loading of product volume per area of membrane referred to as the filter the capacity and measured in l/m$^2$. Facility fit concerns will be around maximum installable area. Operationally an additional constraint of processing time may also be applied to reduce exposure to bioburden as nano-filtration is not considered a sterile processing step.

#### 1.2.1.4 Polishing

Polishing refers to one or a sequence of multiple chromatography steps that are designed to remove those impurities which are present after product capture. These impurities include process related impurities such as host cell proteins (HCP), DNA, non-specifically bound cell culture components from the capture step, endotoxin and product related impurities such as aggregates, fragments and modified or malformed product species. The capture step may also introduce impurities in the form of leached protein A ligand which must be removed. (Carter-Franklin et al., 2007) The polishing steps also have an associated virus removal capability which is added to that of the virus inactivation and the nano-filtration to add to the overall virus reduction capability of the process.

The most common technologies employed during polishing are cation exchange chromatography (CEX), anion exchange chromatography (AEX), hydrophobic interaction chromatography (HIC) and hydroxyapatite (HA).

**Anion exchange (AEX)** uses charge to separate impurities. In flow through mode the pH of the buffer is adjusted to below the isoelectric point (pI) of mAbs of between pH6.1 and 8.5. In this mode the mAb flows through the column and negatively charged impurities bind to the column. Operation above the pI means that the mAb will bind to the column and the impurities will flow through to waste. Given the high pI of

antibodies operation in flow through mode is more common. In this mode impurities such as DNA, HCP and endotoxin are reduced. (Curtis et al., 2003)

**Cation exchange (CEX)** is used for the reduction of HCP and DNA. (Tugcu et al., 2008) For cation exchange the relation to pI is reversed relative to AEX, as such the common mode of operation is bind and elute, where the product binds to the resin and the impurities are allowed to flow through. Cutting of the elution peak can also act to remove aggregates on some CEX steps as elution conditions can be adjusted such that they elute at a different rate than the product. (Aldington and Bonnerjea, 2007)

**Hydroxyapetite (HA)** is particularly used for the clearance of HCP and aggregates. Aggregates may be present from the beginning of the process as they often have intact Fc regions and so co-purify with the monomer on the capture step. Aggregates may also be generated during the process through a variety of mechanisms such as concentration dependent aggregation during UFDF steps. (Wang, 2005)

**Hydrophobic interaction chromatography (HIC)** can also be used for the reduction of HCP, DNA, endotoxin and aggregates. (Aldington and Bonnerjea, 2007) In this step high salt conditions are used to promote binding of the product and elution is achieved using a low salt elution buffer. The often high salt concentration required for binding can result in the precipitation of some mAbs so may limit the application of HIC as a platform technology.

**Scalability and Facility Fit Considerations:** Polishing steps are chromatography based therefore the scalability is the same as that for the product capture step. There may however be additional facility fit concerns regarding elution volumes, specifically when steps are operated in flow through elution where the degree of variability may be higher due to the likelihood of tailing elution peaks.

### 1.2.2 Other Platform Technologies

A presentation given by Martin Wrankmore of Lonza Biologics talked about the effects disposables have had on operations at Lonza. (Wrankmore, 2005) The presentation highlighted the key advantages of disposables technology which include:

- Greater flexibility to adapt to changes in demand generated by increases in prod-

uct concentrations from cell culture processes. i.e greater buffer demands, larger eluates from columns.

- Ability to simplify processes.
- Significant reduction in on-site preparation work, i.e Clean in Place (CIP)/ Steam in place (SIP)
- Reduced reliance on on-site utilities.
- More flexibility.
- Reduced risk of cross contamination.

The presentation goes on to talk about increased challenges that are presented by the development of consumables such as balancing the cost of consumables against costs savings through reduced time and resource demands. The cost aspect has been discussed in various articles. An article in Bioprocess International talks about the design of a concept facility. (Sinclair and Monge, 2002) The facility proposed was designed to take full advantage of disposable technology in all areas of the process. In terms of economics the initial capital outlay for this concept facility is expected to be up to 41% lower, (reduced from £26.3m to £15.4m), than a similar non-disposables based facility. Notable savings predicted by Sinclair are seen in areas such as buffer preparation, harvest, process utilities, solution handling, electrical power and validation. In each of these areas the concept facility is predicted to reduce capital costs by between 66-93%. The only notable area where costs are predicted to increase is in the fitting out of clean-rooms which sees a 26% increase. The reason for this increase is not discussed. A cost of goods analysis carried out by the same authors (Sinclair and Monge, 2005) stated that a traditional stainless steal based facility, producing 15-16kg per annum of a mAb, could see a cost of goods saving of 8% if retrofitted to take advantage of disposable technology.

## 1.3 Bioprocess Modelling

Bioprocess modelling has been employed to support decision making in biopharmaceutical manufacturing in a variety of areas. Many of these models utilise simulation

packages such as Extend (Imagine That! Inc. San Jose, USA), G2 (Gensym Corp. TX, USA.) (Farid et al., 2007) or Aspen Batch (Aspen Technology Inc. MA, USA.) or are mathematical, where the performance of processes is calculated using differential equations. (Groep et al., 2000) The latter has seen limited success in the modelling of biopharmaceutical processes due in part to the high degree of complexity and uncertainty inherent in biological systems.

Applications such as Excel (Microsoft Inc. WA, USA.) and Superpro Designer (Intelligen Inc. NJ USA) are commonly used to carry out investigations into facility throughput, utility sizing and capital cost estimations. As static models, these tools are limited in their ability to take into account the effects of dynamic factors such as resource and utility constraints.(Gosling, 2005) In order to provide a more complete analysis, tools must be able to account for those parameters which are time sensitive. This is especially crucial when considering that two key parameters of interest are process time and cost. With the simplified assumption that any amount of monoclonal antibody (mAb) can be captured from any scale of process given sufficient time, capturing and investigating the trade-offs requires an accurate calculation of both elements.

Previous models have followed common trends in structure and have been constructed using simulation engines such as Extend and a combination of spreadsheets and custom user interfaces to manage the flow and storage of data. (Chhatre et al., 2007) Additional graphical output has also been accomplished by linking the Excel spreadsheets to MATLAB (The Mathworks Inc. MA, USA). (Chhatre et al., 2006) Combining multiple applications in a single framework has the advantage of being able to account for the weaknesses of one application with the strengths of another. Whilst Chhatre et al. demonstrated this advantage, the degree of integration between the components of the framework varied as did the methods for transferring data. Data connectors such as those utilising open database connectivity (ODBC) can be used to link the various elements of the framework. ODBC is an application and platform independent application programming interface that enables a wide array of software tools to exchange data using standardised methods. By utilising ODBC for data exchange the elements of the simulation framework become compatible with a huge array of tools ranging from statistical, graphical and data storage applications.

Many of the existing approaches rely on prior knowledge of the factors to be investigated with a view to creating a model specific to the task. (Chhatre et al., 2007; Farid et al., 2005b; Groep et al., 2000) To provide a framework that can handle different processes simultaneously, enable rapid reconfiguration with minimal effort and have the flexibility to provide data for any number of process development questions to a user with minimal modelling experience a more flexible approach is needed. In addition to a flexible simulation engine, there is also the need for a data structure which allows for the definition of a process and facility whilst remaining flexible enough to be adaptable to scale. Previous research has described a hierarchical approach to structuring bioprocess models (Farid et al., 2005b), this approach could serve as the foundation to aid in the creation of standardised models and is of particular interest in developing a structure for data storage which can be shared between models constructed on different platforms by different developers.

Uncertainty is inherent with all biological processes, the risk of contamination of cell culture, variability in product titres or failure of equipment can impact on process design. Outside the process the possibility of products failing in clinical trials or changes in market demand can affect the amount of product required and whether processes will even appear on the manufacturing schedule. Monte Carlo simulations have been used to generate data on the effects of risk and how different processing options are compared (Lim et al., 2006). As alluded to earlier, the amount of data generated when carrying out Monte Carlo simulation is significantly higher than from deterministic models. Arriving at an optimum configuration will require the simulation framework to assess potentially thousands of options, this must be done rapidly and automatically.

## 1.4 Current Modelling Practices

### 1.4.1 Financial Models

Most companies will at some point attempt to produce financial model to predict costs or to collate information to calculate company matrices such as Cost of Goods, Internal Rate of Return, NPV or simple cash flow analyses. Most often these are static models built in Excel.(Gosling, 2005) The products and use of these models is discussed below.

#### 1.4.1.1 Cost of Goods

Cost of goods (COG) analyses are used to provide a standard metric for manufacturing costs. In the biopharmaceutical industry, COG is generally quoted as /g. In the manufacture of any pharmaceutical it is always advantageous to have the lowest possible cost of goods. For this reason, COG is often one of the factors used to assess the impact of process changes. Pharmaceutical processes are complex and the calculation of cost of goods requires significant amounts of data, data which is not always readily available, even more difficult is the accurate calculation of COG in advance of product manufacture. A common approach to prediction of COG is to use factors to calculate indirect costs based on direct costs. An example of a direct cost would be raw material purchasing costs, this figure is usually readily available. More complex are the additional costs which that raw material accumulates by it being stored in warehouses or cooled. Factors can be used to account for these indirect costs. Farid et al. (2000); Sinnot (1993)

#### 1.4.1.2 Project Feasibility

Many financial models are used in an attempt to assess the feasibility of a project before it is executed, or to compare different projects that may be proposed to solve a similar problem. One of the most common analysis is the discounted cash flow, which is used to calculate the Net Present Value of a project (NPV). (Novais et al., 2001) NPV calculations are useful for rapid analysis for projects however the accuracy of these calculations are limited as NPV is reliant on a discount factor which can be in itself difficult to determine accurately. (Pandey, 2003)

### 1.4.2 Process Models

Process models are those models which are designed for analysis of processes either in part, such as in unit operation models, or as a whole. (Gosling, 2005) In process modelling the aim is to mimic the operation in the real world by using mathematical formulae and logical programming. Key concepts and approaches in process modelling are discussed below.

#### 1.4.2.1 Single Unit Operation Models

Computer models can be produced to predict the performance of single unit operations such a centrifugation. (Varga et al., 2001) In these sorts of models differential equations are generated to account for the changes in the variables over time. (Wai et al., 1996) Once derived, usually based on experimental results, the equations are entered into a mathematical modelling program such as Matlab where the values are calculated and graphical outputs are generated. Models such as these are generally accurate within a reasonable range however they are often quite specific, not only to unit operations, but to individual models of equipment. (Gosling, 2005) This specificity makes these types of models less ideal for entire process models on which to base management decisions.

#### 1.4.2.2 Discrete Event Simulation

A model is a computer representation of a representation of a system or process, a simulation is a model that accounts for the changes that occur within the system or process over time. Most simulations fall into two categories, continuous or discrete event. (Carson, 2005) Discrete event simulations differ from continuous in that discrete event models only calculate the state of the system at certain defined points in time, termed events. In continuous models the state of the system is defined by differential equations which alter continually with respect to time. (Carson, 1993)

When modelling industrial processes discrete event simulation is generally the preferred method as continuous simulations rely on equations which are not always available.

#### 1.4.2.3 Resource Allocation

In the real world tasks within a manufacturing process are constrained by resources and it is often resources shared between multiple processes that increase the complexity of a system and drive the need for simulation. (Law and Haider, 1989; Gosling, 2005) All discrete event simulation platforms provide methods for accounting of resources. As in the real world, resources will impact on the simulation by delaying or stopping events from occurring. When building a simulation it is critical to identify the resources present in a system and how they can effect the system

In many industrial processes, the pharmaceutical industry being no exception, the availability of resources can have a profound effect on the schedule of a process. At the same time many of the resources such as raw materials and equipment are high cost items and over availability can have significant negative effects on the cost of goods. Simulation provides a tool where strategies to maximise the efficiency of resource usage can be quickly developed and tested. (Gosling, 2005)

#### 1.4.2.4 Hierarchical Approach

In order to improve and perhaps begin to standardise the creation of process models, hierarchical programming provides a major design methodology in the creation of simulations; it looks at the process in a series of levels of increasing complexity. (Lim et al., 2004) By taking this approach and utilising the correct model building environment it is possible to take an evolutionary approach to building simulations. Each layer in the hierarchy can be built onto the next as new levels are developed, the model becomes increasingly more complex, and more accurate at representing the system. By taking this approach, the model begins to function and can be run and tested very early on in the development process.

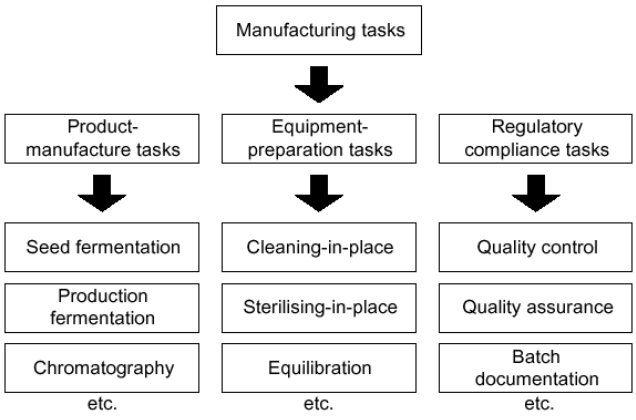



Figure 1.2: An example of an hierarchical framework to accommodate pharmaceutical manufacturing Lim et al. (2004)

There is a further level of complexity that is not represented in Figure 1.2, this level has been termed manufacturing phases (Farid et al., 2000) and represents the subtasks that make up each unit operation.

## 1.5 Simulation Packages

There are currently a number of packages on the market available for the modelling of entire pharmaceutical processes.(Gosling, 2005) These include:

- SuperPro Designer developed by Intelligen Inc.
- Aspen Batch developed by Aspen Technology Inc.
- Extend developed by Imagine That inc.

Some analysis can also be carried out in Microsoft Excel.

Both SuperPro and Aspen Batch provide the user with a bank of unit operations that can be used to build up a process. In both cases information about these operations are stored in libraries that can expanded by the users own data. With differing degrees of accuracy, both programs are also able to predict the effects of scale-up. The graphical user interface of SuperPro provides increased usability over Aspen Batch's command line based structure. Both programs provide scheduling information by considering the availability of resources. (Shanklin et al., 2001; Gosling, 2005)

Extend is a somewhat different package. Instead of having a bank of unit operations, Extend has a library of functions that can be used to build unit operations from scratch. Essentially this means that the user can make the model as simple or complex, as required. This flexibility also has disadvantages in that the model build process in Extend is more complex and time consuming. As with SuperPro, Extend provides a graphical user interface (GUI), greatly adding to it's usability, in addition to the GUI, Extend also provides the ability to modify the blocks in the simulation at the programming code level. The language used is ModL, a variant of C making it relatively easy to learn if required. (Krahl, 2002)

## 1.6 Managing Uncertainty

Many traditional approaches to assessing or predicting the performance of processing strategies have relied on physical metrics; such as cell culture titre, percentage recovery and batch times, and financial metrics such as cost and NPV. Although these can be

good indicators, they do not provide the whole picture. Additional methods have been proposed to provide decision-makers with more information. (Lim et al., 2005) Of particular interest are the methods used to account for and quantify uncertainty. This section discusses some of the proposed methods and how they may be incorporated into existing methods.

#### 1.6.0.5 Monte Carlo

Monte Carlo simulations are used to account for and assess the impact of risk on a system. The Monte Carlo approach works by understanding that certain parameters of a system are not fixed and are subject to a degree of variability. In many cases, although variable, these parameters are not chaotic and instead the variability can be accurately approximated using probability distributions. (Lim et al., 2005) Monte Carlo functionality will greatly increase the run time of the process due to the large number of iterations that are required to produce an accurate outcome. (Farid et al., 2005a) Several applications exist which give MS Excel Monte Carlo functionality, these include @RISK (Palisade Corporation, Newfield, NY) and Crystal Ball (Decisioneering, London, England). It is also possible to build Monte Carlo functionality into some modelling environments such as Extend. (Curry, 2006)

#### 1.6.0.6 Risk Adjusted Values

Another method has been demonstrated where values are adjusted based on their level of risk, an example would be risk adjusted net present value (rNPV). (Stewart et al., 2001) In general, NPV is calculated in the assumption that the project will be a success, this means that a high NPV on its own is meaningless if the chance of success is minimal, rNPV attempts to combine the metrics of Cost, Time and Risk into a single factor. The equation used for the risk adjustment of any value is shown in equation 1.1.

$$rV = PR_0 - \sum_{i=0}^{n} C_i \frac{R_0}{R_i} \tag{1.1}$$

Where: rV = Risk Adjusted Value, P = Payoff, $R_0$ = Current Risk $C_i$ = Associated Cost, $R_i$ = Risk associated with event $i$.

By adjusting the discounted cash flow throughout the project and carrying out a

standard NPV calculation the rNPV can be produced. This is a quick simple way to account for risk in financial factors within a project.

#### 1.6.0.7 Decision Trees

For more complex series of events where there are multiple outcome for various situations decision trees can be constructed. A decision tree is a network of nodes, where each node has a single input with multiple outputs. The probabilities are assigned to each output where the sum of all output probabilities for each node is 1. Each output can also have a cost or revenue associated with it. Decision trees can be analysed using Monte Carlo simulations. (Critchfield and Willard, 1986)

## 1.7 Multivariate Statistical Techniques

Multivariate analysis (MVA) techniques such as PCA have been used in a range of biotechnology applications. These include the use of PCA score plots to identify outlier batches across vaccine production runs. (Thomassen YE, 2010)

Rapid identification of outliers is facilitated when a large number of process variables across multiple batches can be reduced to a plot of two principal components that capture adequate variance in the data; observations outside a defined confidence interval are considered outliers. PCA score plots combined with loading plots have been used to assess process comparability for cell culture runs across different scales of production. (Kirdar AO, 2007)

As well as looking at a wide array of process variables, PCA has been used to reduce the dimensionality of complex and unwieldy datasets such as those generated by near-infrared spectral analysis and chromatograms. This approach has been adopted to analyse the near-infrared spectra of multiple raw material lots so as to understand the impact of raw material variability captured by the spectra on cell culture performance. (Lee HW, 2012; Kirdar AO, 2010)

On the downstream front, PCA has been used to analyse the impact of chromatography operating conditions and scales on chromatogram profiles as well as to generate predictive models for chromatographic separations.(Pate ME, 2004; Malmquist G, 1994; Larson TM, 2003; Edwards-Parton S, 2008; Hou Y, 2011)

Most works on MVA utilise historical datasets. However, the nature of tech transfer activities where a single batch is usually run to identify facility fit issues and resolve them before further batches are operated means that such datasets do not exist for this problem. However, companies with experience in tech transfer activities can identify typical process fluctuations seen from the expected base case performance. Monte Carlo simulation has been used increasingly in various bioprocessing examples such as process economics studies to capture the impact of common manufacturing uncertainties such as yields and batch failures on cost metrics. (Farid et al., 2005b,a; Lim et al., 2005, 2006; Pollock J, 2013)

Other applications include fermentation kinetic modelling studies where the impact of uncertain model parameters on outputs such as biomass generation are accounted for (Sin G, 2009) and portfolio management and capacity planning models where reward-risk characteristics are generated given key technical, clinical, and commercial uncertainties. (George and Farid, 2008; Rajapakse et al., 2005)

## 1.8 Research Focus

This work in this thesis focuses on the capture and purification operation steps which will for the remainder be referred to as downstream processing (DSP). These DSP operations receive material from upstream processing (USP) at a frequency which is determined by the number of reactors and the cell culture time. This frequency in turn determines the DSP slot length. For companies such as contract manufacturers, a key aim during process development is to size equipment to meet the slot length at minimum cost to ensure that DSP operations do not become the bottleneck in the production schedule.

Achieving the optimal trade-off between processing time and cost is a complex problem. As well as the core process operations, as discussed above, there are many ancillary operations which must be taken into account. These operations include the preparation of process buffers and the cleaning and sterilisation of equipment. Both core and ancillary operations interact through a shared set of resources. Equipment resources include chromatography columns and rigs, UF/DF skids as well as hold and process vessels. Material resources include disposable bags, chromatography resins,

filter cartridges, buffer reagents and water for injection.

Many facilities will run multiple processing suites simultaneously where some or all of the above resources are shared between multiple processes. The advantage of multiple suite operations is that the slot length can be increased with multiple batches being processed simultaneously. The disadvantage is the difficulty in predicting how the resource demands from one processing suite will affect the resource availability in another.

To increase complexity further it is also important to consider the inherent uncertainties present when processing biological materials. Cell culture product titre, process yields, filtration flow rates, chromatography binding capacities and elution volumes are just a few of the parameters which can see significant batch-to-batch variation. This variation can lead to some batches falling outside the operating limits of a facility and may result in product loss or the loss of entire batches. An understanding of the effects of this variation can aid in the design of more robust processes.

Process design must be carried out with a view to the changing landscape of biomanufacturing. Predictions are that cell culture product titres will continue to increase for both new products and throughout product life-cycles. This puts increased pressure on purification which currently suffers from equipment size limitations for chromatography columns, space constraints within facilities and time constraints to maintain the production schedule. The option presented to developers is to either intensify existing processes to remove bottlenecks or to investigate alternative technologies with fewer limitations and greater scalability.

Process design should also be carried out whilst considering the entire lifecycle of a drug product. This includes an understanding of the technology transfer activities which will be carried at each phase from pilot to clinical and finally commercial scale manufacture. This is not only a consideration of how processes will scale, but how they will fit into the facilities into which they may eventually be transferred. These facility fit assessments carried out early in the development of a process can highlight potential sub-optimality in later phase processing and probabilities of product loss or batch failure.

The need is for more systematic methods to capture the complexities of operating

multi-product facilities, assess the impact of increasing titres at the process-business interface and carry out facility fit assessments and root cause analysis.

# Chapter 2

# Development of the Simulation Framework

## 2.1 Introduction

This chapter outlines the development of the simulation framework from the definition of the scope to a detailed description of the components within the framework.

## 2.2 Scope

The scope was defined as follows:

- To model multi-suite, multi-product facilities with different process sequences, demands and performances for each product.
- To evaluate the performance of multi-product facilities across a range of cell culture titres and scales based on cost of goods (COG), throughput, resource utilisation and risk metrics.

The types of scenarios that the tool should be able to address were defined as:

- Operational Level
  - Identification of facility limits at higher titres and testing of process intensification strategies.
- Tactical level

  - Selection of optimum purification capacity to meet a schedule.
  - Assessment of robustness of purification capacity to titre fluctuations.

- Strategic Level
  - Prediction of failures upon technology transfer to larger facilities and root cause analysis.
  - Impact of developing alternative purification platforms.

## 2.3 Requirements Specification and Software Selection

Considering the tool scope and research focus a requirements specification for the integrated software platforms was defined. The requirements specification for this framework was adapted from previous work. (Farid et al., 2007) New key drivers for this research included the need to share and manipulate large amounts of data as well as to rapidly reconfigure multiple processes in a multi-product and multi-suite facility whilst maintaining the ability to assess the impact of uncertainty and present the results through novel visualisation techniques. The specification is outlined in Table 2.1.

Table 2.1: Requirements specification.

| **Requirement Type** | **Specification** |
|---|---|
| Representation of declarative knowledge | Tasks and their characteristics |
| | Resources and their characteristics |
| | Material flow and its characteristics |
| | Sequence of tasks |
| | Resource requirements for each task |
| | Calculation procedures for mass balances and costing |
| | Variables for the calculation procedures |
| | Time |
| | Hierarchical views of tasks |

*Continued on next page...*

| **Requirement Type** *(continued)* | **Specification** *(continued)* |
|---|---|
| | Risk/uncertainty: stochastic variables defined using probability distributions |
| | Multi-product facilities |
| | Facility definition |
| | Processing Suites |
| | Demands / Customer orders |
| Dynamic simulation | Dynamic simulation of sequences |
| | Dynamic allocation of resources to tasks |
| | Dynamic invocation of calculation procedure to compute compositions and costs |
| | Dynamic invocation of procedures to compute resource utilisation statistics |
| | Monte Carlo simulation |
| | Single-threaded, multi-threaded and parallel processing |
| Flexible development environment | Graphical user interface |
| | Modular |
| | Extensible |
| | Ability to store large amounts of data |
| | Database driven |

A key aim in the development of the software tool was the creation of a flexible environment enabling users to specify a wide array of process sequences whilst having minimal programming experience, to this end, it was seen as critical to maintain independence between the data and the simulations. Input and output data accessed via a database rather than being embedded in a programming language can provide the user with a familiar environment similar to a spreadsheet or other data entry methods which may be in use in legacy systems. As a result linking a database platform to a discrete-

event simulation was found to be an efficient way of capturing all the requirements.

Multiple process simulations demand more complex data management components than those available in the spreadsheet based simulation frameworks. To address these issues, the MySQL distribution (MySQL AG. Uppsala, Sweden) of structured query language (SQL) was chosen for implementation into this framework.

SQL is a powerful and flexible data storage engine, capable of handling the volume of data and maintaining a logical structure for organising and relating information. This ability to specify strict data structures in the form of related data tables and data type definitions in addition to the large storage potential provides the framework with a clear advantage over spreadsheet based approaches. Validation functions act to maintain the integrity of the dataset by verifying the format of entered values as well as maintaining referential integrity between the tables during update and deletion of data. Key database fields are continually indexed to improve sorting and data retrieval operations to reduce the simulation run times when operating with a large dataset.

Maintaining the database on a dedicated server allows for a more efficient workflow where analysis of data and the planning of future simulations can be carried out while simulation jobs are in progress. Multiple networked computers connected to the server provide the hardware framework to parallelise the simulation engines in the form of a distributed cluster. This is advantageous when performing Monte Carlo simulations where run times may be higher.

Extend (Imagine That! Inc, San Jose, USA) was selected as the discrete-event simulation package to simulate a wide array of processes with dynamic resource allocation and scheduling. Discrete-event simulation models are composed of a series of interconnected blocks between which items containing variables or attributes move. The blocks contain functions which generate simulation events. Events may act to delay the progress of the item through the model such that subsequent events are generated at a later simulation time. This functionality can be used when simulating any activity which takes time. This is valuable when calculating the impact of resource delays (e.g. due to labour constraints) and shift patterns on batch throughput.

SQL data access functionality is not available natively in Extend, rather the functionality was developed using the proprietary compiled modelling language ModL,

unique to Extend, and the Simulation Dynamics Industry Developer Application Programming Interface SDIDAPI, a third party API. (Simulation Dynamics Inc. San Jose, USA). This functionality uses Microsoft Open Database Connectivity (ODBC) as middleware to control communication between SQL and Extend. ODBC functionality was further utilised to connect third party applications such as Microsoft Access and Sigma Plot, for data entry, retrieval and visualisation. These connections are summarised in Figure 2.1.

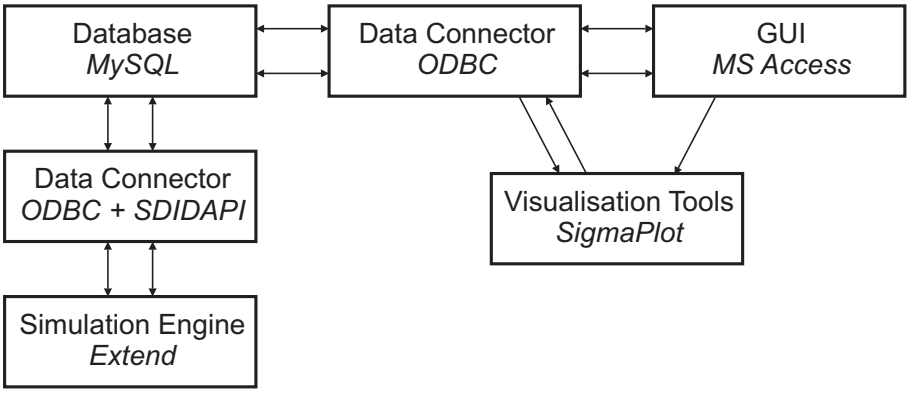



Figure 2.1: Diagram outlining the connection of third party tools to the simulation framework. (ODBC = Open Database Connectivity, SDIDAPI = Simulation Dynamics Industry Developer Application Programming Interface, MS = Microsoft.)

## 2.4 Tool Implementation

### 2.4.1 Hierarchical Representation of Facility and Process Details

The representation of the key features of the manufacturing process addressed by the tool was based on the hierarchical data structure outlined by Farid and co-workers (Farid 2002; Farid et al. 2007a), in which different levels of detail of the process and facility are defined. Figure 2.2 shows the interpretation of this hierarchical structure through the use of a unified modelling language (UML) class diagram, which represents key elements of the process to be implemented in the database and in the simulation engine.

The UML class diagram is very commonly used as a standard language to specify the structure of databases in an object-oriented way. In this diagram the different types

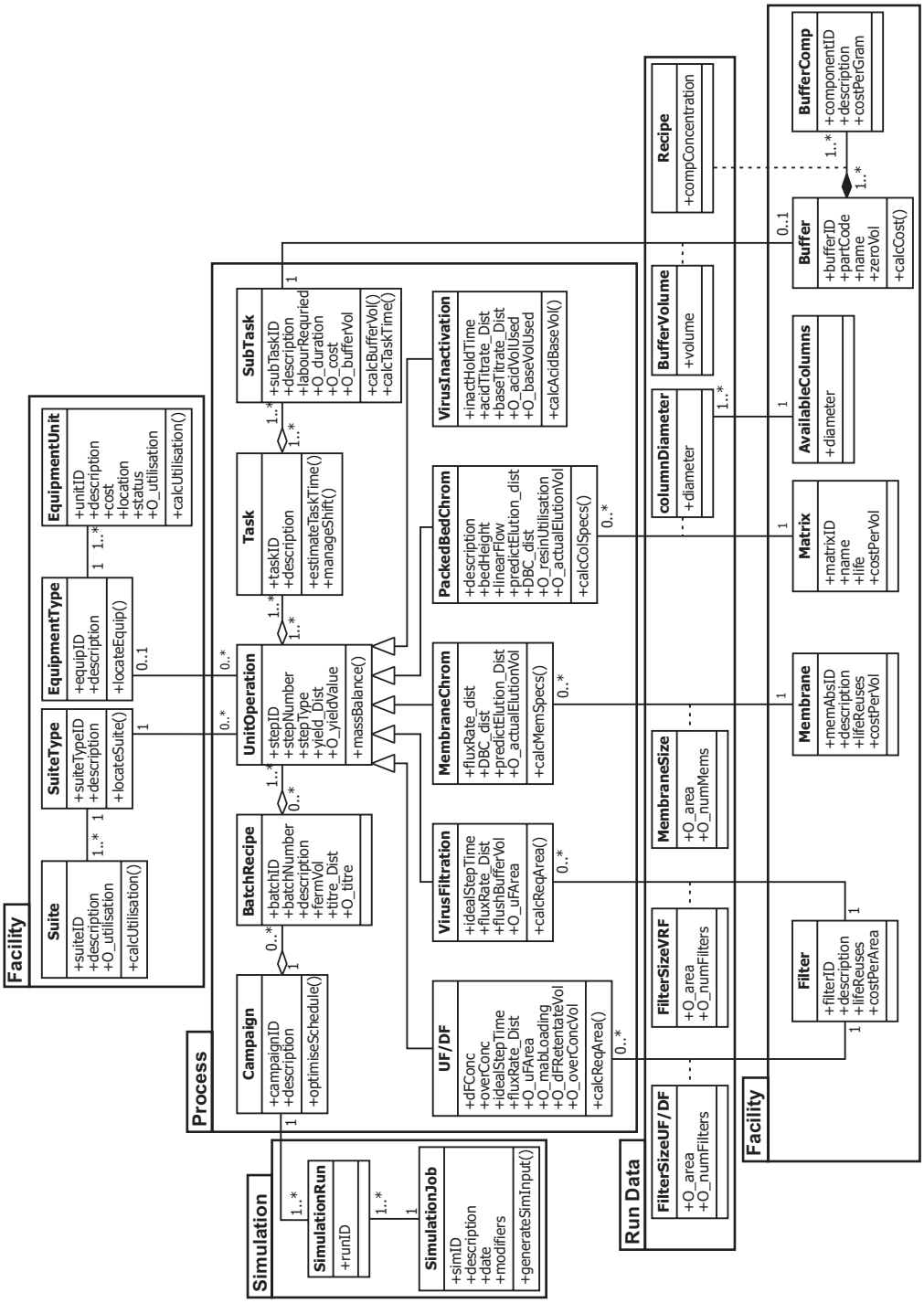



Figure 2.2: UML Class diagram of the simulation framework. Some functions and attributes have been removed for clarity. Each block shows the class name and a list of selected attributes and operations. The various classes have been grouped into packages. The classes in each package have similar or strongly related functionality.

of objects (classes) and their relationships are described.

In Figure 2.2 each block represents a class with a name (e.g. UnitOperation), a list of attributes (e.g. stepID, stepType) and a list of operations (e.g. massBalance). In this diagram a generalisation relationship exists between the class UnitOperation and the classes representing the different types of step (UF/DF, VirusFiltration, etc). It represents a relationship of type "IS A" (e.g. UF/DF is a UnitOperation). Classes with similar functions or themes are grouped into packages (e.g. the Facility package includes the classes to define suites and equipment.)

We can also see aggregation relationships (unfilled diamond and a line), specifying "part-whole" relationships (e.g. a Campaign has BatchRecipes a BatchRecipe has UnitOperations, etc). The multiplicity of an association is represented by the numbers next to the lines (e.g. one Suite has exactly one SuiteType while one SuiteType can have one or more Suites associated with it.). These part-whole relationships establish the hierarchical nature of the framework: campaigns are at the highest level and sub-tasks at the lowest.

## 2.5 The Database

### 2.5.1 Database Structure

The relational structure of the database implemented in SQL derives directly from the class structure outlined in Figure 2.2. Bennett et al. (2005) present a set of rules to correctly map the classes to database tables. In many cases where there are simple one-to-one or one-to-many relationships between classes, these classes can be directly translated to tables where the attributes become the fields and the relationships are enforced using foreign keys. An example using this form of mapping would include the Campaign and BatchRecipe classes with the one-to-many relationship between them. In this case, the BatchRecipe table contains the campaignID field from the campaign table as a foreign key. The result is a single record in the Campaign table linked to multiple records in the BatchRecipe table. A more complex mapping example would be those required for the Buffer, BufferComponent and BufferRecipe classes. The relationship between the Buffer and BufferComponent is a many-to-many relationship

defined by the association class BufferRecipe. In the database these classes become three tables with the BufferRecipe table containing foreign keys from both the other tables. In this case, the relationship is enforced via the intermediate table. This relationship can be viewed graphically in the screenshot shown in Figure 2.3.

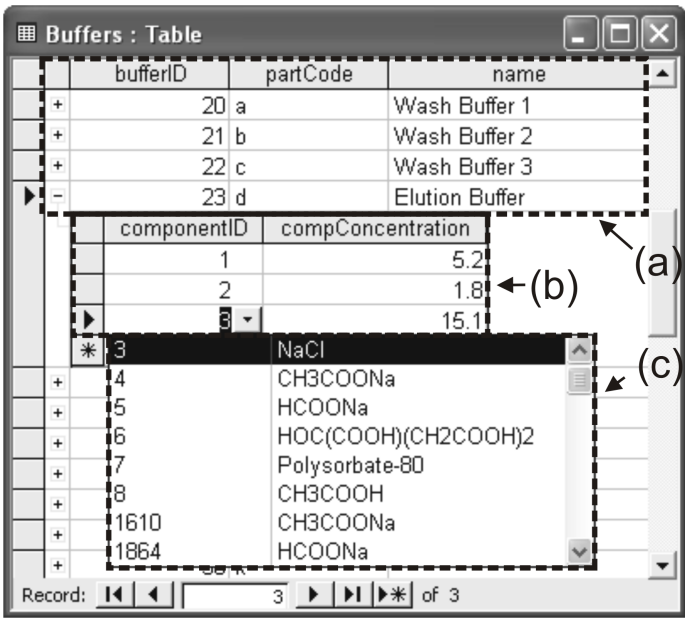



Figure 2.3: Screenshot of the buffer definition user interface. (a) buffer fields, (b) bufferRecipe fields, (c) bufferComponents fields.

The tables in the database can be grouped in a similar way as the packages, however, the relationships between the packages are only realised when the simulation engine brings the elements together in a simulation job. For example, it is during the simulation that a particular equipment unit is assigned to a unit operation.

The tables in the Process package contain the information relative to the description of the process, from the batch name and input titre, at the highest level, to the buffers and labour requirements for each individual sub-task. The Facility package tables contain information regarding the structure of the facility and include the availability of suites and equipment. The Simulation package tables are used to define the conditions of the simulation and how the base case data stored in the database will be modified for each simulation job.

### 2.5.2 Process Definition

Figure 2.2 shows that the Process package contains, amongst others, the five classes which make up the process hierarchy: Campaign, BatchRecipe, UnitOperation, Task and SubTask. Figure 2.4 shows a screenshot of the process hierarchy tables from the BatchRecipe to the SubTask when viewed from within a Microsoft Access (Microsoft Corp, Redmond, WA, USA) database connected to the SQL server. MS Access has been used to demonstrate an example of a data entry interface where the user can drill down through the hierarchy by expanding records to view the information in the level below.

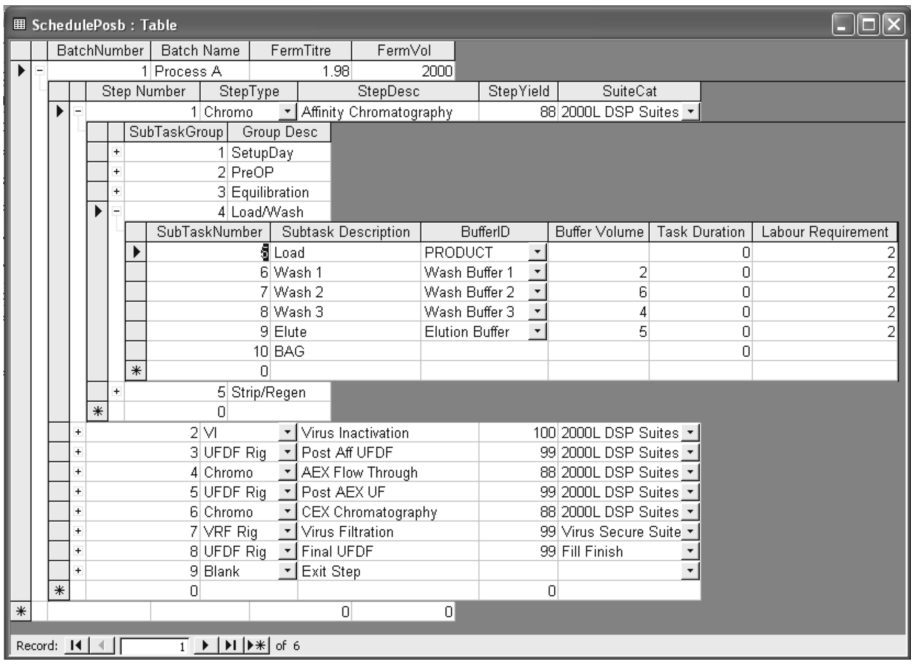



Figure 2.4: Screenshot of the process hierarchy data entry tables. This screenshot was generated using Microsoft Access as the front-end to the SQL database.

In Figure 2.4 a single batch record for Process A has been expanded to show the unit operations. The first of these steps, affinity chromatography, has been further expanded to expose the five tasks and finally the load/wash/elute task has been expanded to view the six sub-tasks contained within. Values such as StepType and BufferID are specified using drop down boxes, which contain values from other tables representing different classes. For example, the BufferID field of the SubTask is the result of a relationship with the Buffer class in the Facility package. Similarly the StepType field

in the UnitOperation level is used to form the generalisation relationship, by selecting the appropriate operation from the available types. This intuitive user interface structure is a direct consequence of the structure defined in SQL and serves to illustrate the advantage of incorporating a relational database into the framework.

The definition of the process is done using scale independent parameters. For example, in Figure 2.4 the buffer volume field on the sub-task is defined in a scale independent parameter relative to the column volume of the step rather than in litres. This is because the scale of the step, i.e the chromatography column volume, is not defined until run time, using this notation ensure that the volume of buffer required scales accordingly. Such scale independent parameters enable a single process to be used in a range of investigations at different scales and with different facility configurations without the need for modification within the database. This functionality is advantageous when the framework is used to assess technology transfer challenges where a largely fixed process will be simulated at a variety of scales from the pilot plant to full scale manufacturing.

### 2.5.3 Facility Definition

The facility definition consists of several elements which define the infrastructure resources such as the suites, vessels, and equipment as well as material resources such as buffer components, filter membranes and chromatography resins. Data are also stored that define labour availability and shift patterns.

In the database structure facility definition is defined independently of the process description allowing the performance of a single process to be assessed in multiple facilities. This is of particular use when considering process fit analyses for tech transfer operations, where the aim is to assess the performance of a process in a variety of facilities with differing capabilities.

Figure 2.3 shows the user interface for the definition of buffers. Buffers are stored over three tables. Each buffer has one buffer recipe and it is composed of a number of raw materials. By expanding each buffer the recipe for that buffer is displayed as a list of part numbers and quantities. Clicking the drop-down box for each part provides a list of available components. In the case of Figure 2.3, 15.1 g/L of NaCl is being added

as a component of an elution buffer. The drop-down list is designed to show a useful summary of the component list to the end user. The full buffer component table also contains cost data which is used when calculating the total cost of the buffer during simulations. This method of defining buffers by their components is a more efficient method than specifying each buffer as an individual raw material. From the perspective of data entry the costs of raw materials and recipes is more readily available information than the costs of a buffer. It also allows for the easier investigation into the effects of changing raw material costs since changing a single component cost will automatically update related buffer costs.

### 2.5.4 Simulation Definition

Simulation jobs are defined using two tables in the database. The first of these provides a unique identifier for the simulation job along with a description and the current status of the job. It is this first table that the simulation engine interrogates to identify if there are any jobs pending.

The second table records the specification of the simulation job. The rows in this table are used to construct update queries which modify specific values in the process and facility tables. The construction of the modifier queries will depend on the type of parameters to be investigated.

The framework can be configured to run a number of simulation types. At the conclusion of all simulations, the results are exported back to the database as a series of archive tables. The results archive contains a copy of all input data tables as well as dedicated results tables. A unique identifier field is added to each table to store the job number such that the data from each simulation can be recalled at a later date

#### 2.5.4.1 Base Case Analysis

The simplest simulation type, the base case analysis, uses data drawn directly from the process and facility tables without modifiers. Simulations of this type are used to provide a set of base case results to which other simulations can be compared. As well as providing useful results, this type of simulation is used to validate the input data. Errors in the input data which were not identified by the database will be reported by the simulation engine. The speed of base case simulations makes them ideal for

debugging operations.

#### 2.5.4.2 Multiple Simulation Analysis

For more complex investigations multiple simulation jobs can be specified by using modifier queries. This allows for the parameters in the base case to be changed automatically without affecting the source data. The modifiers queries are stored in four fields in the database which are combined by the simulation engine to generate a correctly formatted SQL query. This method can be used to define multiple jobs changing a field value for each. During data analysis the jobs can be combined such that output data can be linked directly to the changing input parameter.

#### 2.5.4.3 Monte Carlo Simulation

The framework can also be used to run Monte Carlo simulations. Monte Carlo simulations are defined using a similar method to the multiple simulation jobs with modifier queries being used to define the parameters. There are however differences in how the data in the process and facility specification tables is used and the way in which the simulation engine runs.

In Monte Carlo simulation the sensitised parameters are stored using two fields instead of the single field used in normal simulations. These two fields store the type and bounds (e.g. type: triangular, bounds: minimum, maximum, mode). In Monte Carlo simulations the simulation engine is configured to import the distribution data instead of the single value field and generate a value within the bounds using a random number generator.

The simulation engine runs a number of iterations in a single job using a different random number seed for each. The number of iterations is specified in the simulation tables. The simulation outputs a result set as with other simulations however in addition to the unique job reference an additional field is used to store the run number. This enables the results from runs to be analysed individually as well as together.

## 2.6 The Simulation Engine

In order to maintain the flexibility of the framework, the model is required to be adaptable to a wide array of processes. To this end, a router-based simulation approach was developed. Figure 2.5 shows how this approach was developed and how it differs from traditional practices.

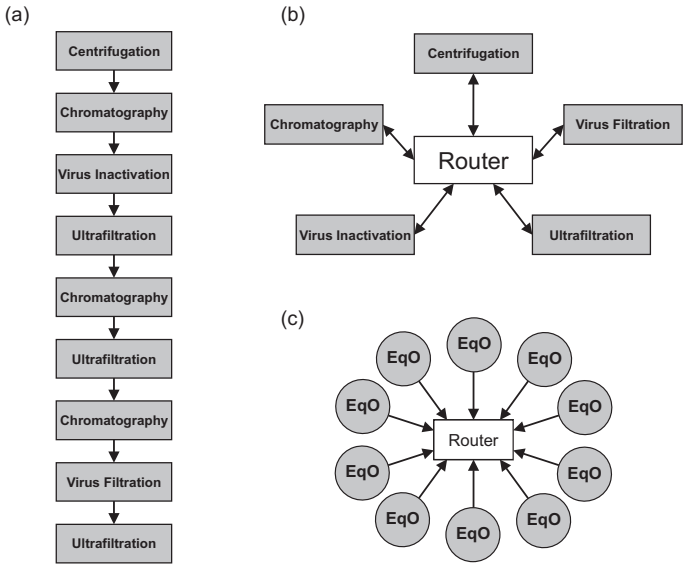



Figure 2.5: Evolution of the simulation structure. (a) Linear, unit operation based simulation approach. (b) Router and pre-defined unit operations. (c) Router and generic equipment operations.

Figure 2.5a represents a more traditional approach, here a process is modelled as a linear sequence of unit operations. Figure 2.5b shows how this linear structure can be modified using a router. The limitation here is that it is difficult to model multiple processes simultaneously as a single unit operation may be used in several processes. The revised router structure shown in Figure 2.5c replaces the traditional unit operation with a more flexible equipment operation. Each equipment operation contains the functions to become any unit operation allowing the same type of operation to be carried out in multiple nodes. In this approach all the nodes are identical and development is simplified. This methodology is in-keeping with the software engineering principle of abstraction which aims to reduce the amount of programming code through the use of standardised functions and procedures.

### 2.6.1 Class Implementation in Extend

ModL, the compiled language used in the Extend simulation, is similar to the more common programming language C; as such there is no native object-oriented programming functionality.

When creating the simulation functions, the SQL database tables or classes, are associated with simulation blocks. Upon import into the simulation, the structure of the tables is maintained however the relationships between the tables are lost. To maintain the integrity of the data after the import into Extend, an SQL function adds a coded variable to the tables. The coded variable enables the correct object, represented by table rows to be referenced by the simulation engine. The coded variable is an eight-digit number assigned to each object in the BatchRecipe, UnitOperation, Task and Subtask classes. The code is generated based on the position of the object within the process as shown in Table 2.2.

Table 2.2: Construction of the coded variable.

| Class | Batch | Unit Op | Task | Subtask | Code |
|---|---|---|---|---|---|
| BatchRecipe | 1 | - | - | - | 01000000 |
| UnitOperation | 1 | 2 | - | - | 01020000 |
| Task | 1 | 2 | 3 | - | 01020400 |
| Subtask | 1 | 2 | 3 | 4 | 01020304 |

Functions in the simulation engine keep track of the current simulation position such that the code can be generated at any point and used to retrieve the attributes for the correct object. To access objects from other classes which do not contain the coded variable, the code is first used to retrieve the primary key from one of the above classes, this value is in turn used to identify the object attribute values in the related table using the foreign key methodology discussed in 2.5.1.

#### 2.6.1.1 The Core Structure

The core of the simulation engine contains the primary elements such as the router and the blocks responsible for simulating the unit operations as shown in Figure 2.6. Elements outside the core contain functionality to simulate ancillary buffer preparation and vessel cleaning.

#### 2.6.1.2 Setup and Initialisation

The simulation begins with an initial setup and initialisation phase and includes the functions of the SimulationJob and SimulationRun classes. These functions generate the dataset for the current instance of the simulation by creating a temporary snapshot of the database, modifying field values using the attributes defined in the Simulation classes and transferring the dataset to storage local to the simulation instance. To avoid conflicts between multiple instances of the simulation engine, the database is locked to a single instance until the complete dataset has been defined.

Initialisation involves defining the simulation elements that will correspond to the buffers, equipment and vessels, in essence configuring the model to represent the facility specified in the database. Using data regarding upstream processing (USP) operations, the frequency of batches entering DSP can be calculated and is used to generate simulation items. Data are stored on items as attributes allowing for the passing of variables between the blocks in the simulation. An attribute on the item is initialised with an increasing batch number used in the generation of the batch level coded variables; this value will remain constant for the life of the item. Additional attributes for calculation of the unit operation, task and subtask coded variables are initialised to 1; unlike the batch level variable these attributes will be incremented as the process progresses through the simulation.

The items are independent and can take different paths through the simulation engine blocks. The path taken by the items is defined jointly by data stored in the database along with on-the-fly decisions made by the simulation. These decisions may be affected by resource, facility and equipment constraints. It is this decision-making ability that enables the simulation engine to simulate a wide array of processes without modification.

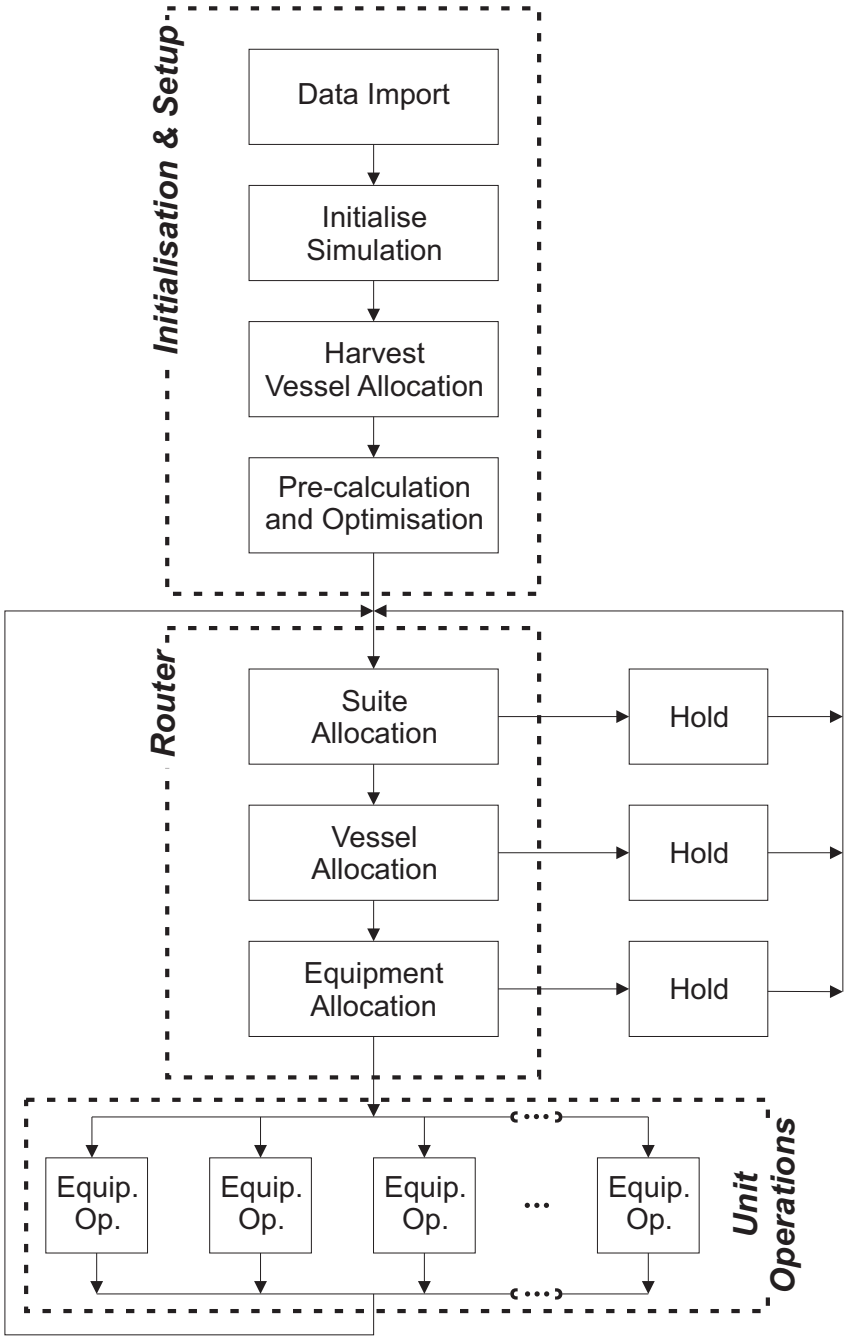



Figure 2.6: Core elements of the simulation engine. This structure enables the route taken by the simulation items to be defined in the database rather than as a fixed sequence of operations. The equipment allocation block carries out mass balance calculations, identifies appropriate equipment and routes the item to the corresponding equipment block. The structure of the equipment operation blocks is shown in Figure 2.7.

The process simulation now begins with vessel allocation. Depending on the process specification the vessel allocated may be stainless steel or a disposable bag. Input times to vessels are recorded in the database and will be used to calculate hold times and vessel utilisation. Management of vessels will be discussed in a later section.

Each item passes through a pre-calculation and optimisation block. Using data from the database the pre-calculation block performs a rapid analysis of the batch to be simulated. The aim of the block is to arrive at the optimum process configuration by identifying the equipment sizes able to process the batch within a desired deadline whilst minimising costs. The pre-calculation block uses brute force optimisation, a decision space of all the equipment size configurations possible is generated and the configuration with the lowest cost and with a process time closest to the manufacturing slot length is selected.

The optimisation routines are initialised by the OptimiseSchedule procedure associated with the Campaign class. This procedure serves a similar function the router element of the simulation engine however instead of routing simulation items; the procedure calls the functions of the simulation engine to estimate the mass balance, buffer costs and task times in the UnitOperation and Subtask classes. These functions are being called programmatically rather than being triggered by simulation events and therefore calculate parameters using an incomplete dataset. For example for the purposes of estimation, all resources and equipment are considered to be unconstrained since the data on resources usage is not available without running the simulation. The result is an estimation of batch cost and time. The process time estimate is improved by an additional procedure which attempts to account for process delays incurred through shift based operation.

Once the cost and time for all the process configurations is complete, a procedure selects the configuration that meets the processing time constraint at the lowest cost.

#### 2.6.1.3 The Router

After the pre-calculation the items move into a series of blocks that determine the correct processing suite and allocate specific items of equipment and further processing vessels. The selection of suites, equipment and vessels is done based on information

stored in the database combined with data on the current status of the facility, i.e. suites and vessels may already be in use and equipment may be utilised elsewhere in the facility. The functionality developed in the router element of the simulation engine is key to the frameworks ability to adapt to any process and facility configuration specified. Establishing a clear set of rules enables the simulation engine to make decisions which would otherwise have to be specified in the input data. This is time consuming as any process or scale change could potentially require the user to manually specify new equipment for each step. By taking an automated approach the performance of a fixed process can be assessed rapidly across a range of facility configurations and scales.

In order to provide enough information for suite allocation, the suites in the facility must first be classed by capability. For example a number of suites could be allocated specifically for chromatography operations because they contain large tanks and chromatography rigs. In the process specification each unit operation is assigned to a suite class. If classifying suites is not appropriate, the user can specify a range of suites in which the step can be performed. This is less advantageous since these values must be changed for each facility. The specific suite will be decided by the model at run-time based on class and availability. If no suites are available, the batch will be routed to a holding area and will be released when a suite becomes available.

After suite assignment, process vessels are allocated. With stainless steel vessels the selection of the correct vessel may be dependent of the item of equipment selected as well as the location of the suite. These limitations can be defined in the database; the vessel allocation block identifies the correct vessel and allocates it to the batch. If a vessel is not currently available the item will be routed to a holding routine until the vessel is released. Allocation of vessels is based on location, availability and required volume. It may also be based on a physical connection to a specific item of equipment as may be the case for filtration retentate vessels.

For equipment allocation the simulation engine will select equipment based on type, availability and a scale parameter. The scale parameter is a volumetric flow rate range for chromatography and ultrafiltration rigs and a maximum process volume for virus filtration. The scale parameter used for the selection of equipment was calculated by the pre-calculation block during the initialisation phase of the simulation. The

equipment allocation block contains further balance calculations for the product across the unit operation. The values generated here are more accurate than those estimated in the pre-calculation since they represent the current state of the process including any losses which may have been incurred previously. This more up to date information is used to determine the correct filter areas and chromatography cycles. These functions form part of the massBalance operation in the unitOperation class and use data from specific unit operation type classes i.e. UFDF, VirusFiltration etc... The specific mass balance calculations used will depend on the unit operation type of the current instance of the unitOperation class.

Where multiple units of equipment are available the model will preferentially allocate any equipment which already resides in the current operating suite. This is an important rule if we consider a process with multiple chromatography steps being carried out in the same suite. If possible the same rig will be used for all operations thus avoiding changeover delays.

Each item of equipment is assigned to an equipment operation block during initialisation of the model. The allocation of equipment directly affects the route taken by the item through the simulation. This functionality is key the frameworks ability to adapt automatically to the process and facility specified.

#### 2.6.1.4 Unit Operations

The term equipment operation is used in the framework to describe those elements of the simulation engine which contain functions to mimic the unit operations.

The equipment operation blocks contain the functions which allocate resources, calculate processing times and handle the scheduling of sub-tasks. Once calculation in these blocks is complete, the items are sent back to the router to be redirected to the next operation.

Figure 2.7 shows a more detailed overview of the equipment operation blocks. The path taken by the simulation items is shown as well as the lines of communications with various resource manager blocks. The triggering of functions within the equipment operation is iterative, with items cycling through the various elements within the sub-task loop shown in Figure 2.7.

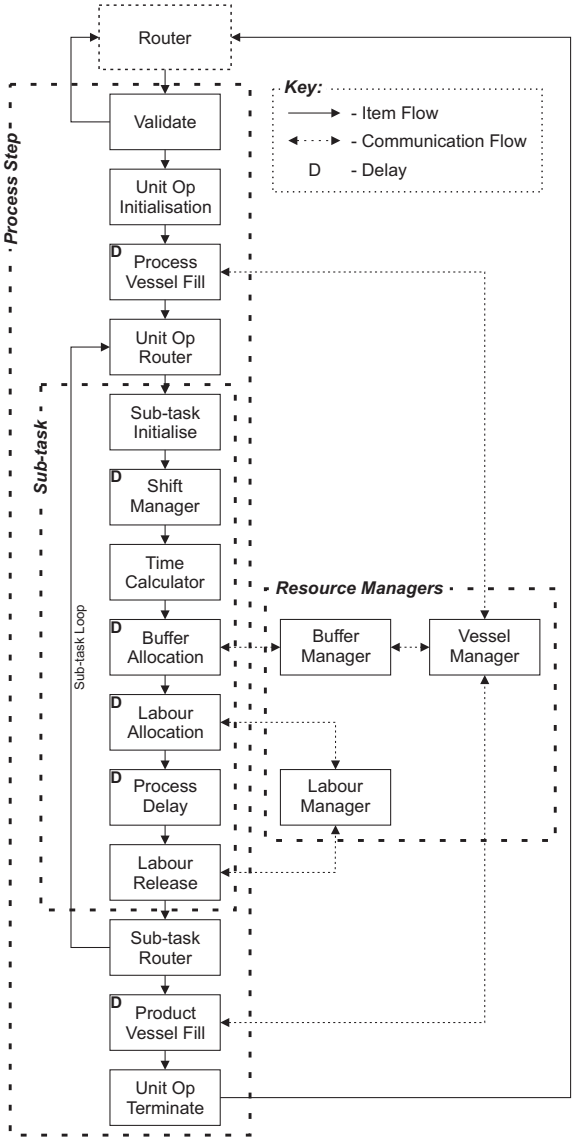



Figure 2.7: Structure of the equipment operation blocks showing the sequence of key functional elements used to simulate the unit operations and sub-tasks. Each block corresponds to a section of programming code in the simulation. The diagram shows the flow simulation items and therefore the order of execution as solid lines. Dashed lines indicate the communication necessary between various blocks of the simulation engine. For example, when the buffer allocation block is triggered by the item, procedures in the buffer manager must be triggered to determine the composition of the buffer and in turn the buffer must be allocated to a vessel. This must be completed before the item moves on to the process delay block which then determines the time taken to complete the subtask.

The number of iterations is determined by the sequence of sub-tasks and in the case of chromatography the number of processing cycles.

**Initialisation** Upon entering the equipment operation blocks the current status of the equipment is retrieved from the database to ensure that the item has not been sent to a piece of equipment that is already in use or which has not yet been cleaned. If the check fails the item is sent back to the router to be re-allocated otherwise the item can continue to initialisation of the unit operation.

Initialisation involves retrieving the parameters from the internal database that have been calculated by blocks earlier in the model. These items will be stored either on the items as attributes or stored in local variables in the blocks for faster access. A process vessel is also assigned at this point.

The item now enters into the sub-task loop. Control of this loop is handled by the unit operation router. This block generates a number of simulation items depending on the number of sub-tasks and cycles required. Items are generated to represent the product as well as the pre and post product operations. This means that the item representing the product is able to leave the unit operation and move on to the next whilst other items continue any operations that are carried out after the product has left, such as cleaning and dismantling the equipment. When multiple cycles of chromatography are required the situation is more complex as an item is generated for each cycle of product. This allows the cycles to continue processing independently if this is required by the process. The allocation of items is shown in Figure 2.8.

On completion of the sub-task the process items are sent back to the router to be either recycled through to the next sub-task or to trigger release of the product item. At the end of processing the product item waits in the router to be released from the unit operation and to be filled into the appropriate vessel. This release may also trigger the next step in the process if desired. The release is triggered by specifically named sub-tasks. In the case shown in Figure 2.8 the product item, which also represents the load sub-task, will be released when the elute sub-task is complete and that item passes through the sub-task router.

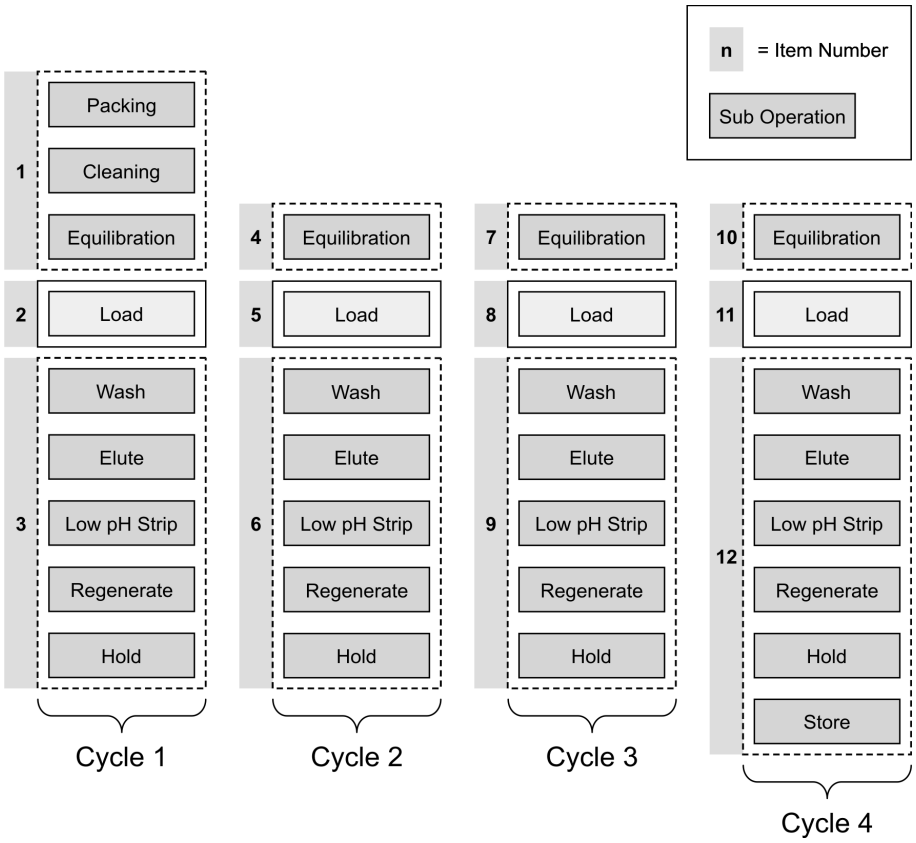



Figure 2.8: The number and allocation of simulation items to sub-tasks for a four cycle bind and elute chromatography step.

**Sub-tasks** The lowest level in the hierarchy of the process specification is the sub-task level. Each unit operation is composed of a number of sub-tasks which take into account the various operations which must be carried out in order to run the step. In order to be classed as a sub-task the operation must require a process material, labour or processing time. Process materials include WFI, buffers or the product. Examples of sub-tasks can be seen in the data entry screenshot in Figure 2.4.

Processing time is calculated for tasks requiring process materials and is based on volumetric flow rates which are defined in the process specification. If no buffers are used, the task time must be recorded in the process specification.

In addition to being defined individually, the sub-tasks are also grouped. Grouping is used to ensure that sub-tasks are carried out without delay. For example all tasks between the loading and elution of a chromatography column will be contained in a single group as it would be undesirable to hold the process at any point whilst product is bound.

Rules relating to the management of groups are maintained by the shift manager element using data on the start and end time of shifts defined in the facility specification. The functions here can result in three outcomes:

1. There is sufficient time left in the shift and processing of the group can continue.

2. There is insufficient time in the shift and the process is held until the beginning of the next shift.

3. There is insufficient time in the shift however the process can continue in overtime.

The third condition will only be met if an overtime allowance has been specified in the facility specification. This condition will result in an increase in labour costs. The shift manager uses data generated by the pre-calculation block as a more accurate task time is not known until the sub-task time is calculated in the next block.

If the process is allowed to continue a more accurate sub-task time is calculated and the resources are allocated.

**Resource Allocation** The resources to be allocated at the sub-task level are process materials and labour. All process materials requests are handled by a resource manager. The resource manager receives resource requests from the sub-task elements and attempts to fulfil them without incurring process delays. In order to fulfil requests the resource manager triggers buffer preparation operations, which include requesting storage vessels from the vessel manager.

Buffer preparation tasks are independent of the main process sequence; however they directly impact shared resources. Data regarding the buffer storage and buffer preparation tanks is stored in the facility specification. These values will affect the rate at which the buffers can be prepared as well as the number and volume which may be stored at any one time. The buffer manager will trigger buffer generation if supplies are low. The buffer preparation operations can be defined such that they do not constrain the process. Here all demands for buffer are met without delay. In this case the requests and profiles of buffer usage become a key output from the simulation.

Labour allocation is controlled by the labour manager. As well as requests to allocate labour to a task, the manager also receives notices that labour has been freed up at the end of an operation. If labour is not immediately available a delay may be incurred. In the sub-task, labour is allocated before the process delay and then released after. The process delay is the point at which the time taken to perform the operation is simulated. This is the time which is calculated by the process time block earlier in the sub-task.

## 2.7 Data Visualisation and Analysis

Basic data analysis tasks can be carried out using SQL queries. SQL queries are particularly useful for pulling together related information from different tables in the database and carrying out a calculation. An example of an SQL query is shown below.

### 2.7.1 SQL Query Example

The SQL queries and results are presented as viewed from the MySQL command line tool. This example uses two simplified tables with sample data. An SQL SELECT query is used to view the contents of the table. This query along with the output table is shown in Figure 2.9.

```
mysql> SELECT * FROM subTasks;
+-----------+-------------+----------+-----------+
| subTaskID | SubTaskDesc | bufferID | bufferVol |
+-----------+-------------+----------+-----------+
|         1 | Equilibrate |        1 |        70 |
|         2 | Load        |     NULL |      NULL |
|         3 | Wash        |        2 |       100 |
|         4 | Elute       |        3 |        60 |
|         5 | Equilibrate |        1 |        70 |
+-----------+-------------+----------+-----------+
```

Figure 2.9: SQL query and output table showing a list of subtasks.

A second query retrieves a second table outlining the buffers in use and is shown in Figure 2.10.

The data in the two tables is of more use when the information is combined to calculate the buffer costs. This is done be using a SELECT query which joins the data and can calculate a new costs field as shown in Figure 2.11

```
mysql> select * from buffers;
+----------+----------------------+----------+
| bufferID | bufferName           | costPerL |
+----------+----------------------+----------+
|        1 | Equilibration Buffer |      0.5 |
|        2 | Wash Buffer          |      0.7 |
|        3 | Elution Buffer       |      0.9 |
+----------+----------------------+----------+
```

Figure 2.10: An SQL query to retrieve a list of buffers.

```
mysql> SELECT subTaskID, subTaskDesc, bufferName, bufferVol, costPerL,
    -> (bufferVol * costPerL) AS bufferCost
    -> FROM subTasks LEFT JOIN buffers USING (bufferID);
+-----------+-------------+----------------------+-----------+----------+------------+
| subTaskID | subTaskDesc | bufferName           | bufferVol | costPerL | bufferCost |
+-----------+-------------+----------------------+-----------+----------+------------+
|         1 | Equilibrate | Equilibration Buffer |        70 |      0.5 |         35 |
|         2 | Load        | NULL                 |      NULL |     NULL |       NULL |
|         3 | Wash        | Wash Buffer          |       100 |      0.7 |         70 |
|         4 | Elute       | Elution Buffer       |        60 |      0.9 |         54 |
|         5 | Equilibrate | Equilibration Buffer |        70 |      0.5 |         35 |
+-----------+-------------+----------------------+-----------+----------+------------+
```

Figure 2.11: An SQL query to combine the data from two table to calculate the buffer costs for set of subtasks.

The above query also carries out a simple calculation to determine the buffer cost per step. A simple query can also be used to determine the buffer cost for all the subtasks. This query is called an aggregation query and is shown in Figure 2.12.

```
mysql> SELECT SUM(buffervol*costperL) as bufferCost
    -> FROM subtasks
    -> LEFT JOIN buffers USING (bufferID);
+------------+
| bufferCost |
+------------+
|        194 |
+------------+
```

Figure 2.12: An SQL calculation query to retrieve the total buffer cost for a batch.

This example is given using simplified sample data however the methods outlined can be used to carry out the same simple calculations on even the most complex data sets. The queries can be stored as functions such that a single word command can be used to retrieve the required information.

### 2.7.2 Third Party Applications

Any application with ODBC functionality can use the MySQL database as a data source. This includes Microsoft Access (Microsoft Corp.) which provides a user-friendly interface for generating complex queries and the ability to build intuitive user interfaces using familiar windows forms. Data retrieved through Access can be copied into other applications, such as Microsoft Excel where more complex analysis can be carried out. This wide ranging third party application compatibility means the framework will in many cases be able to integrate with legacy systems and work practices.

For convenient graphical analysis of the data, the SQL server can be linked to SigmaPlot (Systat Software Inc. San Jose, USA). SQL statements can be stored in the form of SigmaPlot Query Files which can be recalled to provide data for Sigma Plot graphs. Since this method relies on stored queries, the same graphs can be reproduced for different datasets without the need to rewrite the query. The enables the development of a standardised reporting practices to provide consistent visuals and statistics to decision makers.

## 2.8 Using the Tool

This section includes a selection of scenarios which are used to highlight the functionality of the simulation framework. The overall structure of the framework along with the key input and output parameters is shown in Figure 2.13.

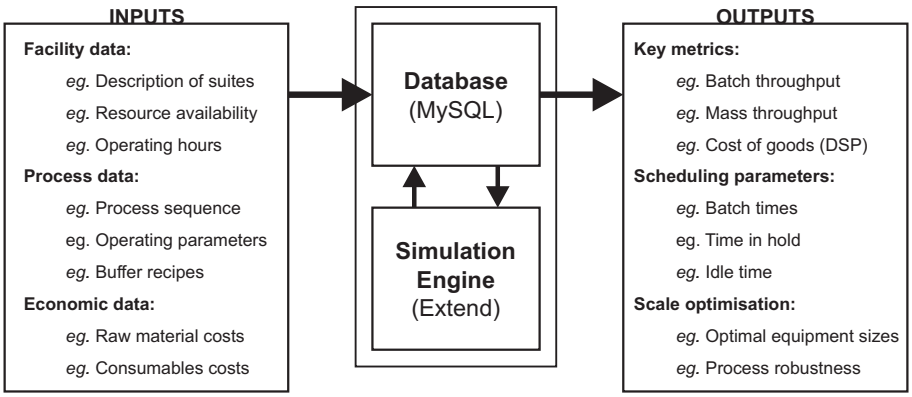



Figure 2.13: Structure of the framework.

### 2.8.1 Beginning an Investigation

The user configures the framework for their simulations by first defining a process and facility definition in the database. In the prototype tool a user interface developed in Microsoft Access helps to maintain the integrity of the data and provides the user with a logical view of the whole process, a screenshot is shown in Figure 2.4. The database validates all data entry and provides the user with feedback if invalid parameters have been entered or if required parameters are missing. An overview of key parameters required in the process specification is shown in Table 2.3.

Table 2.3: A list of key input and output variables to and from the framework.

| **Inputs** | **Outputs** |
|---|---|
| **Batch Parameters** | |
| Cell culture volume (L) | Final output volume (L) |
| Cell culture product titre (g/L) | Final product mass (g) |
| **Unit operations general parameters** | |
| Sequence of tasks | Buffer volume used (L) |
| Buffer type | Calculated duration (hr) |
| Buffer volume per step (CV or L/m$^2$) | |
| Task duration (optional) (2) | |
| Labour requirement (FTE) | |
| Step yields (%) | |
| **Chromatography** | |
| Bed height (m) | Column diameter (m) |
| Linear flow rate (m/hr) | Product load per cycle (g) |
| Packing flow rate (m/hr) | Resin utilisation (%) |
| Dynamic binding capacity (g/L) | |
| Product elution volume (CV) | |
| **Filtration** | |
| Diafiltration concentration (g/L) | Filter Area (m$^2$) |
| Over concentration (g/L) | |

*Continued on next page...*

| **Inputs** *(continued)* | **Ouputs** *(continued)* |
|---|---|
| Cross flow rate (L/m$^2$/hr) | |
| Performance data (3) | |
| **Virus Inactivation** | |
| Acid titration (% of feed vol) | Acid volume (L) |
| Base titration (% of feed vol) | Base volume (L) |
| Inactivation hold time (hr) | |
| **Virus Filtration** | |
| Vmax (L/m$^2$) | Filter area (m$^2$) |
| Average flux (L/m$^2$/hr) | Flush buffer used (L) |
| Max processing time (hr) | |
| Flush buffer requirement (L/m$^2$) | |

Once data entry is complete, a single base case simulation should be defined. On either the same computer or a separate networked client the simulation engine should be activated. The simulation engine will carry out further checks on the data and provide further feedback to the user if errors are present. Correcting the data is iterative and the base case simulation should be re-run until a satisfactory set of results has been obtained. At this point the facility and process specification can be saved as data files offline from the database and can serve as a starting point for all future investigations. Multiple processes can be defined in the same way and schedules of manufacturing campaigns and batches can be defined.

Once a satisfactory base case has been defined, more complex simulation runs can be attempted by defining modifier queries and sensitised parameters for Monte Carlo analyses. To analyse results, the user can execute predefined database queries which calculate standard parameters such as batch costs, processing times, resource utilisation and facility throughput. The simulation engine also maintains a log of key events which occur during the simulation as well as parameters that are associated with these events. Analysis of this log is useful as a starting point for root cause analysis especially when carrying out Monte Carlo simulations. Typical log entries will include vessel overages, resource conflicts and any other events which may result in process delays or material

loss. Combining the values of the sensitised variables to the presence of certain log entries can help identify the probability that these events will occur and when.

## 2.9 Conclusions

A novel framework has been developed which allows users to bring together multiple process specifications with multi-suite facility configurations to provide insight into the operation of modern multi-process biopharmaceutical facilities. A purpose built relational database has been developed and linked directly to a flexible simulation engine through the use of industry standard data transfer middleware. Both simulation inputs and outputs are stored in the same data structure and made available for data analysis. Data analysis and visualisation is achieved using similar middleware to establish a link between a wide array of third party applications, including Sigma Plot, Excel and MS Access, and the archive of simulation results. This wide ranging compatibility also extends to legacy systems where data could be harvested directly from additional sources such as data historians.

Maintaining data independence allows the database to become a central repository of process and facility information and can facilitate efficient collaboration between members of a process development or technology transfer team either locally or across the globe via an SQL compatible web interface.

The structure of the database allows for processes to be specified using scale independent parameters. For each simulation the process specifications are paired to a facility specification in addition to initial starting parameters to define the volume and product titre in the cell culture. The remaining unit operations are sized automatically by the simulation engine using the equipment available in the selected facility. Where multiple process configuration options are available, the simulation framework automatically selects optimum equipment sizes to minimise cost and scheduling bottlenecks where possible.

The simulation engine has been designed to allow for both stochastic and deterministic analysis though the use of Monte Carlo simulation. Unlike many systems, the resolution of the data is maintained during Monte Carlo simulations giving the user access to a complete dataset from every simulation iteration. This provides the user

with the ability to drill down into the data set to identify root causes to potentially undesirable outcomes. Some of the processing overheads of running the large number of iterations can be alleviated by operating multiple simulation engines in parallel as part of a distributed cluster.

The following chapters show example case studies which serve to demonstrate the range of simulation options available and show how by the development of complex SQL queries, a wide array of data analysis operations can be performed.

# Chapter 3

# Assessing Process Robustness and Long-Term Facility Fit Using The Database-Driven Dynamic Simulation Framework

## 3.1 Aim

This chapter demonstrates the use of the database-driven discrete-event simulation tool described in Chapter 2 for assessing process robustness and future facility fit. The case studies focus on the ability of purification suites in an existing (CMO) facility to cope with increased loads as a result of upstream advances in cell culture titres. A platform monoclonal antibody (mAb) purification process is defined for the analysis, as well as the features of a multi-suite clinical scale manufacturing facility.

The first case study focuses on characterising the throughput, economics and robustness of optimal process configurations at typical current titres. The second case study extends this analysis to assess the long term fit of the platform process given a range of equipment sizes available to enable scale intensification efforts. This is investigated over a theoretical 10 year period that sees titres increase from a modest 1g/L to a challenging 10g/L. These case studies harness the benefits of the database-driven approach developed using MySQL (MySQL AG. Uppsala, Sweden) to capture and manipulate the large datasets generated from brute for optimisation over a range of titres.

Novel approaches to visualising process robustness and long-term facility fit are presented.

## 3.2 Introduction

As the biopharmaceutical sector has matured, increased scrutiny has been placed on improving production costs and capacity utilisation. (Farid et al., 2005b; Jagschies, 2008; Kamarck, 2006) Consequently continuous improvements in platform technologies are being sought so as to keep manufacturing off the critical path whilst minimising regulatory burdens. (Kelley, 2007; Davies et al., 2009; Farid, 2009; Sommerfeld and Strube, 2005) This has become even more critical for cell-culture derived products such as mAbs which have seen significant increases in mammalian cell culture titres. This has not been matched by similar improvements in purification capacity.(Aldington and Bonnerjea, 2007; Arunakumari et al., 2009)

As a result conventional chromatography-based purification sequences employed in mammalian cell culture processes pose key capacity challenges as cell culture titres continue to increase. This has shifted the focus of biopharmaceutical process development efforts to re-evaluate the feasibility of conventional purification steps. (Kelley, 2007; Low et al., 2007) Downstream capacity bottlenecks potentially arise with increased titres since they result in greater mass loads on chromatography steps and can, for example, prompt a decision between opting for additional cycles or investment in larger columns which may breach either time or budgetary constraints respectively. Process scale intensification efforts require the ability to rapidly identify facility limits as well as the sequence of optimal equipment sizes that minimise process cost whilst satisfying time constraints. A further challenge is finding process configurations that are both optimal and robust in the face of inherent uncertainties such as titre fluctuations. Capacity planning endeavours are further complicated by facility resource constraints not only in equipment sizes and material availabilities for the unit operations but also for hold steps and ancillary operations including buffer preparation. Restrictions on the number and type of available suites pose a further scheduling challenge. The complexity is further exacerbated when dealing with multi-product facilities and scale-up of processes from a pilot scale facility to commercial manufacture. This chapter

presents the application of the decision-support tool described in Chapter 2 to address these facility fit challenges and hence facilitate in the design of robust and cost-effective manufacturing strategies. More specifically to address the question: how will existing facilities perform with the increasing titres?

Previous work on chromatography sizing optimisation has tended to focus on a single chromatography step, For example Joseph et al. (2006) developed a technique to assess the impact of titre changes on unit operations. Joseph et al. use a mathematical optimisation routine which aims to find the optimum size and linear flow rate of a protein A chromatography column for the purification of a concentrated mAb stream. In contrast, this work focuses on chromatography sizing optimisation for a sequence of three chromatography steps, (Protein A, anion-exchange, cation-exchange) so as to determine the optimal configuration of each step across a range of titres that minimises the overall process rather than a single step cost, whilst satisfying time constraints. With multiple chromatography steps in a process, each with different resin costs and binding capacities, all the process steps must be considered together as one complex optimisation challenge in order to arrive at an optimum configuration across the whole process. The result should in effect be the over-sizing of cheaper resin steps to free up process time to reduce the size of more expensive operations, thus capturing the key trade-offs across the whole process. This chapter demonstrates the key elements of the simulation framework which were specifically designed to overcome some of the functional limitations seen in previous models.

Applications such as Excel (Microsoft Inc. WA, USA.) and Superpro Designer (Intelligen Inc. NJ USA) are commonly used to carry out investigations into facility throughput, utility sizing and capital cost estimations. As static models, these tools are not designed to react to dynamic factors such as resource and utility constraints or handle the large datasets generated by brute force optimisation to determine optimal Pareto frontiers. These two latter features are critical to the case studies in this chapter. Hence

The tool described in chapter 2 builds on previous discrete-event simulation tools created at UCL (Farid et al., 2005b; Lim et al., 2005, 2006). It has been developed to link simulation and optimisation in a dynamic environment, whilst overcoming the

limitations of spreadsheet applications when working with large amounts of data by linking the simulation engine to a relational database. In order to provide a more complete analysis, tools must be able to account for parameters which are time-sensitive. This is especially crucial when considering the case that two key parameters of interest are process time and cost. With the simplified assumption that any amount of mAb can be captured from any scale of process given sufficient time, capturing and investigating the trade-offs requires an accurate calculation of both elements.

## 3.3 Method

A base case process description was developed for the use in the case studies in this chapter. The list of unit operations and information regarding the construction are described in section 3.3.1. Additional information on the process used can be found in Appendix A. The requirement to rescale the process at each titre point also prompted the development of the process optimisation element of the framework which is discussed in section 3.3.2. Further detail on the process optimisation element can be found in chapter 2.

### 3.3.1 Process and Facility Description

The common structural and chemical properties of all mAbs has enabled many manufacturers to develop platform processes for their production. This approach is aimed at reducing development times and cost. A number of variations of these platforms exist usually containing two or three chromatography operations which may or may not be separated by ultrafiltration and diafiltration steps and include at least two methods for virus inactivation and removal.

A typical mAb platform manufacturing process, variants of which can be seen throughout the bioprocessing industry, was developed for use in a series of case studies and is summarised in Figure 3.1 along with key parameters regarding the design of the facility.

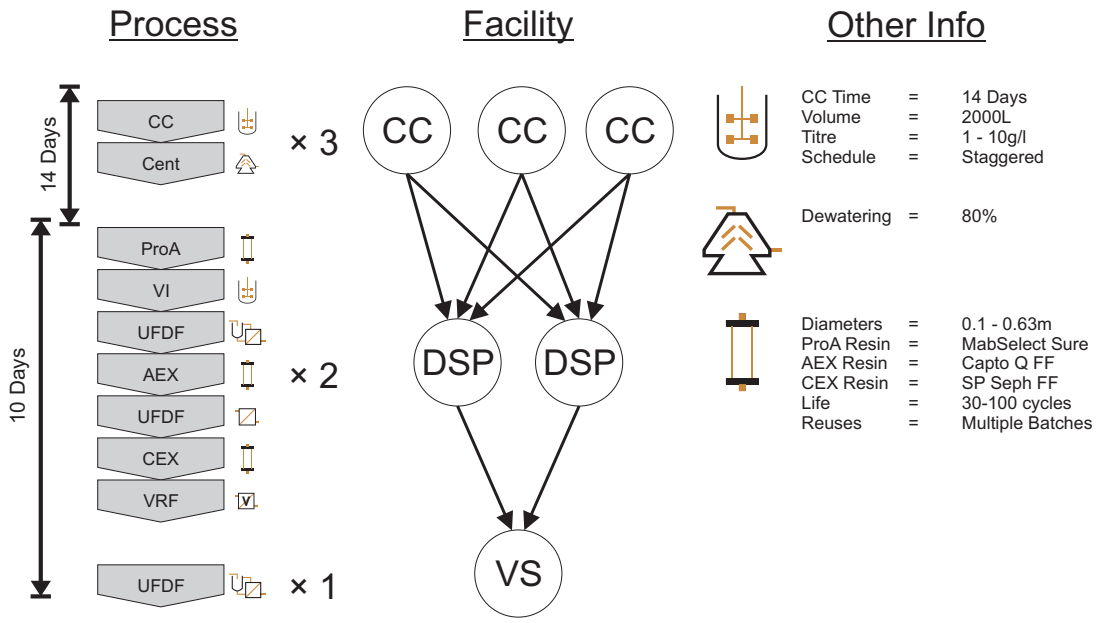



Figure 3.1: Base case process and facility. On the left the process sequence is shown along with the estimated processing times. Note the process is split into three sections with each section aligned to a class of suite. The centre of the diagram shows the number and type of processing suites within the facility. The circles represent the different suites with the arrows showing the route process material can take. CC = cell culture, Cent = centrifugation, ProA = Protein A, VI = virus inactivation, UFDF = Ultrafiltration/Diafiltration, AEX = anion exchange, CEX = cation exchange, VRF = virus retention filtration, DSP = down stream processing, VS = virus secure. On the right additional key information is shown.

For simplicity the manufacturing schedule consists of the single process utilising all suites in a year long campaign. All studies were based on a clinical scale manufacturing facility with 2 x 2000L fermenters operating in a staggered mode, with a new batch entering each of the DSP suites every 5 days. In this study a total of 10 campaigns were run with titres increasing by 1g/L each year from 1-10g/L. This was designed to be analogous to the expected trend of increasing titres over the next decade.

Three key metrics investigated in this analysis are:

- Annual batch throughput (Batches/annum)
- Mass product per annum (Kg/annum)
- Cost of Goods in DSP (RMU/g)

Throughput can be represented in terms of mass of product produced or the number of batches passing through the facility. Batch throughput for a contract manufacturer is of key importance, especially in facilities designed for clinical supply. An increased number of clients passing through the facility at this stage translates into a greater potential for ongoing collaboration at larger scale by increasing the pool of clients and thus mitigating the risk of non-continuation of contracts for products which fail in clinical trials. Mass throughput is of a lesser concern in this instance however it is of interest as it provides the context of scale when considering the third metric, cost of goods.

The case studies were developed based on a process deemed to be a standard platform for the production of mAbs. The case studies were designed to investigate DSP and as such USP operations were not modelled explicitly, Their effect was implied in terms of a frequency of batches being received into DSP and calculated using equation 3.1.

$$f_B = \left\lceil \frac{T_{CC}}{N_R} \right\rceil \tag{3.1}$$

Where: $f_B$ = frequency of batches entering DSP, $T_{CC}$ = cell culture batch time, $N_R$ = number of reactors. In both the case studies discussed, the cell culture batch time was set at 14 days and there were 3 reactors, which resulted in a batch frequency

entering DSP of 5 days. In turn this value as well as the number of DSP suites $N_{DSP}$ was used to calculate the DSP slot length $T_{DSP}$ as shown in equation 3.2. With 2 DSP suites available the slot length in this case was 10 days.

$$T_{DSP} = f_B N_{DSP} \tag{3.2}$$

The slot length was used as the scheduling constraint for the process optimisation module. The decision space for the process optimisation was limited by investigating alternative chromatography column diameters only. Filter areas were sized to process material in a specified time and as such could be calculated. The facility was defined to have a number of column diameters available ranging from 0.10m - 0.63m.

Using the above simple equations it is possible to account for the effects of cell culture operations as well as some aspect of the facility construction, in this case the mismatch between the number of cell culture reactors and DSP suites. Figure 3.1 outlines the USP/DSP mismatch, the sequence of unit operations, the DSP suites and how the process was separated into the available suites. The number and allocation of suites in the DSP operations do not have to be implied in the same as USP as the model was designed to account for these.

#### 3.3.1.1 Key Process Assumptions

In order to arrive at a suitable process certain assumptions were required. These assumptions are detailed in Table 3.1 below and their rationale is discussed in more detail. Further assumptions can be found in appendix A.

Table 3.1: Key Process Assumptions

| **Parameter** | **Step** | **Assumption** |
|---|---|---|
| Resin Reuse | Protein A | 100 cycles |
| | AEX | 50 cycles |
| | CEX | 100 cycles |
| Resin DBC | Protein A | 25 g/L |
| | AEX | 50 g/L |
| | CEX | 15 g/L |
| Resin Bed Height | Protein A | 20 cm |
| | AEX | 25 cm |
| | CEX | 25 cm |
| Max Filtration Time | VRF | 6 h |

The ability to reuse resins is an important factor when calculating process costs. In many cases the high cost of affinity resins can be mitigated by the ability to reuse resins for multiple batches. In the case studies investigated in this chapter, the resin reuse limits indicated in Table 3.1 were assumed. This is valid as the case study also assumed the manufacture of a single product in a year long campaign.

#### 3.3.1.2 Key Process Costs

In order to calculate the cost of goods, costs for key process items had to be assumed. Approximate values were provided by industrial collaborators and through discussion with vendors and are shown in Table 3.2.

Table 3.2: Key Process Costs

| Item | Cost |
|---|---|
| **Chromatography Resins** | |
| Protein A | £8000/L |
| Anion Exchange | £800/L |
| Cation Exchange | £400/L |
| **Filters** | |
| 30kDa UFDF Membrane | £800/$m^2$ |
| Virus Reduction Filter | £1700/$m^2$ |
| **Disposable Bags** | |
| 500L Bag | £450 |
| 200L Bag | £400 |
| 100L Bag | £350 |
| 50L Bag | £50 |
| **Other** | |
| Labour | £30/h |

Of particular note in Table 3.2 is the inclusion of disposable bag costs. The base case process was not designed to maximise the potential for disposable technologies, however it was assumed that bags would be used for the storage of buffers and intermediate product. It can be seen later in this chapter that the cost of disposable bags can represent a significant proportion of the cost of goods for a process.

### 3.3.2 Process Optimisation

The process optimisation element of the framework was specifically developed for the types of analyses covered in this chapter. In a real facility unit operations are scaled appropriately relative to the mass of product and volume of the process. Since the process performance was investigated over a range of titres a method was needed to scale to unit operations accordingly.

A module of the simulation engine was developed to automate certain elements of process scaling. When used to investigate long term scenarios, for example the effects of increasing cell culture titres over 10 years, it is reasonable to assume that the DSP process will be scaled up to meet the demands placed. This is achieved by specifying the process using, where possible, scale-independent parameters, for example defining chromatography buffer volumes in terms of column volumes and filter buffer requirements relative to membrane areas.

Based on equipment availability and the rules of scale-up the simulation engine was able to generate a decision space which included every potential process configuration which could be achieved using the available equipment. In a process with 3 column steps and 9 different column sizes this resulted in 729 process options. Given the run time of the simulation engine it was not practical to simulate all 729 options therefore a pre-calculation module was developed to perform rapid calculations on all the configurations to provide estimations of cost and processing time.

The structure of the pre-calculation module was based on that of the simulation engine however compressed into a single block of programming code rather than the approximately 5000 blocks which make up the simulation engine proper. In order to achieve the processing time savings the functions of the code were concerned only with the calculation of processing time and cost. The structure of the pre-calculation module is shown in Figure 3.2.

The code of the router procedure shown in Figure 3.2 generates the list of process configurations based on the process sequence and availability of equipment as specified in the simulation engine's database. The procedure then called the correct unit operation procedure where basic mass balance calculations were performed. The unit

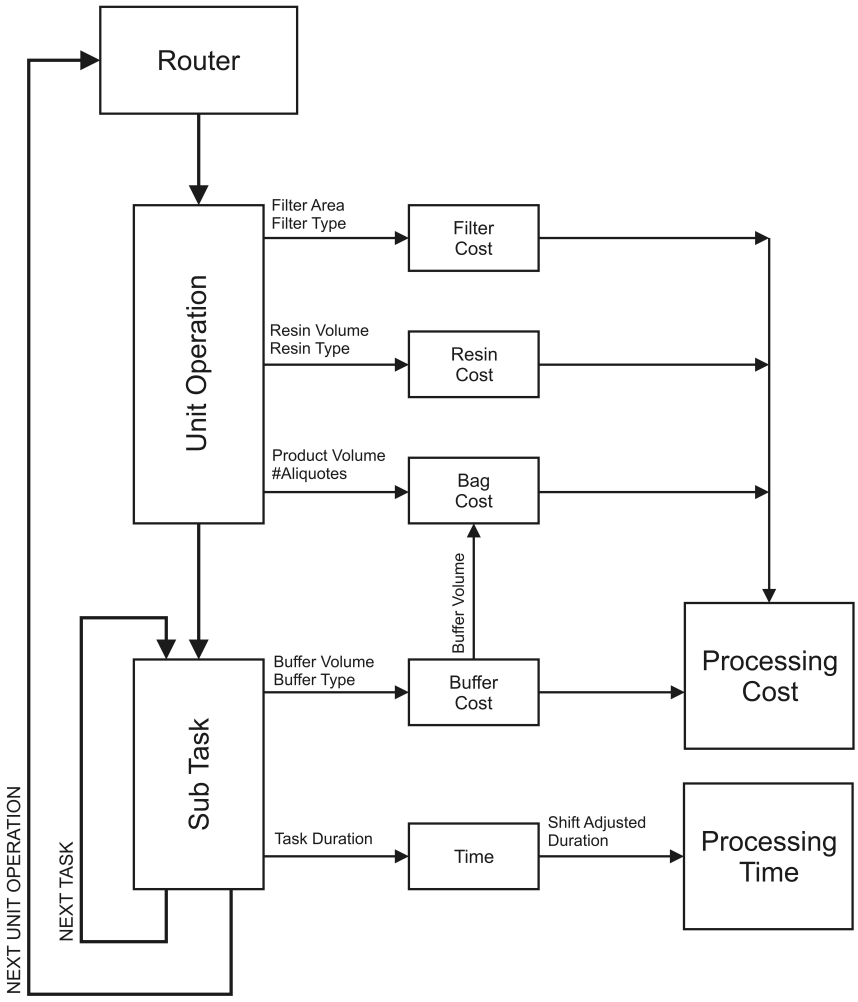



Figure 3.2: Structure of the pre-calculation block. The overall structure is similar to the simulation engine however functions are concerned only with the calculation of processing time and cost. Each box represents a programming procedure or group of related procedures which carry out the calculations required.

operation procedure called functions which calculate filter, resin and bag costs for the step. In turn these costs were added to the global process cost. The bag costs at this point were for the intermediate products. Buffer and process timing information can only be calculated with information from the subtask level of the database therefore a subtask procedure was called which iterates through the subtasks of the unit operation and calculates the buffer requirements and task times. The buffer requirements were passed to a buffer cost procedure which in addition to calculating the buffer cost also

passed information to the bag cost procedure to calculate the cost of the bags required for buffer storage. Task duration information was passed from the sub task procedure to a set of procedures which increment a global clock. The time procedures also take into account shift durations and account for tasks which must be carried out in groups. This for example avoids the potential for a shift change to occur between the time the product is loaded and the time it elutes from a column.

For each process configuration the module calculates the estimated cost and processing time. A set rule is then used to select the configuration with the lowest cost capable of meeting the manufacturing slot length. This rule allows the simulation to select a single process configuration out of the range of options available.

Taking the brute force approach to this optimisation challenge produces satisfactory results as generally the availability of equipment results in a limited number of options, the decision space can be reduced by actively scaling operations to meet a schedule. For example certain operations may be sized to ensure that they are completed within a time limit, i.e a shift. where this is the case the equipment selection for this operation and subsequently the decision space will be limited even further.

### 3.3.3 Model Specification for Case Studies

The simulation engine was configured to execute 10 simulation runs each with increasing titre values between 1 and 10g/L. All other process and facility configuration data were constant for all simulation runs, as a result, the decision space generated by the process optimisation module remained constant for all simulation runs. The output from each simulation run consisted of a table of costs and times for each of the 729 process configurations investigated. Each process configuration was given a unique identifier which was exported along with the cost and time data. In total 10 tables were generated and analysed. To reduce the size of the dataset only processes configurations which fall on the Pareto frontier of a cost versus time space were investigated.

Outputs from the simulation engine were exported to Excel where the Pareto frontiers were automatically extracted using a procedure written in Visual Basic. The procedure identified the Pareto frontier by comparing the point, x(A,B) to all other points, n(A,B) in the decision space and determining if there were any points which have lower

values of both A *and* B. If the test was true for any n(A,B) then x(A,B) cannot fall on the Pareto frontier. Each point in the decision space was tested using this rule allowing all points on the Pareto frontier to be identified. Sigma plot was used to visualise the Pareto frontiers for all simulation runs. The points on each Pareto frontier from the same process configuration were joined to form a series of vertical tie lines to easily identify those processes which appear on multiple frontiers. This is possible since the process and facility configuration and hence, decision space remained constant between each simulation run.

## 3.4 Results

The studies were designed to test the simulation engine and to provide insight into the operation of multi-suite mAb manufacturing facilities and when and how the trend of increasing cell culture titres may affect their design. The process and facility used for the case studies is outlined in Figure 3.1.

The first case study looked at investigating the performance of a current manufacturing facility running an existing mAb process. The aim was to identify the limitations of a typical manufacturing process with respect to increasing titre and where, within the process, these limits are likely to be reached.

The second case study expands on the process optimisation elements of the simulation engine and demonstrates how using the limited information provided by the optimisation module it is possible to arrive at conclusions regarding robust facility design in a future of increasing titres. Specifically, it focused on assessing the robustness of optimal configurations to batch-to-batch titre variability as well as identification of facility limits at high titres.

### 3.4.1 Case Study 1 - Throughput and Process Economic Analysis

The tool was initially used to characterise the throughput bottlenecks in an existing facility originally designed so that the DSP capacity matched harvest loads from cell cultures. However the facility had a range of available chromatography column diameters (0.1-0.63m) for each of the steps in the fixed DSP sequence outlined in Figure 3.1. Hence conventional scale-up could be replaced with scale optimisation to max-

imise throughput whilst minimising cost as titres increase. At each titre in the range 1-10g/L, the tool's brute force optimisation module was used to identify the optimal set of column and filter sizes that minimise the DSP batch materials costs whilst satisfying the 10 day slot length for DSP so as to maintain the batch schedule. These optimal configurations were then run in the discrete-event simulation engine to determine their performance characteristics given possible resource constraints and time delays.

Figure 3.2(a) shows the facility throughput in terms of mass and number of batches per annum across the titre range 1-10g/L at 2000L scale. This highlights the titre levels at which the throughput bottleneck would occur in this facility after scale intensification, as represented by the pointe where the batch throughput drops sharply and the mass throughput levels off. In this case the bottlenecks occur above titres of 6g/L which indicates that the DSP can handle harvest loads of up to 12kg/batch.

The theoretical maximum batch throughput is calculated using the following formula:

$$N_B = \frac{N_D}{f_B} \tag{3.3}$$

Where: $N_B$ = Number of batches per year, $N_D$ = number of continuous operating days per year, It was assumed that the number of operating days was 365 and cell culture time was 14 days with 3 reactors; this results in a maximum theoretical batch throughput of 73 batches per annum. The maximum batch throughput shown in Figure 3.2(a) was slightly lower than the theoretical value as processing on the final 1 or 2 batches was not always complete before the simulation end point. Data is only analysed for complete batches.

The data generated by the simulation was examined to trace factors contributing to the facility limits. The simulation aims to maintain a schedule and as such batch time is strictly controlled through the adjustment of equipment sizing. Since the frequency of batches entering DSP is fixed, for a bottleneck to be present the batch time must have increased above the slot length. Figure 3.2(b) shows that this is the case, where the batch time can be seen to increase dramatically at titres above 6g/L. At lower titres the batch time value reaches the maximum slot length value and is evidence that the optimisation routine is functioning correctly. Below 6g/L the number of cycles and batch time fluctuates around the slot length as only discrete column sizes are available

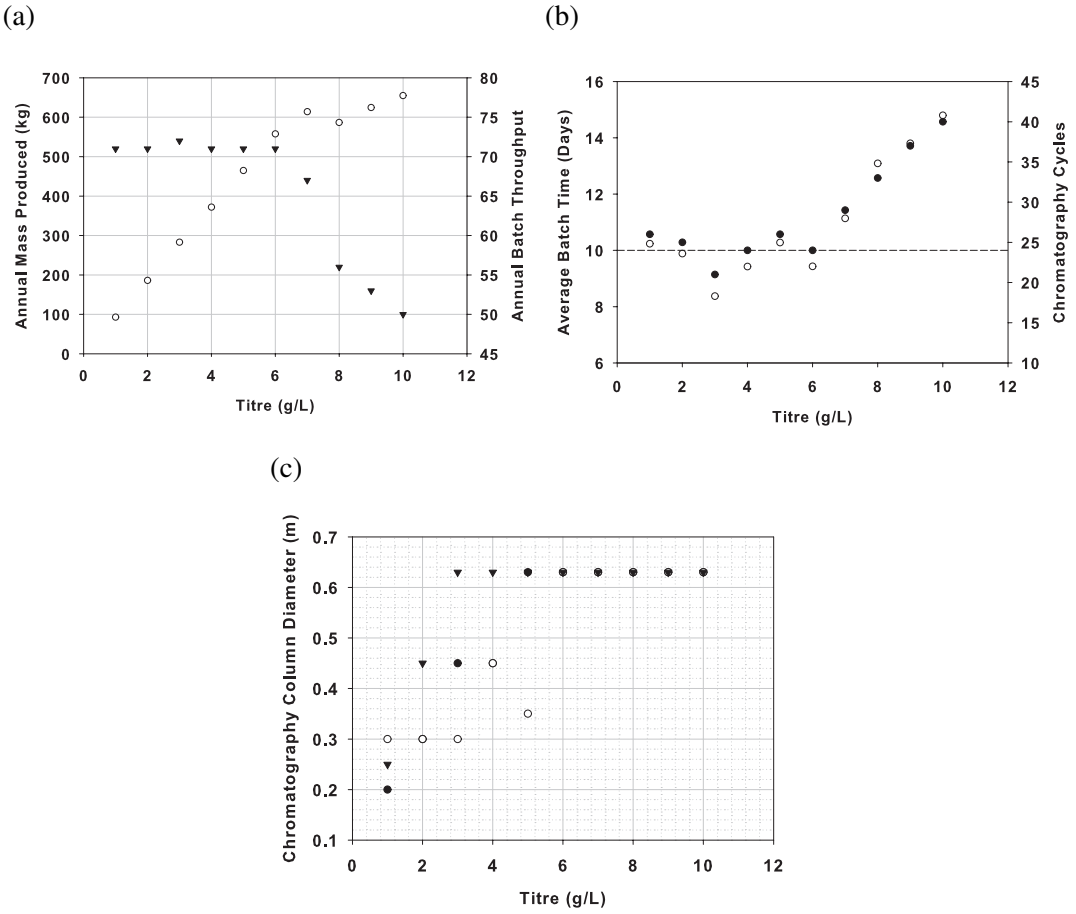



Figure 3.3: Impact of titre on throughput-related parameters: (a) Annual mass produced (○) and batch throughput (▼), (b) Average batch time (○) and number of chromatography cycles (●), (c) column diameters for protein A (●), anion exchange (○) and cation exchange (▼). In (b) The average batch time is the average duration of all completed batches within the year of operation. The chromatography cycles is the sum of the number of cycles for all steps. The horizontal line at 10 days shows the DSP slot length. In (c) the column diameters were selected using logic built into the brute force optimisation module of the simulation engine.

so the number of cycles required is not always directly proportional to titre.

Figure 3.2(b) shows that as titres increase the optimisation routine can no longer locate equipment sizes to maintain the slot length and correlates the batch time to the number of chromatography cycles. This relationship is evidence that chromatography operations are the root cause of the DSP bottleneck in this process, with insufficient

capacity to handle the larger product masses within the desired batch slot length.

The optimisation block has a contingency for when no processes meet the schedule constraint, at this point the fastest process will be selected. This configuration will have the largest columns and lowest number of cycles. At all titres above the bottleneck point the maximum column diameters will be chosen leaving increasing cycles as the only option to handle the increased load. To confirm this, Figure 3.2(c) shows the column diameters selected for each of the chromatography steps. Beyond 6g/L all columns in the process are at 0.63m in diameter since this is the maximum column size available to the process and is the result of a physical facility limitation.

In order to de-bottleneck this process, solutions must either increase the available processing time in the slot, improve other unit operations to make more time for chromatography or improve the chromatography steps to achieve the same separation in less time or with smaller columns.

In addition to throughput, cost is also a significant factor when analysing process performance. To investigate cost the model provides data for the $COG_{DSP}$ for future titres as shown in Figure 3.4. $COG_{DSP}$ includes overheads, labour, raw materials and consumables costs such as disposable bags, resins and filter membranes for all DSP operations. $COG_{DSP}$ is seen to reduce significantly with titre, and up to 6g/L the benefits of increasing titres is clear. Beyond 6g/L the results suggest a change in the relationship between cost and titre and the values remain largely constant, beyond 6g/L there is no cost advantage to increasing titres. Un-constraining the facility by introducing larger columns does not have a significant impact on this value as the base case assumes the reuse of resins over multiple batches, a reasonable assumption for a single product facility. The increase in overhead operating costs which may be a consequence of purchasing the larger columns is not taken into account here.

To formulate a plan for optimising the costing it is useful to look at the breakdown of costs to identify areas of the process where changes will have the largest impact. Figure 3.5 shows the percent breakdown of the main consumables costs for the optimal process configurations in the processes and how the values change with titre. Most notably all costs increase almost linearly with titre up to the bottleneck point of 6g/L, beyond this point the relationship changes and the values remain approximately con-

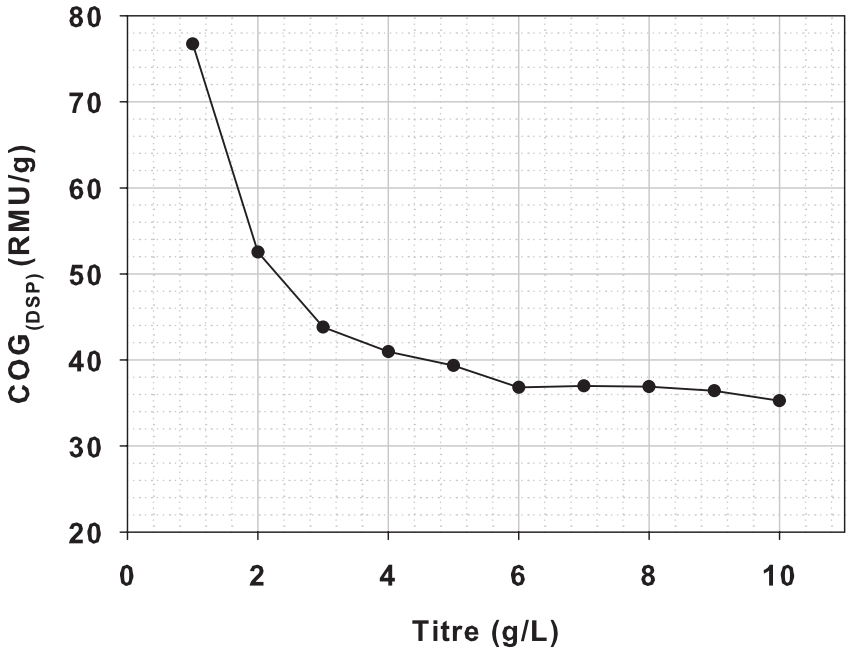



Figure 3.4: Cost of goods in down stream processing ($COG_{DSP}$) vs. titre. $COG_{DSP}$ includes facility overheads, labour, chromatography resin, filter membranes, disposable bags and buffer components including Water for Injection (WFI) (at 1.50 RMU/L) for all unit operations occurring post harvest.

stant.

The same costs this time as a percentage of total annual material costs are shown in Figure 3.6.

The reducing proportion of resin costs can be attributed to more cycles in chromatography achieving better utilisation of resins. The larger resins and increased number of cycles is the largest contribution to the increased proportion of buffer costs. Finally, the proportion of bag costs plateaus as the larger requirement for each buffer translates into increased use of the larger bags; the bag cost per litre of buffer in a full 100L bag is 3 times greater than a full 500L.

Because of a general lack of information as to the true cost of WFI, Figure 3.6 shows the same data at two WFI costs. At either rate, the data points towards the buffer costs being most significant at all but the lowest titres and highlight an area for further investigation. A useful metric calculated by the simulation engine is the amount of buffer required per gram of product. In this process and facility studied the value

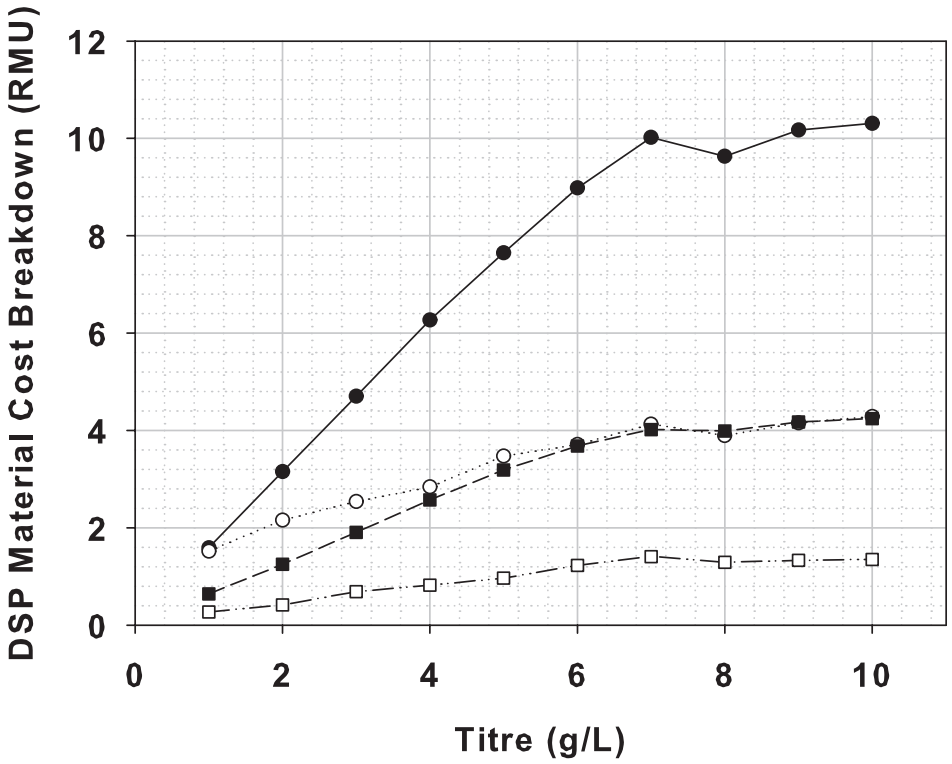



Figure 3.5: Actual annual material costs vs. titre for base case process. Costs have been separated into disposable bags (■), buffers (●), chromatography matrices (○) and filter membranes (□). Buffer costs include WFI at 1.50 RMU/L.

was calculated at 5.2±0.3 L/g and was constant across the whole range of titres. This presents another issue in that a 10 fold increase in titre and hence output, will result in a 10 fold increase in required buffer capacity.

As titres increase the process volumes increase, this results in increased diafiltration needs leading to an increase in membrane area needed to process the material at higher flow rates in an attempt maintain the schedule, it also accounts for some of the increases in buffer costs as there is an increased need for diafiltration buffers. The overall percentage cost of membranes however does not change with titre.

To start an optimisation study, it is useful to identify where in the process the buffers are consumed. When buffer use per step is expressed as a percentage of buffer consumption across the whole process the values remain constant across a range of titres. The process data was extracted from the database and is summarised in Figure 3.7. The steps with the largest consumption in this case are protein A and anion ex-

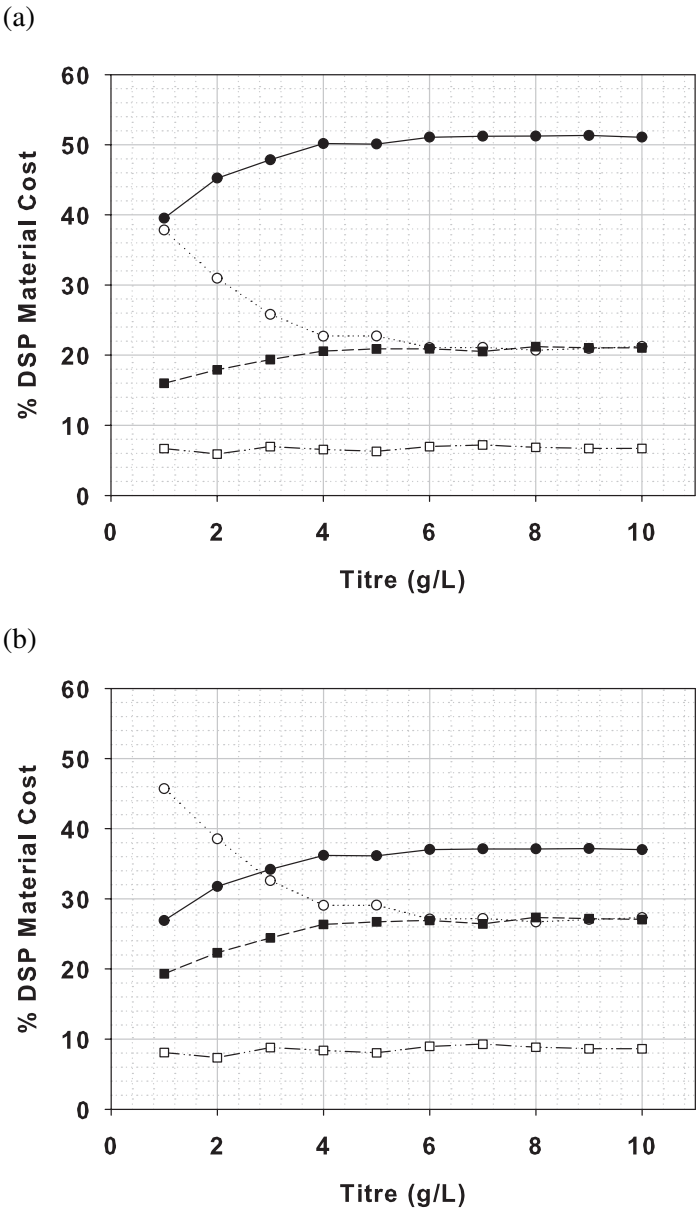



Figure 3.6: Impact of titre on percentage breakdown of material costs at 2 WFI rates for base case process: (a) WFI cost = 1.50 RMU/L, (b) WFI cost 0.10 RMU/L. Costs have been separated into disposable bags (■), buffers (●), chromatography matrices (○) and filter membranes (□).

change chromatography and can therefore be considered as targets for optimisation. In this example the two steps utilise 78% of all process buffers.

This result is not surprising given their lower resin dynamic binding capacities (Table 1) combined with the need for higher numbers of buffer CVs per cycle; for example, the adoption of 27 CVs/cycle in Protein A versus 1820 CVs/cycle for ion-exchange steps is largely due to the more complex wash steps that are often adopted in

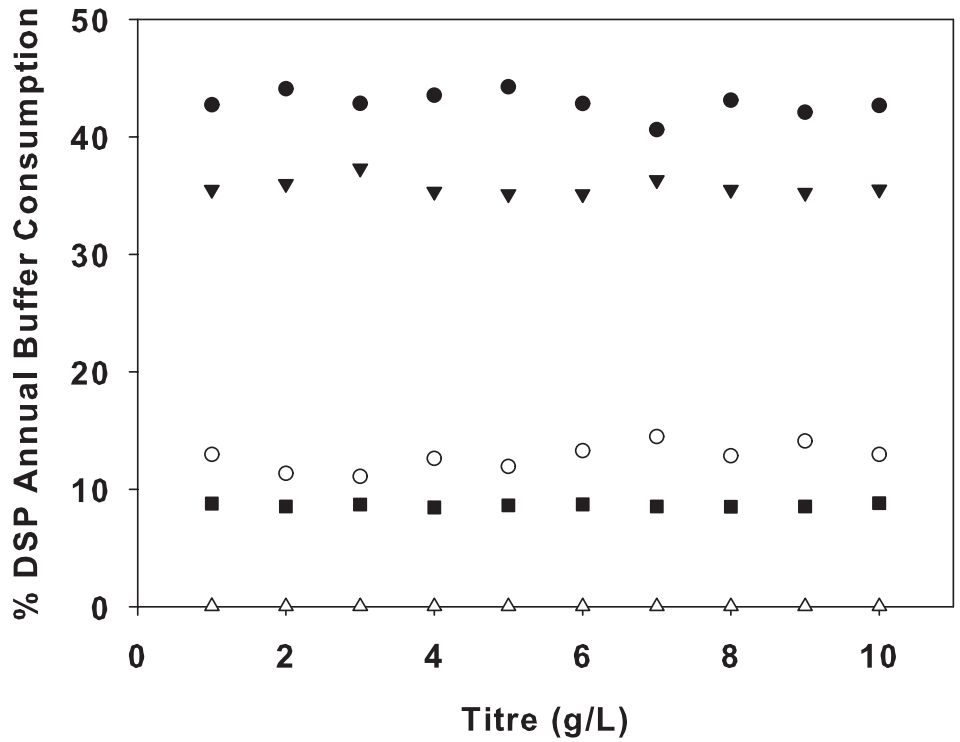



Figure 3.7: Buffer volume required per annum for selected unit operations. Percentages are shown for protein A chromatography (•), anion exchange chromatography (○), cation exchange chromatography (▼), virus reduction filtration (△) and ultrafiltration/diafiltration (■). The process contains three ultrafiltration diafiltration operations which are combined here into a single result set. Total annual buffer consumption includes the buffer used for all unit operations.

Protein A so as to maximise yields.

The long terms effects have the potential to be significant and may lead to a situation where the buffer demands cannot be met at large scale. Potential solutions exist and may include inline buffer dilution. Whilst reducing the size of buffer prep and storage tanks, inline dilution requires a reliable, constant supply of water for injection (WFI) and the potential exists for a shortfall in WFI generation rates to delay processes.

### 3.4.2 Case Study 2 - Process Robustness Under Uncertainty

The data generated by the process optimisation routines used to reconfigure the process at different titres can itself be analysed to capture the robustness of process configurations to titre variances. Using this approach the same information can be used to not

only optimise the process selection for a single titre value, but can also be used to select a configuration which is optimum over a range of values.

Figure 3.8 shows the decisions space for all the process configurations considered by the model for a set titre of 2g/L. Each point represents the key objectives/perfor-

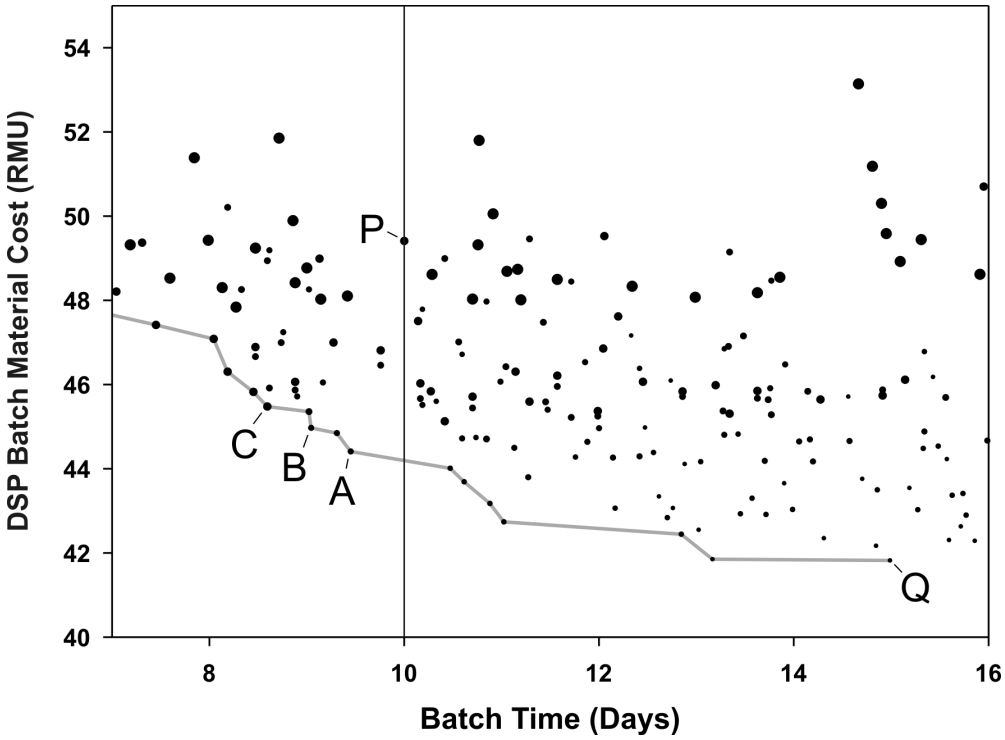



Figure 3.8: Batch cost vs. batch time for number of process configurations considered by the model framework. In this case, the framework is attempting to optimise the sizing of the columns for the 3 chromatography operations in the process. Also shown is the Pareto frontier on which the optimum configuration should lie and the ideal processing time is shown as a vertical line at 10 days. The size of the point symbol is proportional to the affinity column diameter.

mance metrics to optimise, DSP batch material cost and batch time for each process configuration. The Pareto frontier shows those configurations which are neither cost or time dominant and is a method to reduce the process choices to those which are feasible. A vertical line also shows the maximum batch time (10 days) and is an additional constraint. The optimisation routine aims to find the lowest cost process below the slot length, represented in this case by point A. Arguments can be made for additional logic which could arrive at different points. For example, point P may be desirable since the process cost is higher however the process fits better into the schedule, minimising

downtime. From a purely cost perspective point Q is more appealing. Figure 3.8 also provides some additional information on the sizing of the affinity step where the size of the point is proportional to the size of the column. The trend is for column sizes to decrease as processing time increases as indicated by the larger points size on the Pareto frontier a the low processing times relative to the high processing time. Interestingly the lowest cost process to operate contains the smallest affinity column step highlighting that protein A is a key cost driver in mAb processes, backing up the finding from the first case study.

Although valuable insights can be gained from analysing the options at a fixed titre, the data does not provide an indication of the robustness of the optimal configuration to expected batch-to-batch variability. The analysis was therefore extended to provide a novel method for visualising the robustness of process configurations to typical titre fluctuations. Similar decision spaces were generated at 1.5g/L and 2.5g/L to represent $\pm$0.5g/L titre fluctuations. To simplify the visualisation, only the Pareto optimal fronts were plotted (Figure 3.9). In figure 3.9 the points labelled A - C are the same process configurations as labelled in 3.8.

The location of each configuration on each frontier was identified and connected with a linear tie-line, since DSP batch materials cost and time were found to vary linearly with respect to titre. To be considered robust, the selected configuration should now span the 1.5-2.5g/L range without exceeding the maximum slot length. Further Pareto frontiers were generated with the titre fluctuation range with 100 titres selected randomly from a triangular titre distribution, Tr(1.5,2,2.5) so as to determine the likelihood of a configuration exceeding the batch slot length. The enhanced dataset in Figure 3.9 illustrates that although configuration A is optimal at 2g/L. as the titre increases towards the upper fluctuation boundary of 2.5g/L it's slot time exceeds the max slot length. Configuration A was found to be feasible for only 80% of batches given the expected titre fluctuations. Process r is more capable of handling the increase in titre; however it ceases to be on the Pareto frontier at low titres, this suggests that although this process would still be feasible at the lower titres there would be a more optimal solution in this region. Configuration B would be an optimal solution for 96% of batches at only an average increase in cost over configuration A of 1.3% and a processing time

Table 3.3: Chromatography column configuration for processes selected in Figure 3.9. Change in costs and time are quoted relative to process A. Titre = 2g/L, Harvest = 4kg/batch.

| | Column Diameter (m) | | | | | |
|---|---|---|---|---|---|---|
| Process | Affinity | CEX | AEX | % Feasible | Δ Cost (%) | Δ Time (%) |
| A | 0.30 | 0.30 | 0.45 | 79 | 0.00 | 0.00 |
| r | 0.30 | 0.35 | 0.45 | 69 | +0.98 | -1.51 |
| B | 0.30 | 0.30 | 0.63 | 96 | +1.26 | -4.31 |
| s | 0.35 | 0.30 | 0.45 | 71 | +2.13 | -4.53 |
| C | 0.45 | 0.30 | 0.45 | 100 | +2.40 | -9.07 |

reduced by 4.5%. In order to handle 100% of the processes within the expected titres configuration C would be the most robust option. Configuration A would incur a 2.4% increase in cost over A with a 9.1% reduction in processing time. Configurations r and s shown in figure 3.9 are examples of processes which beyond certain titres have drifted away from the Pareto front and are therefore not present on the Pareto front across the whole titre span. As only those process on the front are shown, the tie lines are curtailed.

Visualising the robustness of the configurations to titre fluctuations in this manor allows identification of alternatives to Configuration A that may ultimately be better selections. Decision-makers can then assess the level of desired robustness versus the probability of either delays or discarding product to meed the slot length.

The method used above can be taken a step further to provide information to drive future facility design. Figure 3.9(a) again shows the Pareto frontiers for a range of titres to represent the 10 year titre trend of 1-10g/L.

Given an existing facility and specifications for a process this figure allows the user to rapidly assess the limits in which the facility is able to operate and how sensitive the material costs and batch time are to changing titre for each of the configurations shown. In this case a 10% increase in titre will result in an increase in batch time of between 6.9-7.4% and an increase in cost of between 5.2-8.7% for a fixed process configuration and scale. These values cover all optimal configurations within the facility and can be

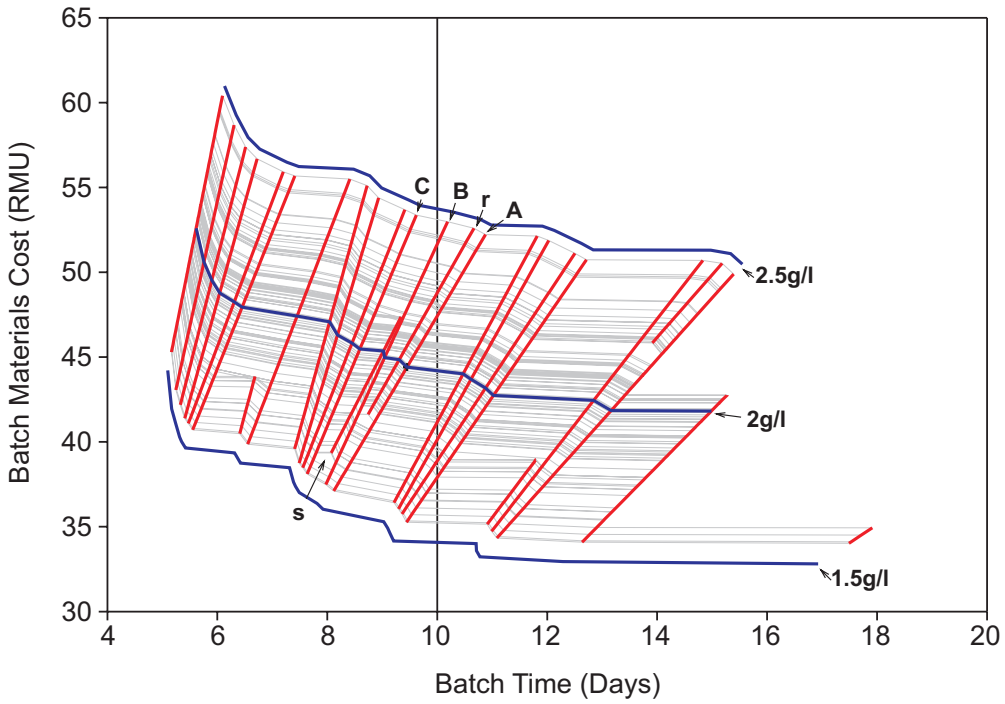



Figure 3.9: Horizontal curves are the Pareto frontiers of data generated after simulating 100 processes. Each process has a differing titre varying randomly within a triangular distribution. The triangular distribution had a minimum of 1.5g/L, a maximum of 2.5g/L and a mode of 2g/L. Identical process configurations on each Pareto frontier have been joined as shown by the red lines. The gradient of these line show how a set process configuration is sensitive to changes in titre with respect to batch cost and process time. Selected processes have been labelled and Pareto frontiers for 1.5, 2 and 2.5g/L have been added for clarity. A vertical line at a batch time of 10 days represents the ideal maximum processing time.

useful for assessing the impact of changes in USP on DSP.

The figure also allows the user to assess the impact of imposing scheduling constraints. In this case an operating time of 10 days is shown by the vertical line. Now the region of operation is reduced to the area to the left of the line. It is also possible to see that the imposed schedule constraint does not allow the facility to reach the maximum investigated titre of 10g/L. In this instance the maximum titre achievable is 6g/L. With the additional simulation data available it is possible to identify the configuration able to achieve the maximum titre and investigate further. As has already been established in the previous case study this facility is limited by the maximum available chromatog-

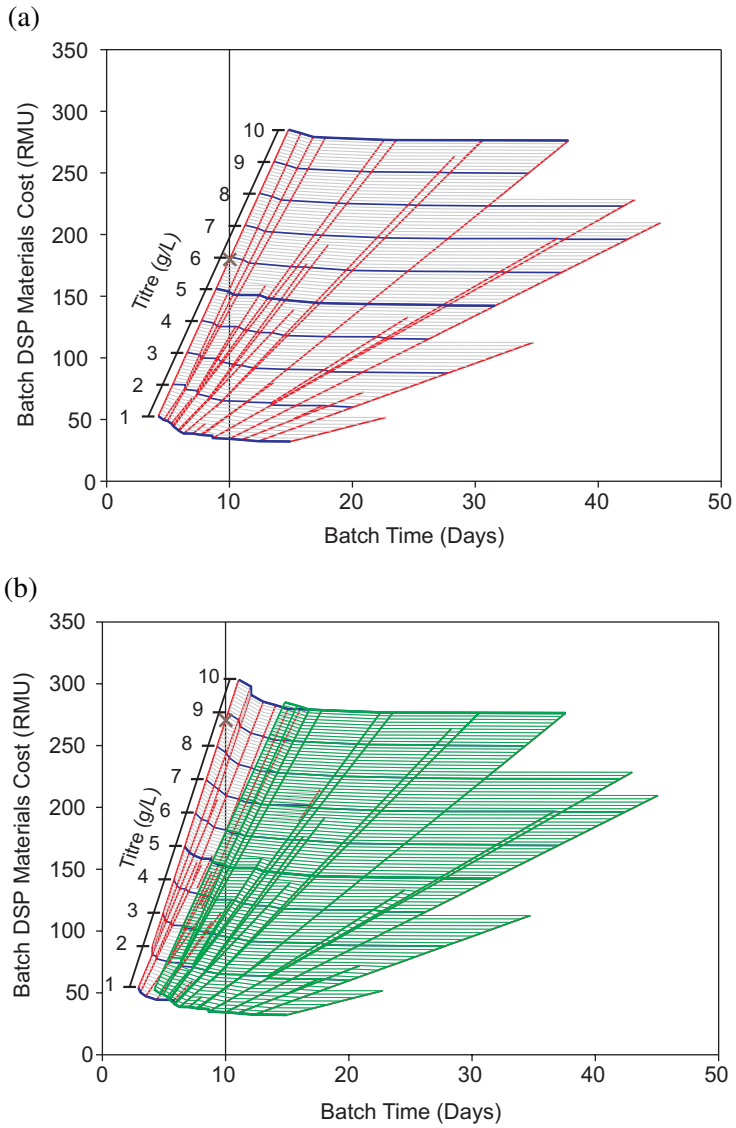



Figure 3.10: (a) Pareto frontiers for titres between 1-10g/L. As in Figure 3.9 straight lines show the changing cost and batch time of a process configuration. (a) Data from (a) is overlaid against data obtained from an investigation into the impact of having chromatography column with larger diameters available for processing.

raphy column diameter due to space constraints. A further scenario investigated the impact of making larger columns available. Figure 3.9(b) shows the impact of increasing the maximum column diameter from 0.63m to 0.75m. The impact of adding the new column is to increase the area of the operating region and raise the maximum titre to 8.8 g/L (17.6kg harvest/batch). It also shows that if the slot length could be increased by just a single day, a configuration is available to process a 10g/L batch. It is impor-

tant to note that increasing the column diameter will not have this effect if there is no packing or operating skid capable of handling the increased volumetric flow rates. In this case the simulation engine assesses the availability of additional equipment when considering process configurations.

Where the aim is to provide insight on facility design, instead of providing data on equipment availability within a facility, information on commercially available equipment can be used. With knowledge of the expected scale of operation, additional information on schedule constraints can be used to narrow the region further if need be. The result will be a list of optimal configurations, that can be cross-referenced in the simulation database to identify the equipment needed to meet to design a facility to operate within the specified limits.

## 3.5 Conclusions

This chapter has outlined and demonstrated an approach for developing a flexible modelling tool to aid in process development. The tool was used to investigate a standard mAb manufacturing process at 2000L scale and has provided insight into the operation of such processes in an environment of increasing titres. The simulation engine provided data to identify and investigate facility bottlenecks and assess the impact of the bottlenecks on key performance parameters including costs and throughput. Novel methods were developed to graphically represent the robustness of a large number of process configurations on a single two dimensional plot. These methods were further expanded to visualise the effect of expanding the capability of a facility and process to improve long term robustness in an environment of increasing titres.

The widely held belief that affinity chromatography in its current state will become the bottleneck of the process, has been supported. It is worthy of comment that the bottleneck in many facilities is going be to felt well before columns reach the current maximum of 2m. More likely, the facilities will reach a point at which they are unable to store the volumes of buffer required for processing material. Alternative buffer preparation methods, such as inline dilution, may compensate for shortfalls in the installed tankage however, this places increased pressure on WFI generation, with generator failures now far closer to the critical process path.

Alternatively, purification processes that actively seek to reduce buffer demands may be necessary such as the adoption of next generation resins with higher resin binding capacities or alternatives to packed-bed chromatography such as single-use membrane chromatography. These process changes may be particularly relevant to facilities that have been designed with higher ratios of USP to DSP trains, and hence shorter DSP slot lengths, than presented in this study where scale intensification alone may not be sufficient to cope with increasing titres.

# Chapter 4

# Integration of Stochastic Simulation with Multivariate Analysis: Short Term Facility Fit Prediction

## 4.1 Aim

The previous chapter outlined a method to visualise long term facility fit and investigated the effects of increasing titre. This chapter describes a decision-support tool that integrates Monte Carlo simulation data derived using a stochastic discrete-event simulation model to mimic process fluctuations with advanced multivariate statistical techniques to help pinpoint the potential root causes of sub-optimal short term facility fit issues. Principal component analysis combined with clustering algorithms was used to analyse the complex datasets from complete industrial batch processes for biopharmaceuticals. The challenge of visualising the multidimensional nature of the dataset was addressed using hierarchical and k-means clustering as well as parallel co-ordinate plots to help identify process fingerprints and characteristics of clusters leading to sub-optimal facility fit issues. Industrially-relevant case studies are presented that focus on technology transfer challenges for therapeutic antibodies moving from early phase to late phase clinical trials. The case study details how sub-optimal facility fit can be alleviated by allocating alternative product pool tanks. The impact of this operational change is then assessed by reviewing an updated process fingerprint.

## 4.2 Introduction

Long term facility fit issues are generally concerned with the future proofing of manufacturing facilities and platform processes (Chapter 3). Any challenges highlighted can be addressed over time with the development of new technologies and large capital projects. Short-term facility fit issues are those that arise due to the batch-to-batch variability associated with a single process. There may be insufficient time to make significant changes to facilities so early identification of issues is crucial.

Technology transfer of pilot scale processes into facilities during late phase clinical trials of a drug candidate can often lead to short term facility fit issues. Hence, it is common practice in industry to perform a facility fit assessment during the initial stages of tech transfer of a manufacturing process into a larger commercial scale facility.

This chapter builds on the flexible database-driven simulation platform described in Chapters 2 and 3 that captures the mass balances, equipment sizing, dynamic resource allocation and process economics of purification sequences in monoclonal antibody manufacturing processes. Chapter 3 discusses the use of the tool to perform a small number of simulation runs to generate sufficient data to assess the impact of a single parameter change. This chapter describes the extension of the tool to mimic the stochastic nature of industrial batch processes when transferred to large-scale facilities and to identify the potential root causes of short term facility fit issues. This was achieved by building in capabilities to run Monte Carlo simulations and exploring how best to integrate stochastic results into advanced multivariate statistical analysis techniques.

Monte Carlo simulations result in very large datasets and hence visualising the results of the MVA becomes more challenging. Although PCA might reduce hundreds of datasets to a few principal components, it does not automatically identify clusters of batches with similar characteristics for further examination. Hence in this article, algorithms adapted from Thornhill et al.(Thornhill NF, 2006) are used to achieve hierarchical and k-means clustering of the datasets so as to identify significant clusters of batches. Multidimensional visualisation of each clusters characteristics in terms of the raw data (e.g. product titre in cell culture) is achieved through the generation of novel

multiple stacked parallel co-ordinate plots; this is in contrast to other works (Wang et al., 2004) where parallel co-ordinate plots are used to plot PC scores rather than reverting to the actual process data. The resulting facility fingerprints enhance process understanding of interactions between variables in an informative and clear manner.

## 4.3 Problem Domain

Upon technology transfer of a process from pilot scale to large scale, a current good manufacturing practice (cGMP) engineering batch is normally carried out to test performance and identify facility fit issues. Until this point, data is only available from the laboratory and pilot scales of the process. The first batch at large scale is subject to a much greater degree of uncertainty that would normally be expected between subsequent batches. This uncertainty is due in part to scale effects such as variability introduced by operating larger chromatography columns or increased product holdup in systems. The impact of this variability becomes more exaggerated at large scale where any equipment limitations are difficult to adapt to. As a result, the first batch at large scale is more difficult to predict than any other batch. THree key parameters were identified as key to the variability at scale: product titre, step yields and chromatography elution volumes. These were derived through extensive discussions with industrial practitioners involved in tech transfer as well as literature sources. Representative triangular distributions were derived through these discussion with industrial experts (Table 4.1).

Whilst sensible ranges in process variability were sought for each of the parameters, the primary aim of this chapter is to demonstrate the application of the proposed methodology to perform more rigorous and predictive facility fit assessments by leveraging process fluctuation datasets to determine both the likelihood and root causes of product loss. Hence, the actual inputs and answers should not be seen as definitive but an illustration of how to approach such an assessment. Triangular distributions were chosen due the limited availability of empirical data on which to base a more data driven decision. These three sources of variability are discussed further below.

**Product Titre.** Variation in the total amount of product entering into the purification process is felt across all chromatography steps since the number of cycles is determined

by the mass load of product, making it an important parameter to capture. Batch-to-batch variability as well as variability in titres across scales is commonly seen due to the inherent uncertainty associated with biological systems. A value of $\pm 10\%$ was determined through discussions with industrial advisors and represents a conservative level of batch-to-batch titre variability.(Legmann et al., 2009) However, a wider range of titre fluctuations may need to be considered for some products, especially where the differences in scale upon tech transfer are large and where tech transfer occurs before the detailed process characterisation scale-down studies. For example, published reports show small-scale bioreactors predicting titre profiles within $\pm 20\%$ of the historical average of large scale runs.(Amanullah et al., 2010; Abu-Absi et al., 2010) Contributing factors include: changes in vessel geometry and subsequent hydrodynamic affects, raw material sourcing, the differing levels of control of critical factors such as pH, temperature, inoculum transfer times, and gas mixtures within different reactors. The impact of these factors is complex and results in varying levels of uncertainty for different products and cell lines.

**Step Yields.** Variation in yield losses across steps are typically small as represented by the relatively narrow ranges specified in Table 4.1. The case studies in this chapter are concerned with a hypothetical first batch at scale where fine tuning with respect to minimising yield losses such as optimising flush volumes to minimise losses through holdup may not have been fully identified. Hence this variation may be reduced further over subsequent batches.

**Chromatography Elution Volumes.** Variability in the eluate volumes from the chromatography operations was highlighted as a significant factor impacting facility fit at scale during discussions with industry experts. When using collection criteria based on UV traces, a small variation in the position of the peak can have a large effect on the volume collected particularly for steps where significant leading or tailing on elution peaks may occur. Certain chromatography steps, for example, may be highly sensitive to the pH and conductivity of the elution buffer. When considering a process at scale which may use inline dilution for buffers, the control over pH and conductivity is generally less precise than can be achieved at the laboratory scale. Different batches of buffers can also influence the binding and elution profiles. Furthermore, when tech

transfer activities are planned before the process limits evaluation validation studies during Phase III clinical trials, most of the manufacturing data would be from lab-scale or small-scale experiments. Predicting variability in eluate volumes at large scale from data generated by small laboratory columns is complicated by several factors such as differences in dispersion and retention volumes between the two scales of operation (Hutchinson et al., 2009) as well as fluctuations in operating conditions (e.g. buffer pH and conductivity). As a result, a value of $\pm$50% in eluate volumes was considered to reflect this uncertainty as a worst case scenario for most processes.

Table 4.1: Variable Distribution Ranges

| Variable | Min | Most Likely | Max |
|---|---|---|---|
| Product Titre | -10% | Base Case | +10% |
| Elution Volumes | -50% | Base Case | +50% |
| Filter Flux Rates | -10% | Base Case | +10% |
| **Step Yields** | | | |
| Chromatography Steps | 83% | 88% | 93% |
| Virus Inactivation | 98% | 99% | 100% |
| Ultrafiltration / Diafiltration | 90% | 95% | 99% |
| Virus Retention Filtration | 90% | 95% | 99% |

## 4.4 Method

The database-driven discrete-event simulation tool described in Chapters 2 and 3 for modelling the logistics and process economics of antibody process was used as the core evaluation engine for this study. It was adapted to perform Monte Carlo simulations and handle the larger datasets required so as to mimic the impact of process fluctuations on the key outputs. A series of multivariate and visualisation analyses were then explored to examine the stochastic simulation outputs. These are elaborated upon further in the following sections.

### 4.4.1 Database Configuration for the Monte Carlo Simulations

The simulation database was not originally designed to store the required inputs and outputs from stochastic simulations. The data structure was modified to handle the more complex datasets. Where before the values were stored as single constant values, stochastic simulations require an ability to define distributions. In this chapter only triangular distributions were used, but the new data structure was designed to allow different distribution functions to be defined in the future.

In some cases, the distribution was not used to calculate the final sensitised value, rather it was used to determine a multiplication factor. In turn, this factor was used to modify a parameter which was only calculated during the simulation. In this instance although the distribution was the same shape, the simulation needed to know that the value generated must be used in a subsequent calculation. This method was used to sensitise the filter flux rate. The final flux rate is dependent on filtration area and product concentration, parameters which are only available after they have been calculated during the simulation.

The solution was to use two fields in the database tables where the distributions were to be defined. The fields were defined in addition to the existing data so as to allow the simulation to be operated in deterministic mode without modification. The first field was a numerical identifier which relates to the shape of the distribution. The second field was a coded variable containing the parameters for the bounds of the distribution. The value in the identifier field determined would function will be used by the simulation and the coded variable stored the inputs to the function as a set of comma separated variables.

This approach to capturing stochastic inputs enabled modifications to be made that limited effect on the existing data structure whilst allowing for the maximum degree of flexibility.

As well as defining a data structure for the input parameters, the results from multiple iterations had to be stored. The existing database described in Chapters 2 and 3 stored a single set of results from each simulation across the archive tables off the database. Each complete simulation was assigned a unique ID number such that the

results from any one simulation could be collated at a later date. During Monte Carlo simulations multiple result sets are generated for each simulation so a single field could no longer be used to identify individual results. The solution was to add an additional iteration ID field. The combination of simulation ID and iteration ID allowed any single result set to be identified and analysed. By being able to separate and isolate individual iterations from Monte Carlo result sets it was possible to carry out a more in-depth analysis on the root causes of the outputs observed.

### 4.4.2 Stochastic Discrete-Event Simulation Engine

The discrete-event simulation engine described in Chapters 2 and 3 was configured to run Monte Carlo simulation by tying a random number generator into new functions which could generate the values within the distributions described in the database. This is the simplest method of implementing Monte Carlo simulations but alternatives such as Latin Hypercube Sampling (Helton and Davis, 2003) can be more efficient and result in faster CPU times.

The key challenges were as follows:

- Enabling the simulation to identify where a parameter was sensitised.
- Importing and converting the coded variable into usable parameters.
- Generating the values within the defined distribution.
- Reconfiguring the functions which manage the input and output from the database to handle the more complex datasets.
- Achieving the above without significant modifications to the existing simulation structure.

The numerical identifier field was used to determine if a distribution should be used. A value of 1 directs the simulation engine to use the original deterministic (point) value. Values other than 1 correspond to different shapes of distribution and whether the calculated value is to be used directly or as a multiplication factor. For example a

value of 2 triggers a function to generate values within a triangular distribution using equation 4.1.

$$x = \begin{cases} a + \sqrt{U\,(b-a)\,(c-a)} & \text{if } U \leq \left(\dfrac{c-a}{b-a}\right) \\ b - \sqrt{(1-U)\,(b-a)\,(b-c)} & \text{if } U > \left(\dfrac{c-a}{b-a}\right) \end{cases} \tag{4.1}$$

where $a =$ Minimum, $b =$ Maximum, $c =$ Mode, $U =$ random number between 0-1.

In the above case, the values for the minimum, maximum and mode were retrieved from the comma separated coded variable stored in the database. The random number was provided by a random number generator built into the ExtendSim software.

### 4.4.3 Principal Component Analysis Method

Methods for principal component analysis have been widely documented. The method used in this thesis was derived from Thornhill et al. (2006) and uses singular value decomposition. The output data from the simulations were first transformed by mean centring and stored as a comma separated variable file which was then imported. The PCA analysis methods were initially coded in Mathematica (See Appendix B) However, to allow for a greater degree of automation the analysis was subsequently transferred to SPSS Statistics (IBM Corp., NY).

The main aim of principal component analysis is to reduce the dimensionality of a dataset whilst minimising the loss of information. It was first developed for use in the behavioural sciences to determine a smaller number of behavioural traits from a large number of behavioural observations. (Rummel, 1970)

The primary output of the analysis is a new data set where the original variables have been replaced by principal components and the values for each variable are transformed into scores within each principal component. The weighting of the original variables within the principal components will determine how the scores relate back to the original data. However, it can be said that the structure of the scores will represent the structure of the original dataset and if successful, the structure will be evident in a

lower dimension than the original data and thus easier to visualise. (Jackson, 1991)

Eigenvalues are used to determine which principal components are of key importance. Several methods are used to test for significance. (Valle et al., 1999; Zwick, 1986) The most common of these is to divide each eigenvalue by the mean of all eigenvalues and consider all principal components with an eigenvalue greater than 1 as significant. The eigenvalues can also be plotted for each principal component, the result is a scree plot as shown in Figure 4.4. Visual analysis of the scree plot may determine that the average eigenvalue method is not suitable if the data is not highly correlated. Instead a visual analysis of the scree is required to identify significant changes in gradient. The ideal scree plot will consist of a small number of high value eigenvalues followed by a sudden drop and a gradual tailing of the remaining eigenvalues. The principal components before the drop are considered significant and those after have less statistical value. (Cattell, 1966; Rozett, 1975)

Upon identification of the key principal components the scores from each component can be analysed using cluster analysis. SPSS was used to assess the performance of two clustering methods. These were hierarchical clustering, and k-means clustering. Hierarchical clustering was used by Thornhill et al. (2006) with a data set of similar complexity and structure and whilst various methods were used to improve the method, hierarchical clustering was not able to satisfactorily elucidate the clusters within the dataset generated in this analysis. An example dendrogram is shown in Appendix B. Instead k-means clustering was used. The k-means clustering algorithm used in this thesis was a function within SPSS.

SPSS was also used to generate graphical outputs in the form of scatter plot matrices and parallel co-ordinate plots. Both methods were used to visualise the higher dimensional datasets generated as part of this analysis and are discussed in more detail throughout this chapter.

## 4.5 Results

### 4.5.1 Case Study Aim

The case study looks at the first batch at scale. The process description used in Chapter 3 and detailed in Appendix A was combined with a hypothetical 10,000L (10K) facility. The aim was to identify potential short term facility fit issues upon tech transfer and the probability that they would be realised. In addition to facility fit issues, the case study also highlights how the sensitivity of a process to uncertainty can change with respect to facility design.

The simulation engine was configured to run Monte Carlo simulations. The results were first analysed to investigate potential facility fit issues. Modifications were then made to the simulated process (referred to herein as the modified 10k Facility) to improve facility fit and the Monte Carlo simulations were repeated with the improved process. The complexity of each dataset was then reduced using principle component analysis combined with clustering to reduce the dimensionality and eliminate noise (Thornhill et al. 2006). The results were compared to assess the impact of the process change.

The appropriate number of principal components were selected by a visual analysis of the eigenvalues plotted for each principal component. After selection of the principal components, clusters in the dataset were identified using k-means clustering. The clusters were generated by analysing the component scores. Finally, the input parameter distributions for each cluster was visualised on vertical parallel co-ordinate plots. The Monte Carlo simulation was run for 1000 iterations to generate the following results.

### 4.5.2 Identifying Sub-optimal Facility Fit

#### 4.5.2.1 Mass Throughput Profile

One of the key parameters of interest is the mass throughput of the facility (kg/batch). In many cases the planning of manufacturing campaigns will be determined well in advance of the first batches at scale, therefore it is important to have an accurate pre-

diction of facility throughput early on in the development lifecycle. The stochastic tool was used to predict the likelihood of product loss upon tech transfer using Monte Carlo simulation, given the expected fluctuations in key performance indicators and purification operating parameters indicated in Table 4.1.

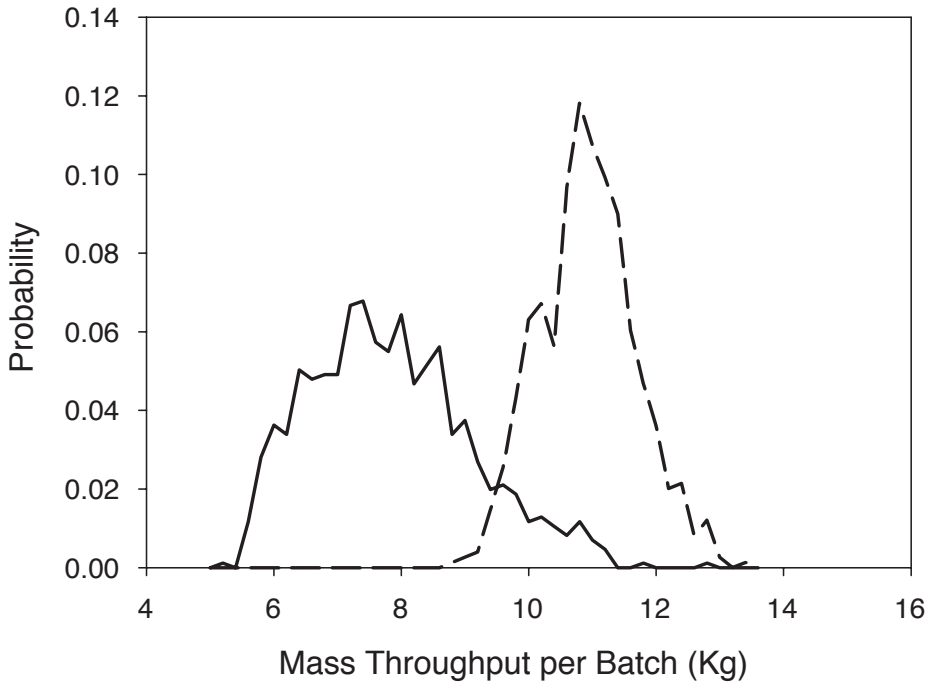



Figure 4.1: Probability distributions for the throughput predicted from both the base case (——) and modified (- - -) 10K facility. The product titre at point of harvest was 2g/L yielding 20kg of product from the reactor. (n=1000)

Figure 4.1 shows the predicted facility throughput for the processes running in the 10k facility. Based on deterministic values, where only the most likely value is considered for each parameter (e.g. product titre = 2 g/L, overall process yield = 55%) and the 10,000L, fermenter scale, the predicted facility throughput would be calculated at 11Kg/batch. The values predicted by the simulation fall well short of this value. A very small proportion of batches meet the expected throughput. This is suggestive of facility fit issues and prompts further investigation. As will be discussed later, upon further investigation a facility fit issue was identified and corrected, resulting in the modified 10K facility probability profile.

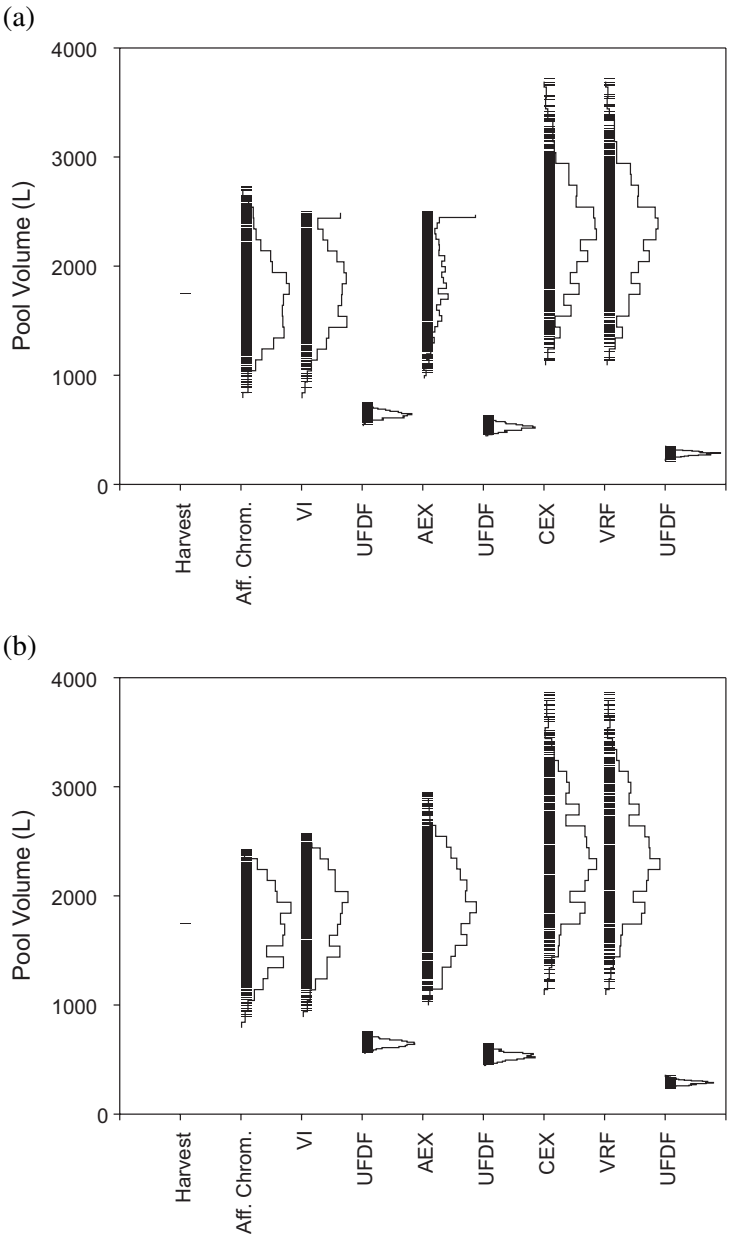



Figure 4.2: Distribution of pool volumes for each step in the (a) base case and (b) modified 10K facility data. Vertical bars are composed of a series of horizontal dashes, each representing the pool volume from a single simulation iteration. For clarity the distribution of values is shown by the histogram alongside each bar. (n=1000)

#### 4.5.2.2 Risk Hotspots for Facility Fit

The Monte Carlo simulation data for the pool volumes after each step in the purification process were examined to identify the location of the equipment limitations causing the facility fit issues. The simulation engine maintained a log of events that occurred dur-

ing each simulation run. A section of this log was dedicated to vessel operations and an error event was recorded when the amount of material exceeded the tank's maximum volume. Review of the log showed that an over-fill event occurred in two of the product pool tanks in a number of runs. Since step yields and elution volumes are stochastic variables some variability of pool volumes was expected. Figure 4.2 shows the distribution of the pool volumes at each step. With the exception of the harvest pool which in this simulation is defined as a fixed value entering the process, variation in the pool volumes is evident. Figure 4.2(a) identifies the virus inactivation and anion exchange chromatography pool volumes as risk hotspots.

The vertical histogram plotted alongside each column of data points shows a spike in the distributions at 2500L for both of these tanks. This is due to the fact that the largest volume that can be stored in these tanks was 2500L. Surplus volume was diverted to waste and the product was lost impacting throughput on a large number of batches. This is a facility fit issue which must be addressed. The action was taken to review tank allocation within the facility on which the simulation was based and additional tankage, not routinely available for product storage was identified. The specification for these tanks were added to the database and therefore became an option for the simulation engine. The simulation was re-run and with the additional tanks available, the fit issue was resolved. This is shown in Figure 4.2(b).

With the tank volumes increased to handle the predicted pools, the volume of the spike in the distribution was removed. The result of this modification would be to increase the volume entering into subsequent steps and could also move the bottleneck downstream. In this instance however sufficient capacity was installed and no additional tank issues were apparent. The impact of this facility fix on the facility throughput can be seen in Figure 4.1 (dotted line), with the throughput closer to the predicted value of 11kg/batch.

#### 4.5.2.3 Probability of Meeting Demand

The data shown in Figure 4.1 can be used to generate probabilities of being able to manufacture sufficient material within a number of batches and hence better manage manufacturing risk. Figure 4.3 shows the probability of being able to meet varying

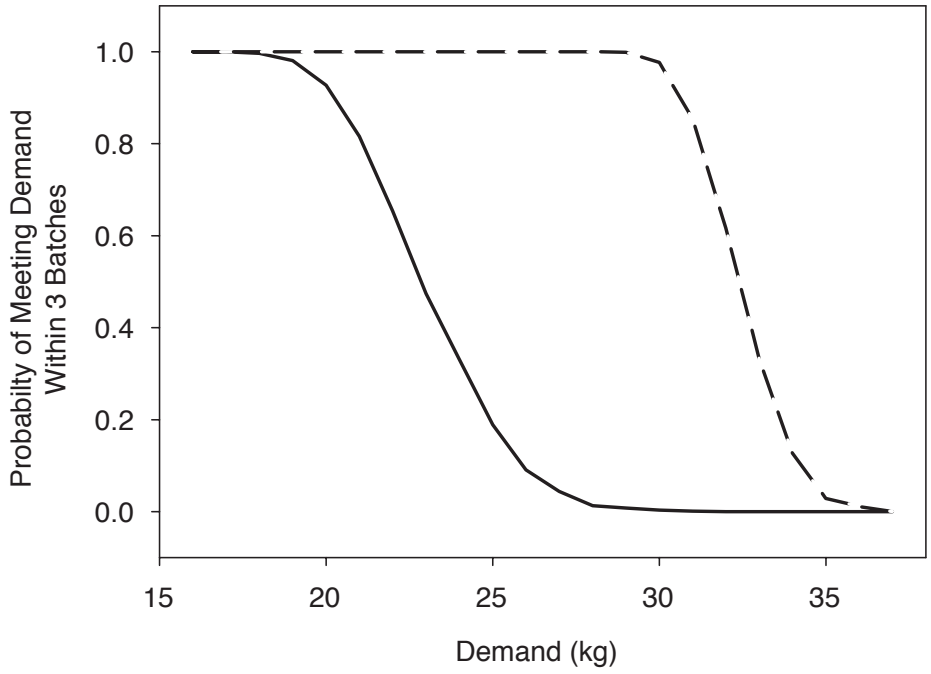


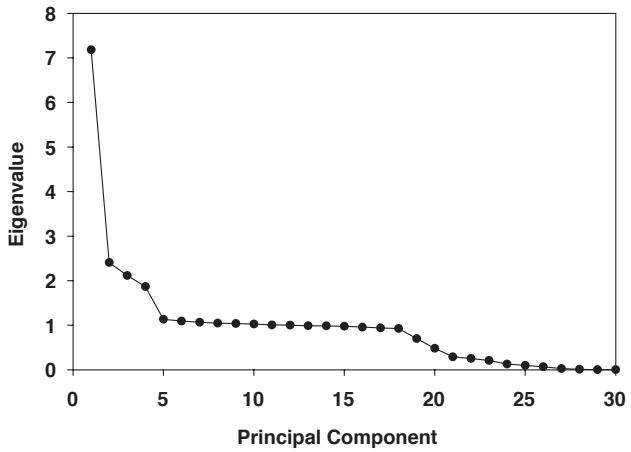

Figure 4.3: Probability plot showing the probability of meeting a range of demands within three batches using either the base case (——) and modified (- - -) 10K facility. (n=1000)

demands within three batches. This illustrates that the minimum guaranteed demand for the modified facility (Approx. 29kg) is almost double that of the base case facility (Approx. 15kg). This is because not only is the throughput of the modified facility higher, it is less variable.

### 4.5.3 Principal Component Analysis

In order to gain more insight into the performance of the process in the 10K, facility principal component analysis was used to reduce the complexity of the dataset. The two datasets from the base case and modified facility were analysed individually using the same procedure. First the number of significant principal components was identified by plotting their eigenvalues. This was followed by determining the number of significant clusters and their membership. Finally, stacked parallel co-ordinate plots were created to determine the combination of batch characteristics that led to product losses.

(a)




(b)

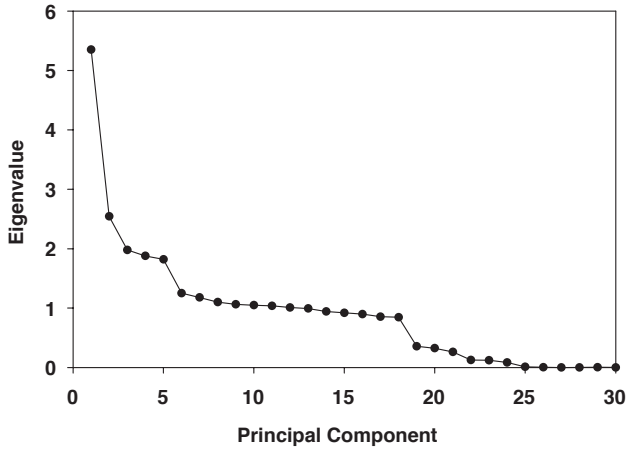



Figure 4.4: Scree plots generated from the principal component analysis of the (a) base case and (b) modified 10K facility data.

#### 4.5.3.1 Principal Component Selection

The scree plot shown in Figure 4.4 was used to determine the number of significant principal components. The average eigenvalue method, which defines significant components as having eigenvalues greater than 1 is not suitable for these datasets as this would include a number of principal components which add little value. Instead the appropriate number of principal components was selected by a visual analysis of the dataset. (Zwick, 1986; Cattell, 1966) In this case for the base case the first 5 principal components were selected and for the modified facility the first 6. In both cases selection of any additional components added little to the integrity of the dataset.

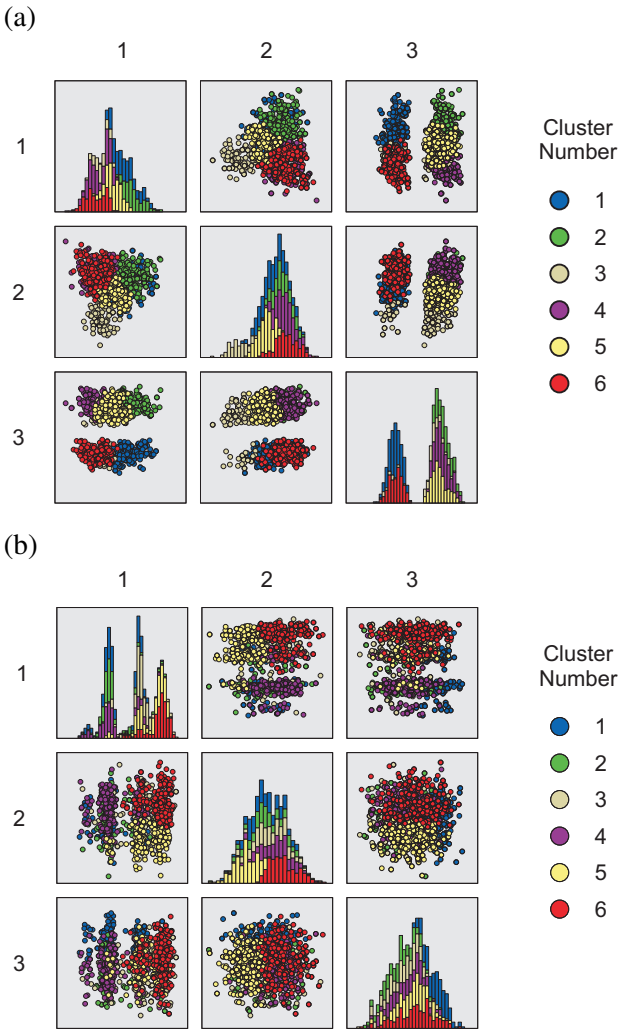



Figure 4.5: Clustered scatter matrices showing the score values for the first three principal components generated by the PCA of the (a) base case and (b) modified 10K facility data. k-means clustering was used on the first 5 principal components, 3 are shown for clarity. (n=1000)

#### 4.5.3.2 Cluster Identification

After selection of the principal components, hierarchical clustering was used to determine the number of significant clusters by visual inspection of the resultant dendrogram. For the base case facility data the dendrogram is shown in Figure 4.6. In this instance the clusters were defined at an average distance between the clusters of 20 units. Further branching leads to less distinct clusters.

The method used for hierarchical clustering did not allow for the extraction of cluster membership at specific average distances hence, k-means clustering was then

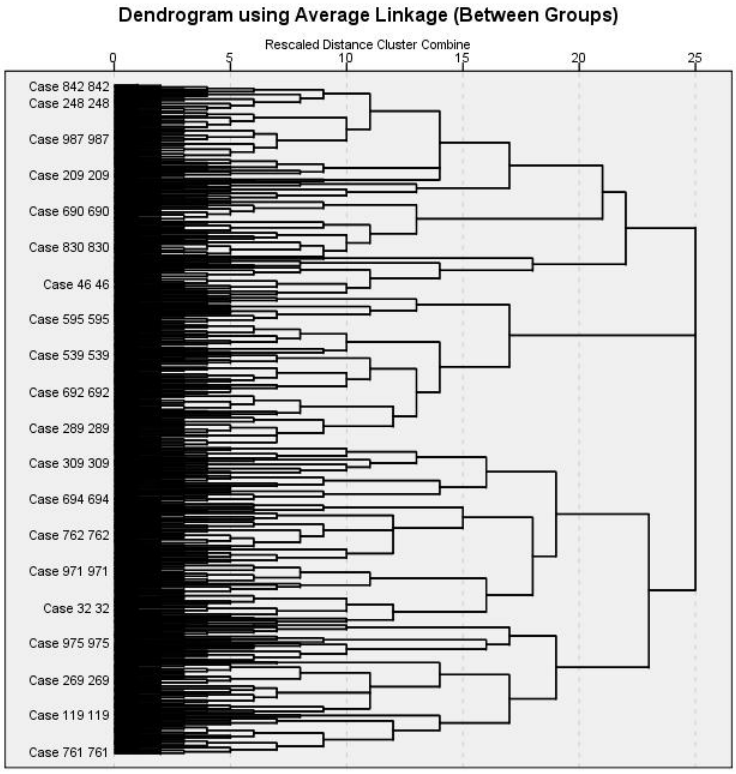



Figure 4.6: Dendrogram generated from the hierarchical clustering of principal component analysis of base case 10K facility data.

used to identify the cluster membership of the six distinct clusters. This method was repeated for the modified facility which also identified 6 distinct clusters at a similar average distance. To aid visualisation, the key principal components were plotted against each other on a scatter plot matrix (Figure 4.5). Each of the cells in the scatter plot matrices shown in Figure 4.5 were generated by plotting the component scores from each principal component against each other. For example, all the charts in the first row show principal component 1 scores plotted against: themselves in column 1, principal component 2 scores in column 2 and principal component 3 scores in column 3. Since the plots on the diagonal are the principal components plotted against themselves the data is represented as a histogram to show the distribution of the scores within each principal component.

In addition to the scatter plot matrices the clusters identified by k-means clustering are shown as different coloured points.

For example, the scatter plots in Figure 4.7(a) for PC3 vs. PC1 clearly indicate the presence of two distinct and nonoverlapping clusters. Each of these clusters can

be split into further non-overlapping clusters as indicated in Figure 4.7(a) as a result of k-means clustering in higher dimensions. By allowing the clusters to be visualised in the PCA score space, these scatter plots allow verification of the success of the k-means clustering for these datasets. By carrying out the principle component analysis first we have removed those parameters which have less influence on the structures within the dataset thereby reducing noise and the dimensionality of the data.

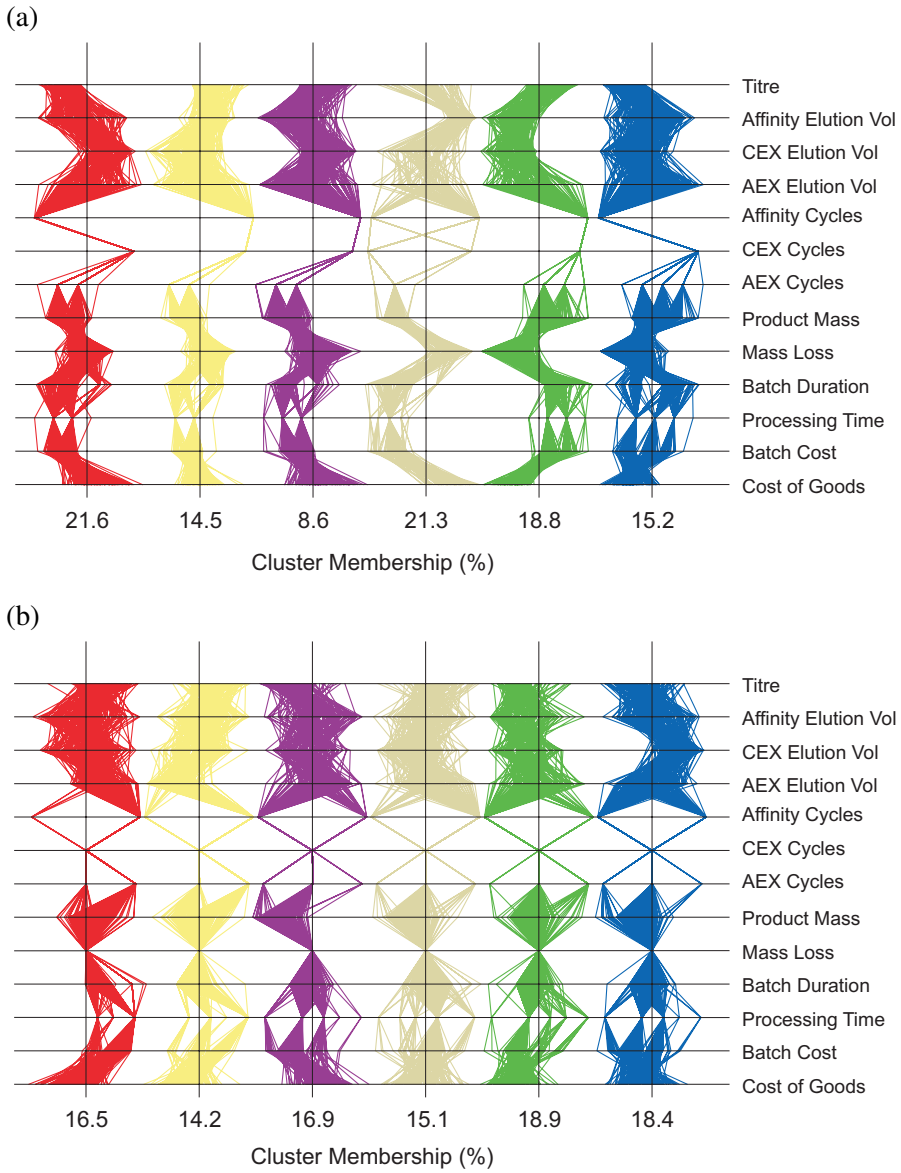



Figure 4.7: Vertical parallel co-ordinate plot showing PCA clustered data for the (a) base case and (b) modified 10K facility data. (n=1000)

#### 4.5.3.3 Parallel Co-ordinate Visualisation

Next, the clustered data was converted back to values which have more meaning. This was done by separating the input dataset into the clusters identified by the k-means algorithm and this time plotting the original variables instead of the component scores on a series of stacked parallel co-ordinate plots. This data is shown in Figure 4.7. Figure 4.7(a) shows the data from the base case 10K facility. Thirty parameters were input into the PCA algorithm however only the parameters of interest are shown here. For clarity each parameter is normalised between -1 and 1 such that they can be easily compared. The result is a facility fingerprint. By comparing Figures 4.7(a) and (b) the wider impact of changing the tank volume can be quickly assessed. However, it should be noted that the magnitude of the distributions in the two sets of parallel coordinate plots are different as they are normalised independently.

Each cluster of batches in Figure 4.7 has common characteristics that combined lead to different outcomes such as high mass losses. For example, an examination of cluster 4 in Figure 4.7(a) indicates that despite high titres, the cluster also exhibits high cost of goods. This can be attributed to a high average level of mass loss that was strongly linked to a high average elution volume on the affinity step.

Figures 4.7(a) and 4.7(b) can be compared together to understand how the facility fingerprint changes after the facility fix. Before the modification (Figure 4.7(a)), all clusters of batches exhibit significant variance in product mass loss. After the fix (Figure 4.7(b)), the variance in mass loss can be seen to be removed as indicated by the pinch point formed across all clusters. Further comparison links this to the reduced variability in the number of chromatography cycles (affinity, AEX and CEX). This represents a significant improvement in the process schedules ability to absorb the uncertainty. The number of cycles overall may be higher in the modified facility, however they are more predictable with is often of greater importance.

## 4.6 Conclusions

This chapter illustrates the insights that can be gained by integrating stochastic simulation data with advanced multivariate statistical techniques for predicting and under-

standing facility fit issues upon tech transfer. The Monte Carlo data enabled a greater degree of understanding of the impact of process fluctuations and subsequent facility modifications on the likelihood of meeting manufacturing targets. An early appreciation of risk allows for the planning of risk avoidance strategies such as contingency batches or process changes. The effect of the facility modification identified was analysed in more detail with multivariate statistical analysis techniques that harnessed principal component analysis, clustering algorithms and high dimensional visualisation techniques combined to generate facility fingerprints. These allowed for rapid identification of process robustness and characteristics of clusters of batches that resulted in product losses and hence suboptimal facility fit.

# Chapter 5

# Validation

## 5.1 Introduction

This chapter discusses the regulatory aspects of pharmaceutical manufacture and focuses on the place of decision support tools in the validation of bioprocesses. This chapter draws on guidance provided by the Food and Drug Administration and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals (ICH).

### 5.1.1 Quality by Design

"Currently, manufacturing process selection and development are usually based on engineers knowledge and experience. Process simulation tools can help identify optimal and efficient processes and facilitate process scale up from exhibit batches to commercial manufacturing." (Center for Drug Evaluation and Research, 2007)

The above quote is from a technical report published by the FDA outlining key areas for consideration within the QbD framework for the development and approval of generic drugs. Since this report was published the FDA has confirmed its stance of the position of QbD within regulatory filings, by requiring that all submissions for generic drugs made after January 2013 must demonstrate QbD principles have been used. (Rosencrance, 2011)

Generic drug manufacture is the current area of focus for the FDA and other regulatory authorities as they attempt to provide legislation and guidance for what is still an emerging area of the industry. It is therefore likely that this same guidance will be

implemented for all submissions in the near future.

The traditional approach to quality management within drug manufacture is for quality assurance oversight of a fixed manufacturing process and quality control release testing of the finished product. Where the process deviates from the fixed process in-process testing is used to justify the impact on product quality.

The move to QbD acts to ensure that the quality is built into the final product by developing the process with an understanding of critical quality attributes and critical process parameters. Manufacturer's must therefore define these attributes and parameters and the ranges of normal operation to form a QbD design space. In principle, with the QbD framework, the emphasis on release testing is reduced as a process operating within the design space will, by design, produce product of the required quality.

Quality by design (QbD) focuses on the robustness of a process design where variation is understood, accepted and managed. Simulation tools provide the potential to investigate the effects of process variation and make design decisions accordingly. For example for products where stability issues are critical, hold times may become critical quality attributes. In this case simulation tools can be used to understand the variation in hold times that may be expected in a process as a result of resource constraints, where the times exceed critical limits decisions can be made to alleviate bottlenecks. Where resource utilisation changes for a process for example due to equipment failure or supplier issues, simulations could be used throughout a process to determine the effects and plan accordingly.

## 5.2 Conclusions

The future of process simulation may be routine use in in-online decision making, simulating potential outcomes of scenarios before implementation and then changing the process accordingly. This may eventually be seen as one way to adapt in a controlled manor to the inherent variability of biological processes. An example would be to adjust the harvest time of a reactor based on cell growth and productivity with a view to optimise primary recovery operations; provide more favourable material to DSP or to better fit the facility schedule.

The FDA and other regulatory authorities are pushing pharmaceutical manufac-

turers to display a greater understanding of their processes. This includes the use of statistical analysis when analysing process data and to demonstrate that a process is operating consistently and in control. Primary to this are the analysis of process performance qualification (PPQ) batches, also known as process validation series or consistency lots. Although there is no binding recommendation as to the number of batches, typically 3 or more are carried out. This presents a potential problem for pharmaceutical companies, as sound statistical analysis on a sample size of only 3 is difficult. One solution regularly employed is to use data generated during process development to increase the samples size and provide a more complete picture to the regulatory authorities. Process simulations could also be an important source of data and could provide a potential wealth of useful information.

Whatever the future for decisional tools in bioprocessing, an insight into the demands of computer systems validation may prove useful for the long term future proofing of systems developed today and the selection of validatable platforms which do not rely on Excel and similar third party tools my be pivotal in establishing a foothold in the industry.

# Chapter 6

# Conclusions and Future Work

## 6.1 Conclusions

The project sponsor was Lonza Biologics plc., as such the main focus of this project was antibody manufacturing processes. Over the course of the EngD supervisors changed and the project moved between different departments, this resulted in a shift in focus which shaped the direction of the project.

The initial remit was to develop a simulation tool that could be used to assess facility fit of manufacturing processes and to provide a greater understanding of the limitations of the manufacturing facilities currently in operation. This was set against the backdrop of an environment where there have been large advances in upstream processing (USP) technology with titres increasing from milligram to multi-gram quantities, in the same timeframe there has been relatively limited progress being made in down stream processing (DSP).

At the start of the project in 2005 a high titre was considered to be 2g/L, at the end, 5 years later, industry is achieving 5g/L routinely and processes achieving titres of close to 10g/L are already becoming a reality. In contrast the platform process in DSP has remained largely unchanged. A large majority of processes can be described by the 3 step platform, named because of the 3 chrome steps that form the backbone of the process. Typically we see a protein A affinity step with two further column polishing steps, virus inactivation and filtrations steps and a typically two ultrafiltration diafiltation (UFDF) steps one for buffer exchange post protein A and one to transition the product into it's final formulation.

Whilst there are a few variations, perhaps hydrophobic interaction chromatography (HIC) in place of the final ion exchange step and more recently membrane based chromatography, none of these truly represents a paradigm shift in manufacturing. Based on this history, the central question for the first part of this thesis was, how long will it be before a facility developed around the 3 step platform process becomes bottlenecked? By this we mean at which point will the process not be able to reap the rewards of the upstream process improvements? This techniques required to address this question were discussed in chapter 3.

After the first two years the original project moved in a new direction as the supervisor, who was based in DSP manufacturing, moved on from Lonza. The new supervisor, now based in process and technology transfer, was more interested in how process uncertainty would effect facility fit. Instead of investigating long term facility fit the focus shifted to short term facility fit. The question now became; how will the predicted process variation between batches within a campaign result in issues? This formed the basis of chapter 4.

In order to answer these questions the need was identified for the development of a flexible simulation framework with the capability of stochastic simulation. This took the form of a discrete event simulation engine and a complimentary relational database. The selection of software tools and the development of the framework was discussed in chapter 2.

Often, the approach to simulation is to develop what is essentially an expert system, a model which is designed around a single largely fixed process. This allows you to design highly efficient and accurate simulations however, if there is a process change the simulation must be rewritten or redeveloped, for example to account for a change in scale. To address the first question, that of long term facility fit in an environment of increasing titres, the technology of the platform process remains constant, and the scale changes to accommodate the increasing upstream titres. To efficiently model the problem, a simulation engine was designed to scale any given process within the limitations of the facility. This required a new approach to the structure of discrete event models. In this case the simulation was built using a novel router based structure that was able to adapt to any process specified in the database. In addition an optimisation

module was able to automatically scale the process using established rules of scale up before feeding that process into the simulation.

Once the project moved into looking at short term facility fit, the simulation engine was further developed to carry out Monte Carlo simulation. Now the process and scale were fixed, but key parameters of the process were no longer defined as fixed values but instead defined as triangular distributions. New functionality was developed to enable the simulation engine to use a random number generator to randomly select values from within distributions defined in the database rather than use the previously fixed values. When running Monte Carlo simulations the simulation engine is connected to a powerful database to facilitate the large datasets generated for each simulation run. This means that it was not only possible to see the impact that uncertainty has on key process variables such as cost time or product yield, it is also possible to investigate circumstances which led to the output that is observed.

The enhanced datasets are however massive and complex and more sophisticated statistical analysis is needed to determine the structure of the data. This led to the development of a set of methods using principal component analysis to simplify the dataset and clustering algorithms to identify key process types, the data are then presented using parallel co-ordinate plots to generate novel process fingerprint which can be used to visualise process robustness. Comparison of these fingerprints can also be used to assess the impact of process changes.

## 6.2 Future Work

This section outlines possible future directions for the research discussed in this thesis to address shortcomings and to expand functionality.

### 6.2.1 Unit Operation Pallet

The simulation engine has the functionality to simulate those unit operations which form part of a generic platform process for the purification of monoclonal antibodies. At the time of development, these unit operations represented the standard. Newer technologies such as membrane chromatography are now seeing an increased use within antibody manufacturing and they present different challenges for facility fit. New unit

operation blocks will need to be constructed to assess these newer technologies. In addition the unit operation pallet could be extended to cover those unit operations used for the production of non-antibody products and include operations such as protein refolding steps or conjugation reactions.

This project focused on whole process simulation and the unit operation models themselves are only designed to perform mass balance, resource and scheduling calculations. More complex models are available to simulate, for example the removal of impurities, filtration flux decay rates or other factors relating to the performance of steps which may be affected by those parameters which were sensitised within the framework such as product concentration. The framework could serve as a basis to link a number of the more advanced single unit operation models together to provide a greater insight into the performance of steps and the effects of these model outputs on the wider process.

### 6.2.2 Ancillary Processes

Ancillary activities are those which are required to support the process, but do not directly interact with the product stream. This includes cleaning and sterilisation processes and buffer prep. Taking the example of buffer prep, the simulation engine provides information on buffer quantities required and associated raw materials, however buffer prep operations do not act to constrain the process schedule. This is because in order to do this buffer prep would need to be predicted in advance of the requirement to provide a realistic representation of how buffer prep activities are scheduled in a real facility. The pre-scheduler element of the simulation framework was initially developed with this functionality in mind, however it could not be successfully implemented to provide accurate enough predictions of the buffer schedule. Further work is needed to build functionality to enable prediction of activities such as buffer prep, clean in place and steam in place such that the impact of these steps can be included in the overall facility fit assessment.

### 6.2.3 Continuous Processing

The simulation engine is focussed on batch processing. Continuos processing presents a number of different challenges which cannot currently be addressed within the frame-

work. Researchers at UCL have demonstrated that the framework can be expanded to include the capability of continuos processing and this presents an interest avenue for further investigation.(Pollock et al., 2013)

### 6.2.4 Optimisation Methods

The prescheduler module which is designed to determine optimum process configurations based the availability of equipment and process variables used brute force optimisation. This method was found to be acceptable with respect to processing times and the robustness of determining optimum process configurations within the bounds of processing time and costs. This method is however not scalable as the number of process options increases exponential with the number of dimensions. More scalable methods such as latin hypercube sampling or genetic algorithms will be required to maintain an acceptable runtime should the optimisation challenges become more complex.

### 6.2.5 Automated Clustering

Two clustering methods were investigated hierarchical and k-means. Other clustering methods exist and could be investigated further for the analysis of the data. The disadvantage of k-means clustering is that the algorithm requires that the number of clusters be known prior to running the algorithm, in the case studies, hierarchical clustering was therefore used first to determine the number of clusters which could then be fed into the k-means algorithm. This method was not automated and required a manual analysis of the hierarchical clustering data.

### 6.2.6 Graphical User Interface

Currently multiple applications are required to allow the user to interact with the simulation engine and the results generated. The development of a single graphical user interface could greatly enhance the usability of the simulation framework. This is an activity that would need to be completed to consider rolling out the framework for use by industry.

# Appendix A

# Base Case Process Description

## A.1 Process Description

The tables in this appendix detail the base case process parameters used throughout this thesis. The process is defined using scale independent process parameters such that the same process can be investigated at a range of scales as required.

### A.1.1 Protein A Chromatography (Bind and Elute)

Table A.1: Protein A Affinity Chromatography Specifications

| Parameter | Value |
|---|---|
| Principle | Bind and Elute Affinity Chromatography |
| Resin Type | Protein A Affinity |
| Max Resin Cycle Number | 100 |
| Packing Flow Rate (cm/h) | 750 |
| Operational Flow Rate (cm/h) | 450 |
| Expected Step Yield (%) | 88 |

Table A.2: Protein A Affinity Chromatography Operation

| Parameter | Value |
|---|---|
| **Clean** | |
| Buffer | Protein A Cleaning Buffer |
| Volume (CV) | 3 |
| Hold Time (h) | 0.25 |
| Frequency | Immediately before each cycle. |
| **Equilibration** | |
| Buffer | Protein A Equilibration Buffer |
| Volume (CV) | 5 |
| **Load** | |
| Dynamic Binding Capacity (g/L) | 25 |
| Volume (CV) | Variable |
| **Post Load Wash 1** | |
| Buffer | Protein A Wash Buffer A |
| Volume (CV) | 2 |
| **Post Load Wash 2** | |
| Buffer | Protein A Wash Buffer B |
| Volume (CV) | 6 |
| **Post Load Wash 3** | |
| Buffer | Protein A Wash Buffer A |
| Volume (CV) | 4 |
| **Elution** | |
| Buffer | Protein A Elution Buffer |
| Elution Buffer Volume (CV) | 5 |
| Volume Collected (CV) | 2 |
| **Strip** | |
| Buffer | Protein A Strip Buffer |
| Volume (CV) | 2 |
| **Regeneration** | |

*Continued on next page...*

| **Parameter** *(continued)* | **Value** *(continued)* |
|---|---|
| Buffer | Protein A Cleaning Buffer |
| Volume (CV) | 3 |

### A.1.2 Low pH Virus Inactivation

Table A.3: Virus Inactivation

| **Parameter** | **Value** |
|---|---|
| Principle | Virus inactivation through low pH hold. |
| Hold Time (h) | 1 |
| Acid Vol (ml/L) | 16 |
| Base Vol (ml/L) | 42 |
| Expected Step Yield (%) | 100 |

### A.1.3 Post Protein A Ultrafiltration/diafiltration

Table A.4: Post Protein A UFDF Specifications

| **Parameter** | **Value** |
|---|---|
| Principle | Buffer exchange and product concentration using cross-flow filtration. |
| Pore Size (kDa) | 30 |
| DF Concentration (g/L) | 30 |
| Final Concentration (g/L) | 25 |
| Diafiltraiton Volumes | 3 |

*Continued on next page...*

| **Parameter** *(continued)* | **Value** *(continued)* |
|---|---|
| Diafiltration Buffer | Anion Exchange Equilibration Buffer |
| $\Omega_1$[1] | -59.217 |
| $\Omega_2$[1] | 253.33 |
| Max Step Time (h) | 6 |
| Expected Step Yield | 99% |

### A.1.4 Anion Exchange Chromatography (Flow Through)

Table A.5: Anion Exchange Chromatography Specifications

| **Parameter** | **Value** |
|---|---|
| Principle | Product flows through column and contaminants remain bound. |
| Resin Type | Anion Exchange |
| Max Resin Cycle Number | 50 |
| Packing Flow Rate (cm/h) | 700 |
| Operational Flow Rate (cm/h) | 450 |
| Expected Step Yield | 88% |

Table A.6: Anion Exchange Chromatography Operation

| **Parameter** | **Value** |
|---|---|
| **Clean** | |
| Buffer | Anion Exchange Cleaning Buffer |

*Continued on next page...*

[1] $\Omega_1$ and $\Omega_2$ are experimentally derived parameters to estimate flux at various concentrations.

| **Parameter** *(continued)* | **Value** *(continued)* |
|---|---|
| Volume (CV) | 3 |
| Hold Time (h) | 1 |
| Frequency | Immediately before each cycle. |
| **Equilibration** | |
| Buffer | Anion Exchange Equilibration Buffer |
| Volume (CV) | 5 |
| **Load** | |
| Dynamic Binding Capacity (g/L) | 50 |
| Volume (CV) | Variable |
| Volume Collected (Load Vols) | 3 |
| **Post Load Wash 1** | |
| Buffer | Anion Exchange Equilibration Buffer |
| Volume (CV) | 8 |
| **Post Load Wash 2** | |
| Buffer | Anion Exchange Wash Buffer |
| Volume (CV) | 2 |
| **Regeneration** | |
| Buffer | Cation Exchange Cleaning Buffer |
| Volume (CV) | 3 |

### A.1.5 Post Anion Exchange Ultrafiltration

Table A.7: Post Anion Exchange Chromatography UFDF Specifications

| **Parameter** | **Value** |
|---|---|
| Principle | Product concentration using cross-flow filtration. |

*Continued on next page...*

| **Parameter** *(continued)* | **Value** *(continued)* |
|---|---|
| Pore Size (kDa) | 30 |
| Final Concentration (g/L) | 25 |
| $\Omega_1$[2] | -59.217 |
| $\Omega_2$[2] | 253.33 |
| Max Step Time (h) | 6 |
| Expected Step Yield (%) | 99 |

### A.1.6 Cation Exchange Chromatography (Bind and Elute)

Table A.8: Cation Exchange Chromatography Specifications

| **Parameter** | **Value** |
|---|---|
| Principle | Bind and elute cation exchange chromatography. |
| Resin Type | Cation Exchange Resin |
| Max Resin Cycle Number | 100 |
| Packing Flow Rate (cm/h) | 200 |
| Operational Flow Rate (cm/h) | 140 |
| Expected Step Yield (%) | 88 |

Table A.9: Cation Exchange Chromatography Operation

| **Parameter** | **Value** |
|---|---|
| **Clean** | |
| Buffer | Cation Exchange Cleaning Buffer |
| Volume (CV) | 3 |
| Hold Time (h) | 1 |

*Continued on next page...*

[2] $\Omega_1$ and $\Omega_2$ are experimentally derived parameters to estimate flux at various concentrations.

| **Parameter** *(continued)* | **Value** *(continued)* |
|---|---|
| Frequency | Immediately before each cycle. |
| **Equilibration** | |
| Buffer | Cation Exchange Equilibration Buffer |
| Volume (CV) | 5 |
| **Load** | |
| Dynamic Binding Capacity (g/L) | 15 |
| Volume (CV) | Variable |
| **Post Load Wash 1** | |
| Buffer | Cation Exchange Equilibration Buffer |
| Volume (CV) | 2 |
| **Elution** | |
| Buffer | Cation Exchange Elution Buffer |
| Elution Buffer Volume (CV) | 5 |
| Volume Collected (CV) | 2.5 |
| **Strip** | |
| Buffer | Cation Exchange Strip Buffer |
| Volume (CV) | 2 |
| **Regeneration** | |
| Buffer | Cation Exchange Cleaning Buffer |
| Volume (CV) | 3 |

### A.1.7 Virus Reduction Filtration

Table A.10: Virus Reduction Filtration

| **Parameter** | **Value** |
|---|---|
| Principle | Nanofiltration used to remove virus particles. |
| Filter Type | 20nm Virus Reduction Filter |

*Continued on next page...*

| **Parameter** *(continued)* | **Value** *(continued)* |
|---|---|
| Flush Buffer | VRF Flush Buffer |
| Flush Volume (L/m$^2$) | 8 |
| V$_{max}$ (L/m$^2$) | 4778 |
| Flux Rate (LMH) | 48 |
| Max Processing Time (h) | 6 |
| Expected Step Yield (%) | 99 |

### A.1.8 Final UFDF

Table A.11: Final UFDF Specifications

| **Parameter** | **Value** |
|---|---|
| Principle | Buffer exchange and product concentration using cross-flow filtration. |
| Pore Size (kDa) | 30 |
| DF Concentration (g/L) | 35 |
| Final Concentration (g/L) | 38 |
| Diafiltraiton Volumes | 10 |
| Diafiltration Buffer | Final formulation buffer |
| $\Omega_1$[3] | -48.47 |
| $\Omega_2$[3] | 253.33 |
| Max Step Time (h) | 6 |
| Expected Step Yield (%) | 99 |

[3] $\Omega_1$ and $\Omega_2$ are experimentally derived parameters to estimate flux at various concentrations.

## A.2 Key Costs

Table A.12: Key Process Costs

| Item | Cost |
|---|---|
| **Chromatography Resins** | |
| Protein A | £8000/L |
| Anion Exchange | £800/L |
| Cation Exchange | £400/L |
| **Filters** | |
| 30kDa Membrane | £800/$m^2$ |
| Virus Reduction Filter | £1700/$m^2$ |
| **Disposable Bags** | |
| 500L Bag | £450 |
| 200L Bag | £300 |
| 100L Bag | £350 |
| 50L Bag | £50 |
| **Other** | |
| Labour | £30/h |

# Appendix B

# Multivariate Statistical Analysis

## B.1 Mathematica Code for PCA

The following code was used to implement the principal component method in Mathematica.

```
a = Import["PCAMatrixdataS.txt", "Table"]; (*Import data from text file into a*)
{u, d, v} = SingularValueDecomposition[a]; (*Perfom SVD on a, export to u, d, v*)
T = u.d; (*Mulitply matrix u by d*)
Wt = Transpose[v]; (*Transpose Matrix v*)
Scores = Transpose[T]; (*Transpose matrix T*)
Eigens = Eigenvalues[Transpose[a].a]; (*Determines Eigen values*)
NormEigens = Eigens/Mean[Eigens] (*Mean centre Eigenvalues*)
```

## Appendix C

# Publications by The Author

For reasons of copyright, papers previously included in this section have been removed. References to the original publishers are included below:

Adam Stonier, Martin Smith, Nick Hutchinson, Suzanne S. Farid. (2009) Dynamic Simulation Framework for Design of Lean Biopharmaceutical Manufacturing Operations. In: Jezowski, J and Thullie, T, (eds.) 19th European Symposium on Computer Aided Process Engineering. p.1069-1073 Elsevier Science BV

Adam Stonier, David Pain, Ashley Westlake, Nick Hutchinson, Nina F. Thornhill, Suzanne S. Farid, (2011) Integration of stochastic simulation with advanced multivariate and visualisation analyses for rapid prediction of facility fit issues in biopharmaceutical processes. In: Pistikopoulos, EN and Georgiadis, MC and Kokossis, AC, (eds.) 21ST European Symposium on Computer Aided Process Engineering. p.1356-1360 Elsevier Science BV

Adam Stonier, David Pain, Ashley Westlake, Nicholas Hutchinson, Nina F. Thornhill, Suzanne S. Farid. (2013) Integration of Stochastic Simulation with Multivariate Analysis: Short-Term Facility Fit Prediction. Biotechnol. Prog., 29(2) p.368-377

Adam Stonier, Ana Sofia Simaria, Martin Smith, Suzanne S. Farid (2012) Decisional Tool to Assess Current and Future Process Robustness in an Antibody Purification Facility. Biotechnol. Prog., 28(4) p.1019-1028

# Bibliography

Abu-Absi, S. F., Yang, L., Thompson, P., Jiang, C., Kandula, S., Schilling, B. and Shukla, A. A. (2010), Defining process design space for monoclonal antibody cell culture, *Biotechnology and Bioengineering* **106**(6), 894–905.

Aldington, S. and Bonnerjea, J. (2007), Scale-up of monoclonal antibody purification processes, *J.Chromatogr.B Analyt.Technol.Biomed.Life Sci.* **848**(1), 64–78.

Amanullah, A., Otero, J. M., Mikola, M., Hsu, A., Zhang, J., Aunins, J., Schreyer, H. B., Hope, J. A. and Russo, A. P. (2010), Novel micro-bioreactor high throughput technology for cell culture process development: Reproducibility and scalability assessment of fed-batch CHO cultures, *Biotechnology and Bioengineering* **106**(1), 57–67.

Arunakumari, A., Wang, J. and Ferreira, G. (2009), Advances in Non-Protein A Purification Processes for Human Monoclonal Antibodies, *Biopharm International* pp.22–26.

Bennett, S., McRobb, S. and Farmer, R. (2005), *Object Oriented Systems Analysis and Design Using UML*, Vol. 2nd Edition, McGraw Hill Higher Education.

Brorson, K., Krejci, S., Lee, K., Hamilton, E., Stein, K. and Xu, Y. (2003), Bracketed generic inactivation of rodent retroviruses by low pH treatment for monoclonal antibodies and recombinant proteins, *Biotechnol Bioeng* **82**(3), 321–9.

Carson, J. S. (1993), Modeling and simulation worldviews, *in Proceedings of the Winter Simulation Conference, 1993*, WSC '93, ACM, New York, NY, USA, pp.18–23.

Carson, J.S., I. (2005), Introduction to modeling and simulation, *in Proceedings of the Winter Simulation Conference, 2005*, p.8 pp.

Carter-Franklin, J. N., Victa, C., McDonald, P. and Fahrner, R. (2007), Fragments of protein A eluted during protein A affinity chromatography., *Journal of Chromatography A* **1163**(1-2), 105–111.

Cattell, R. B. (1966), The scree test for the number of factors., *Multivariate Behav. Res.* pp.245–276.

Center for Drug Evaluation and Research (2007), Critical Path Opportunities for Generic Drugs, Technical Report, Food and Drug Administration.

Chhatre, S., Francis, R., O'Donovan, K., Titchener-Hooker, N. J., Newcombe, A. R. and Keshavarz-Moore, E. (2007), A decision-support model for evaluating changes in biopharmaceutical manufacturing processes, *Bioprocess and Biosystems Engineering* **30**(1), 1–11.

Chhatre, S., Jones, C., Francis, R., O'Donovan, K., Titchener-Hooker, N., Newcombe, A. and Keshavarz-Moore, E. (2006), The integrated simulation and assessment of the impacts of process change in biotherapeutic antibody production, *Biotechnology Progress* **22**(6), 1612–1620.

Critchfield, G. C. and Willard, K. E. (1986), Probabilistic analysis of decision trees using Monte Carlo simulation, *Med.Decis.Making* **6**(2), 85–92.

Curry, J. (2006), APICS Seminar: Simulation Modelling In Lean Programs, *in 2006 APICS Intrenationional Conferences Proceedings*, The Educational Societ for Resource Management, APICS.

Curtis, S., Lee, K., Blank, G. S., Brorson, K. and Xu, Y. (2003), Generic/matrix evaluation of SV40 clearance by anion exchange chromatography in flow-through mode, *Biotechnol Bioeng* **84**(2), 179–86.

Davies, J., Smith, M. and Bonnerjea, J. (2009), The Influence of Scale of Operation on Purification Process Design.

Edwards-Parton S, Thornhill NF, B. D. L. J. T.-H. N. (2008), Principal component score modeling for the rapid description of chromatographic separations., *Biotechnol Prog* **24**, 202–208.

Fahrner, R. L., Knudsen, H. L., Basey, C. D., Galan, W., Feuerhelm, D., Vanderlaan, M. and Blank, G. S. (2001), Industrial purification of pharmaceutical antibodies: development, operation, and validation of chromatography processes, *Biotechnol Genet Eng Rev* **18**, 301–27.

Farid, S. S. (2009), Economic Drivers and Trade-Offs in Antibody Purification Processes, *Biopharm International* pp.37–42.

Farid, S. S., Novais, J. L., Karri, S., Washbrook, J. and Titchener-Hooker, N. J. (2000), A tool for modeling strategic decisions in cell culture manufacturing, *Biotechnol.Prog.* **16**(5), 829–836.

Farid, S. S., Washbrook, J. and Titchener-Hooker, N. J. (2005a), Combining multiple quantitative and qualitative goals when assessing biomanufacturing strategies under uncertainty, *Biotechnol.Prog.* **21**(4), 1183–1191.

Farid, S. S., Washbrook, J. and Titchener-Hooker, N. J. (2005b), Decision-support tool for assessing biomanufacturing strategies under uncertainty: stainless steel versus disposable equipment for clinical trial material preparation., *Biotechnol Prog* **21**(2), 486–497.

Farid, S. S., Washbrook, J. and Titchener-Hooker, N. J. (2007), Modelling biopharmaceutical manufacture: Design and implementation of SimBiopharma, *Computers and Chemical Engineering* **31**(9), 1141–1158.

George, E. D. and Farid, S. S. (2008), Strategic biopharmaceutical portfolio development: An analysis of constraint-induced implications, *Biotechnology Progress* **24**(3), 698–713.

Girot, P., Moroux, Y., Duteil, X. P., Nguyen, C. and Boschetti, E. (1990), Composite affinity sorbents and their cleaning in place, *J Chromatogr* **510**, 213–23.

Gosling, I. (2005), Process simulation and modeling for industrial bioprocessing: Tools and techniques, *Industrial Biotechnology* **1**(2), 106–109.

Groep, M. E., Gregory, M. E., Kershenbaum, L. S. and Bogle, I. D. (2000), Performance modeling and simulation of biochemical process sequences with interacting unit operations, *Biotechnol.Bioeng.* **67**(3), 300–311.

Gülich, S., Uhlén, M. and Hober, S. (2000), Protein engineering of an IgG-binding domain allows milder elution conditions during affinity chromatography, *J Biotechnol* **76**(2-3), 233–44.

Hahn, R., Shimahara, K., Steindl, F. and Jungbauer, A. (2006), Comparison of protein A affinity sorbents III. Life time study, *Journal of Chromatography A* **1102**(1-2), 224–231.

Hale, G., Drumm, A., Harrison, P. and Phillips, J. (1994), Repeated cleaning of protein A affinity column with sodium hydroxide, *J Immunol Methods* **171**(1), 15–21.

Helton, J. and Davis, F. (2003), Latin hypercube sampling and the propagation of uncertainty in analyses of complex systems, *Reliability Engineering  System Safety* **81**(1), 23 – 69.

Hober, S., Nord, K. and Linhult, M. (2007), Protein A chromatography for antibody purification, *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **848**(1), 40–47.

Hou Y, Jiang C, S. A. C. S. (2011), Improved process analytical technology for protein A chromatography using predictive principal component analysis tools., *Biotechnol Bioeng.* **108**, 59–68.

Hutchinson, N., Chhatre, S., Baldascini, H., Davies, J. L., Bracewell, D. G. and Hoare, M. (2009), Ultra scale-down approach to correct dispersive and retentive effects in small-scale columns when predicting larger scale elution profiles, *Biotechnology Progress* **25**(4), 1103–1110.

ICH (1999), *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*, Vol. ICH Q5A(R1), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Jackson, J. E. (1991), *A User's Guide to Principal Components*, Wiley-Interscience: New York.

Jagschies, G. (2008), Where is Biopharmaceutical Manufacturing Heading?, *Biopharm International* **21**(10), 72–75.

Joseph, J. R., Sinclair, A., Titchener-Hooker, N. J. and Zhou, Y. H. (2006), A framework for assessing the solutions in chromatographic process design and operation for large-scale manufacture, *Journal of Chemical Technology and Biotechnology* **81**(6), 1009–1020.

Kamarck, M. E. (2006), Building biomanufacturing capacity - the chapter and verse, *Nature Biotechnology* **24**(5), 503–505.

Kelley, B. (2007), Very large scale monoclonal antibody purification: The case for conventional unit operations, *Biotechnology Progress* **23**(5), 995–1008.

Kirdar AO, Chen G, W. J. R. A. (2010), Application of near infrared (NIR) spectroscopy for screening of raw materials used in the cell culture medium for the production of a recombi- nant therapeutic protein., *Biotechnol Prog.* **26**, 527–531.

Kirdar AO, Conner JS, B. J. R. A. (2007), Application of multivariate analysis toward biotech processes: case study of a cell culture unit operation., *Biotechnol Prog.* **23**, 61–67.

Krahl, D. (2002), The Extend simulation environment, *in Proceedings of the Winter Simulation Conference, 2002*, Vol. 1, pp.205 – 213 vol.1.

Larson TM, Davis J, L. H. C. J. (2003), Use of process data to assess chromatographic performance in production-scale protein purification columns., *Biotechnol Prog* **19**, 485–492.

Law, A. and Haider, S. (1989), Selecting Simulation Software For Manufacturing Applications, *in Proceedings of the Winter Simulation Conference, 1989*, pp.29 –32.

Lee HW, Christie A, L. J. Y. S. (2012), Estimation of raw material performance in mammalian cell culture using near infrared spec- tra combined with chemometrics approaches., *Biotechnol Prog.* **28**, 824–832.

Legmann, R., Schreyer, H. B., Combs, R. G., McCormick, E. L., Russo, A. P. and Rodgers, S. T. (2009), A predictive high-throughput scale-down model of monoclonal antibody production in CHO cells, *Biotechnology and Bioengineering* **104**, 1107–1120.

Lim, A. C., Washbrook, J., Titchener-Hooker, N. J. and Farid, S. S. (2006), A computer-aided approach to compare the production economics of fed-batch and perfusion culture under uncertainty, *Biotechnology and Bioengineering* **93**(4), 687–697.

Lim, A. C., Zhou, Y. H., Washbrook, J., Titchener-Hooker, N. J. and Farid, S. (2004), A decisional-support tool to model the impact of regulatory compliance activities in the biomanufacturing industry, *Computers and Chemical Engineering* **28**(5), 727–735.

Lim, A. C., Zhou, Y., Washbrook, J., Sinclair, A., Fish, B., Francis, R., Titchener-Hooker, N. J. and Farid, S. S. (2005), Application of a decision-support tool to assess pooling strategies in perfusion culture processes under uncertainty, *Biotechnol.Prog.* **21**(4), 1231–1242.

Low, D., O'Leary, R. and Pujar, N. S. (2007), Future of antibody purification, *J.Chromatogr.B Analyt.Technol.Biomed.Life Sci.* **848**(1), 48–63.

Malmquist G, D. R. (1994), Alignment of chromatographic profiles for principal component analysis - A prerequisite for fingerprinting methods., *J Chromatogr A.* **687**, 71–88.

Novais, J. L., Titchener-Hooker, N. J. and Hoare, M. (2001), Economic comparison between conventional and disposables-based technology for the production of bio-pharmaceuticals, *Biotechnology and Bioengineering* **75**(2), 143–153.

Pandey, M. (2003), Investment decisions in pharmaceutical R and D projects, *Drug Discovery Today* **8**(21), 968–971.

Pate ME, Turner MK, T. N. T.-H. N. (2004), Principal component analysis of nonlinear chromatography., *Biotechnol Prog.* **20**, 215–222.

Pollock, J., Ho, S. V. and Farid, S. S. (2013), Fed-batch and perfusion culture processes: economic, environmental, and operational feasibility under uncertainty, *Biotechnol Bioeng* **110**(1), 206–19.

Pollock J, Ho SV, F. S. (2013), Fed-batch and perfusion culture processes: operational, economic and environmental feasibility under uncertainty., *Biotechnol Bioeng.* **110**, 206–219.

Rajapakse, A., Titchener-Hooker, N. J. and Farid, S. S. (2005), Modelling of the biopharmaceutical drug development pathway and portfolio management, *Computers and Chemical Engineering* **29**(6), 1357–1368.

Rosenberg, M. (2000), Development costs and process economics of recombinant proteins produced in E.coli, mammalian cell culture and natural product derived products., San Deago, CA.

Rosencrance, S. (2011), QbD Status Update Generic Drugs, GPhA Fall Technical Conference, Food and Drug Administration, Office of Generic Drugs.

Rozett, R. W.; Petersen, E. M. (1975), Methods of factor analysis of mass spectra., *Anal. Chem.* **47**(8), 1301–1308.

Rummel, R. J. (1970), *Applied Factor Analysis*, Northwestern University Press, Evanston.

Shanklin, T., Roper, K., Yegneswaran, P. K. and Marten, M. R. (2001), Selection of bioprocess simulation software for industrial applications, *Biotechnol.Bioeng.* **72**(4), 483–489.

Shukla, A. A., Hubbard, B., Tressel, T., Guhan, S. and Low, D. (2007), Downstream processing of monoclonal antibodies–application of platform approaches, *J.Chromatogr.B Analyt.Technol.Biomed.Life Sci.* **848**(1), 28–39.

Sin G, Gernaey KV, E. L. A. (2009), Good modelling practice (GMoP) for PAT applications: Propagation of input uncertainty and sensitivity analysis., *Biotechnol Prog* **25**, 1043–1053.

Sinclair, A. and Monge, M. (2002), Quantitative Economic Evaluation of Single-Use Disposables in Bioprocessing, *Pharmaceutical Engineering* **2**(3).

Sinclair, A. and Monge, M. (2005), Biomanufacturing for the 21st Century: Designing a Concept Facility Based on Single-Use Systems, **2**(9), s26–s31.

Sinnot, R. K. (1993), *Coulson and Richardson's Chemical Engineering: Chemical Engineering Design*, Pergamon Press.

Sommerfeld, S. and Strube, J. (2005), Challenges in biotechnology production - generic processes and process optimization for monoclonal antibodies, *Chemical Engineering and Processing* **44**(10), 1123–1137.

Stewart, J. J., Allison, P. N. and Johnson, R. S. (2001), Putting a price on biotechnology, *Nat.Biotechnol.* **19**(9), 813–817.

Teeters, M., Bezila, D., Benner, T., Alfonso, P. and Alred, P. (2011), Predicting diafiltration solution compositions for final ultrafiltration/diafiltration steps of monoclonal antibodies, *Biotechnol Bioeng* **108**(6), 1338–46.

Thomassen YE, van Sprang ENM, v. d. P. L. B. W. (2010), Multivariate data analysis on historical IPV production data for better process understanding and future improvements., *Biotechnol Bioeng.* **107**, 96–104.

Thornhill NF, Melbï H, W. J. (2006), Multi-dimensional visualization and clustering of historical process data., *Ind Eng Chem Res.* **45**, 5971–5985.

Tufts (2005), Tufts CSDD Outlook Report 2005, Technical Report, Tufts Center for the Study of Drug Development.

Tugcu, N., Roush, D. J. and Goklen, K. E. (2008), Maximizing productivity of chromatography steps for purification of monoclonal antibodies, *Biotechnol.Bioeng.* **99**(3), 599–613.

Valle, S., Li, W. and Qin, J. S. (1999), Selection of the Number of Principal Components: The Variance of the Reconstruction Error Criterion with a Comparison to Other Methods, *Ind. Eng. Chem. Res.* **38**, 4389–4401.

Varga, E. G., Titchener-Hooker, N. J. and Dunnill, P. (2001), Prediction of the pilot-scale recovery of a recombinant yeast enzyme using integrated models, *Biotechnol.Bioeng.* **74**(2), 96–107.

Vunnum, S., Vedantham, G. and Hubbard, B. (2008), *Protein A-Based Affinity Chromatography*, John Wiley and Sons, Inc., Chapter 4, pp.79–102.

Wai, P. P. C., Bogle, I. D. L., Bagherpour, K. and Gani, R. (1996), Process synthesis and simulation strategies for integrated biochemical process design, *Computers and Chemical Engineering* **20**, S357–S362.

Wang, W. (2005), Protein aggregation and its inhibition in biopharmaceutics, *International Journal of Pharmaceutics* **289**(1-2), 1 – 30.

Wang, X. Z., Medasani, S., Marhoon, F. and Albazzaz, H. (2004), Multidimensional Visualization of Principal Component Scores for Process Historical Data Analysis, *Industrial Engineering Chemistry Research* **43**(22), 7036–7048.

Wrankmore, M. (2005), Applications of Disposable Technologies in Biopharmaceutical Manufacturing, *in BMD Summit, Disposables for Biopharm Production*.

Zabriskie, D. W. and Sofer, G. (2000), Regulatory Trends Related to Process Validation, *in Biopharmaceutical Process Validation*, Vol. 1, Marcel Dekker, Inc., New York, pp.1–16.

Zhou, J. X., Solamo, F., Hong, T., Shearer, M. and Tressel, T. (2008), Viral clearance using disposable systems in monoclonal antibody commercial downstream processing, *Biotechnol Bioeng* **100**(3), 488–96.

Zwick, W. R.; Velicer, W. F. (1986), Comparison of five rules for determining the number of components to retain., *Psychol. Bull.* **99**(3), 432–442.