A Structured Analysis of the Novel Technologies available to Alleviate Bottlenecks in Biologics Manufacturing.

By

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This project is submitted in part fulfilment of the HETAC requirements for the award of Master of Science in Biopharmaceutical Science.

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Declaration Page

Title: A Structured Analysis of the Novel Technologies used to alleviate bottlenecks in Biopharmaceutical Manufacturing

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Declaration:

"I hereby declare that this project is entirely my own work and that it has not been submitted for any other academic award, or part thereof, at this or any other education establishment".

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Abstract:

Biopharmaceutical companies are under increasing pressure to meet the market demand from patients. Despite a high growth sector within the life sciences sector, the same types of manufacturing methods have been employed since the first approval of biologic in the early 1980's. The bottleneck in biologics processing was originally in the upstream are of manufacturing, because of the low titres achievable in the early biologics cell cultures. However due to advancements in genetic engineering, the improvements in media formulations for cell culture and the introduction of single use technology, as a platform for bioreactors, the bottleneck has swung towards downstream processing. Purification technologies have not evolved at a rate as quickly as upstream technologies and it is now difficult to setup downstream suites in the conventional way, to accept the levels of titres seen in upstream processing. However some novel downstream technologies are emerging to address these concerns, despite the hesitancy from the biopharma industry to engage with them. The following thesis attempts to determine the root causes of the bottle neck shifting to downstream processing, the reasons for the hesitancy to move away from the conventional methods, as well as an analysis of some of the novel downstream technologies available to biologics manufacturing. Furthermore a detailed overview is presented on buffer preparation and the use of In-Line Conditioning Technology as an alternative, to alleviate this bottle neck.

Key Words: Downstream Purification, In-line Conditioning, Single Use Technology, Continuous processing, Bottle Necks, Cell Titres.

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1. Chapter 1: Introduction

A biopharmaceutical drug is a pharmaceutical product that is manufactured using live organisms and has an active ingredient that is biological in nature. There is an increasing demand for biopharmaceutical drugs, as a result of their efficacy and safety in use in patients, compared to traditionally manufactured chemical "small molecule" compounds. The world market value for Biopharmaceuticals is now greater than $200 Billion, which is growing at a rate of approximately 15% annually, forecasted to reach $305 Billion by 2020.

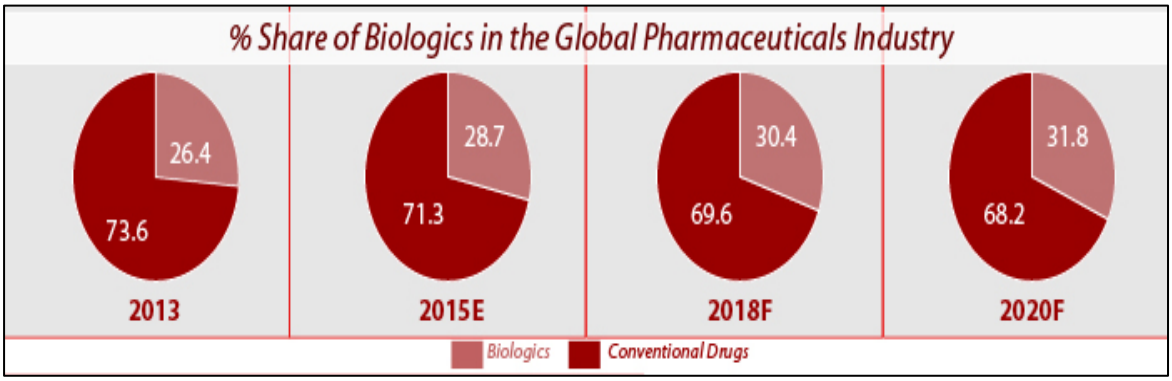

Figure 1 Percentage Share of Biologics in the Global Pharmaceuticals Industry (Global Industry Analysts Inc. Biopharmaceuticals Global strategic business report).

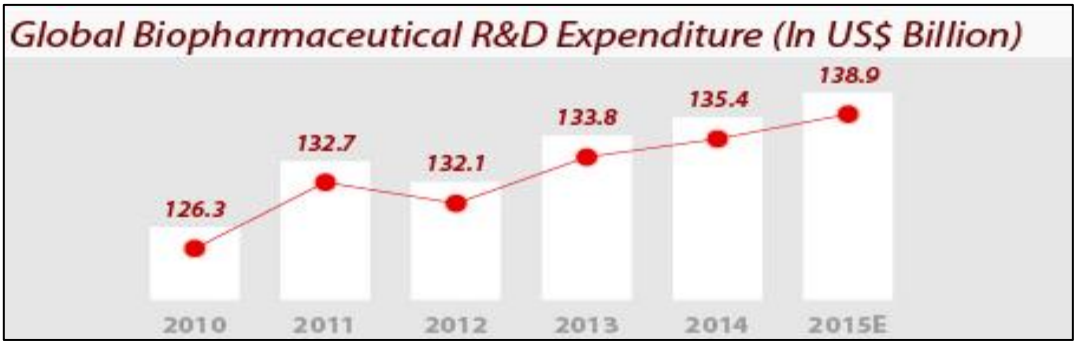

Figure 2 Global Industry Analysts Inc. Biopharmaceuticals Global strategic business report.

With this growth rate, the share of biologics in the global pharmaceuticals market is increasing yearly and is forecast to exceed 30% of the market by 2020 (figure 1). Furthermore the expenditure on research and development has increased year on year since 2010.

The industry is in a state of flux currently. A number of factors are contributing to the intensity, in the demand for bio-therapeutic drugs. These factors are subsequently

contributing to the ever increasing pressure, faced by manufacturers, to increase the speed of delivery of products to market, while at the same time maintaining an interrupted supply chain of drugs of sufficient efficacy.

1.1. Path to Regulatory Approval:

The approval process for a biologic drug is a long and expensive process. It can cost approximately up to $2 billion dollars to bring a new drug through the full development process. The steps from preclinical studies, through to approval by a regulatory body, takes anything up to 20 years. Despite the numerous scientific, technological and operational advances in research and development, a significant number of clinical trials still fail to produce new, effective and safe medicines. The overall succession rate for a drug candidate to succeed from phase I clinical trials, through to achieving regulatory approval is 9.6%. The chart below illustrated the success rate from phase I, II and III clinical trials, as well as the overall likelihood of approval for all four modalities.

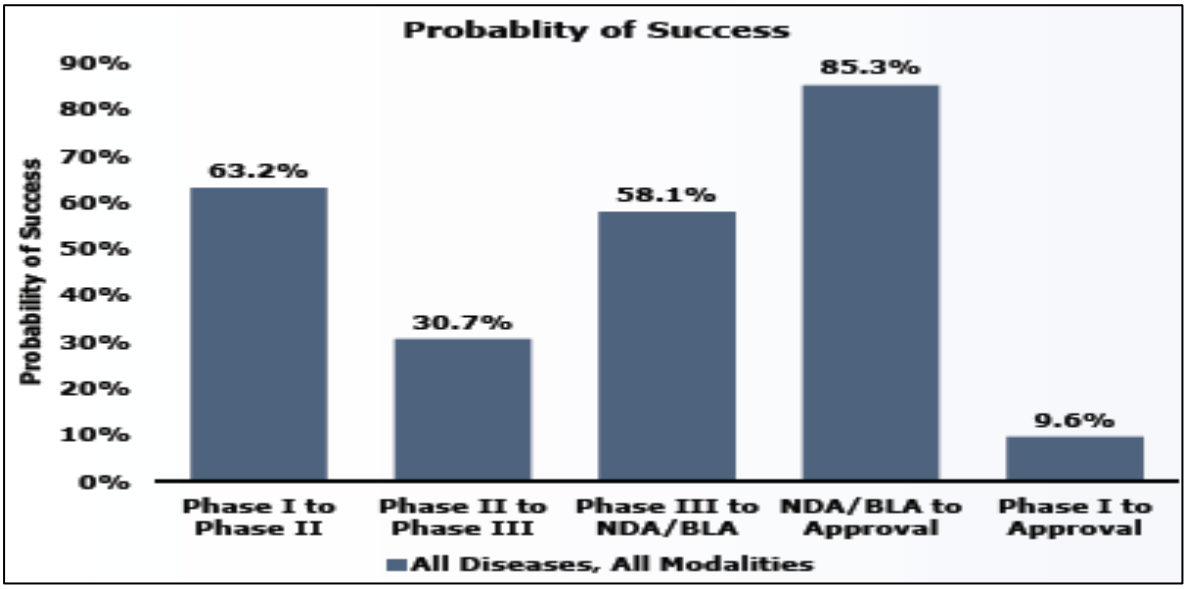

Figure 3 Clinical Development Success Rates 2006-2015 (Biotechnology Innovation Organisation)

Besides the loss of revenue resulting from being unable to manufacture and sell the planned drug, there are many other consequences to a biologic failing late clinical trials. These may include a drop in share price, termination of jobs in all sectors including R&D and manufacturing, and more importantly the impact to patient life.

1.2. Biosimilars:

Additional complexity and challenges arise due to the expiration of patents and the emergence of “biosimilars” results in increased pressures on bio pharmaceutical

manufacturing companies. The current allowance for patent lifetime is twenty years. On expiration of a patent, rival companies are free to a generic of a reference biopharmaceutical drug once the patient is not infringed on. A biosimlar is a biopharmaceutical drug that is highly similar in molecular make up to an existing biologics drug; however it will have the same efficacy in treatment of disease as the existing approved biological drug. A biosimilar is a biologic medicine that is approved based its similarity to an existing approved innovative biological product, known as a reference product. However, unlike generic medicines in which the active ingredients are identical to the reference small–molecule drugs, biosimilars will not be identical to the reference biologics due to several components, including the inherent complexity of biologics and the proprietary details of the reference product. Biosimilars made by different manufacturers will differ from the reference product and from each other, making each biosimilar a unique therapeutic option for patients.The FDA along with the European medicines board have established pathways for companies to develop biologic drugs, without infringing on the existing patents of the original biologic. Again the introduction of biosimilars is also adding to the pressures, on manufacturing, to maximise on the time afforded to them to produce the reference product and show a return on its investment, before patent expiry and potential replacement with a biologic. Given the complexity and cost of development and manufacturing, biosimilars are expected to result in more affordable therapeutic options but are not expected to generate the same level of cost savings as generics. A biosimilar will cost $100 to $200 million to bring to market and take eight to ten years to develop. The world's best-selling drug is a biologic produced by Abbvie, its marketed trade name is "Humira". Its global sales for 2015 amounted to $14 billion with expected increases up to $20 billion by 2020. However the patent protecting the drug expired in December 2016. With competitors such as Allergan, Amgen and others, ready to release biosimilars to tap into this market, some analysts are predicting a drop in Abbvie revenues from $20 billion to $6 billion by 2022. At the time of writing there have been seven biosimilars approved by the FDA and thirteen biosimilars approved by the European Medicines Agency (EMA).

Furthermore there is increasing pressure on pharmaceutical corporations to reduce the list price of drugs for patients. Much media attention has been given to the substantial costs of some courses of treatments using biotherapeutic drugs. An example being BioMarin Pharmaceuticals who recently have been given approval by the FDA for treatment of an ultra-rare paediatric brain disorder, a form of battens disease. A biweekly infusion of the drug will

cost $27,000, over the course of a year, this amounts to $702,000. This is the extreme case, for a drug that treats rare brain disorders but doesn't take away from the fact that to treat certain diseases, prices to the patient are now approaching the one million dollar mark.

The above contributing factors, vis a vis, pathway to regulatory approval, patent expiry, introduction of biologics as well as the peer pressure pharmaceutical companies are experiencing from governments over costs to patients, all contributes to the pressures faced by manufacturing divisions, to provide an interrupted supply of efficacious biopharmaceutical drugs to the markets.

Inevitably a reaction is coming from pharmaceutical companies, to combat the above contributing factors to pressure, by investing in systems to alleviate stresses and bottle necks currently experienced in manufacturing. There is an increasing trend towards an "operational excellence" approach, rather than employing operating regimes associated with historical blockbuster-orientated models, for example focusing on building capacity. Operational excellence includes embracing novel technologies in manufacturing. The likes of the automotive industry, were the first to pioneer this approach, introducing both human and automation solutions to reduce error in manufacturing. The "Toyota Production system" is an example of one such model, which aims to eliminate the seven "MUDA" wastes defined in industry – over production, waiting, transporting, Inappropriate processing, unnecessary inventory, excess Motion and defects. The pharmaceutical industry in comparison has been slow to implement such systems.

The aim of this thesis is to discuss the constraints experienced in manufacturing at present, as well as investigating the technologies being invested in, by some of the major biopharmaceutical players to alleviate such bottle necks. Particularly the thesis will focus on the use of in-line conditioning technology, for preparation of buffers to support downstream purification processing.

2. Chapter 2: Literature Review

2.1. Biopharmaceutical Manufacturing Overview:

A typical Biopharmaceutical process to produce a biologics product will be compromised of upstream, harvesting and downstream unit operations, contained within a sterile cGMP facility. In general a facility can be classified into a number of areas as per table 1 and illustrated in figure 4.

Table 1 General Process steps in production of biopharmaceutical products.

Process Step	Description	Equipment	Desired Outcome
Cell Culture	Generate cells to produce desired protein	Bioreactor	Critical mass of protein producing cells with target protein concentration
Harvest	Separate protein from cell matter	Centrifuge/Depth filter	Proteins separated from cell matter
Purification	Separate protein From other impurities	Chromatography Columns / Filtration skids.	Purified protein in solution
Formulation	Add compounds to stabilize and adjust potency	Compounding Vessel Bulk Drug Substance Fill	Potent and effective biopharmaceutical
Fill/Finish	Segregate into separate doses (freeze if necessary)	Filling machine, freeze dryer	Properly dosed and frozen vials

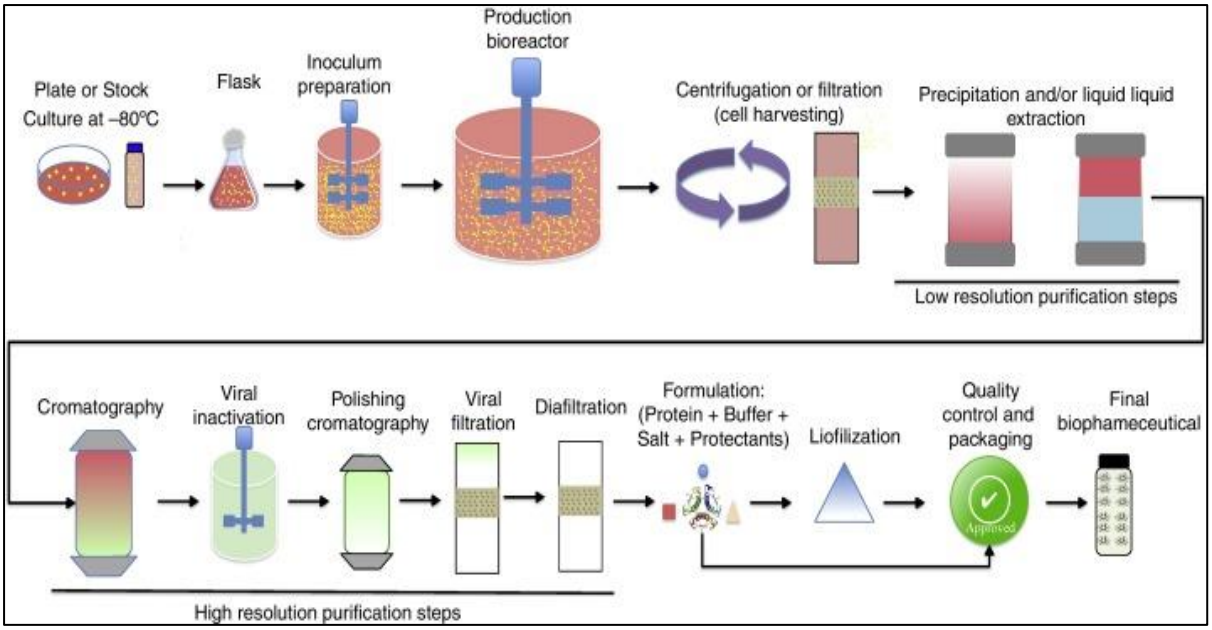

Figure 4 Biopharmaceutical manufacturing flowchart (Jozala, et al., 2016)

Upstream typically encompasses a train of seed bioreactors in series, that have been inoculated with a vial of cell culture from a cell bank. This allows growth of cells in a culture medium. These seed reactors will vary in volumes from twenty to several hundred litres, feeding production reactors which may be up to sixty thousand litres total in volume as can be seen in fermentation processes. Once the cell growth is complete the cell culture will be transferred to a harvesting unit and then onto various downstream processing units, for purification and final formulation of the bulk product. Additional services are required for both upstream and downstream operations such as cell culture media, buffering agents, ultra-pure water such as water for injection and various utilities required for servicing the units. These facilities were traditionally built with multiple stainless steel vessels, interconnected with complicated fixed piping systems for delivery of the services mentioned above. However the use of single use technology or a hybrid mixture of single use and stainless steel systems, are now being used in combination to manufacture biopharmaceutical drugs (Eibl R, 2010).

2.2. Upstream Process Developments:

The following sections will discuss the advancements that have been made in upstream manufacturing. This is in reaction to the demand for biologics products and the

pressures faced by manufacturing divisions to produce an interrupted supply of product to market.

2.2.1. Cell Titre Improvements and the downstream bottle neck:

One of the most significant developments in biopharmaceutical processing in recent years has been the dramatic increase in the number of cells grown in a culture medium, particularly mammalian cell cultures for monoclonal antibody expression (mAb's). Titre is the amount of an expressed agent, generally a protein in aqueous solution relative to the volume of total upstream produced broth containing the agent of interest (Rader & Langer, 2015). Titre is measured in grams of product per litre of cell culture produced, grams per litre. However titre is not the same as product yield. Yield in terms of bioprocessing can be defined as the amount of protein recovered from a bioreactor, after the various downstream harvesting and purification steps have been completed (Yusuf Christi, 1994).

When commercial scale biopharmaceutical manufacturing began in the middle of the 1980's, the average titre started out at 0.5 g/L. Now however the current average reported titre is averaging at greater than 3g/L, with top end values reaching 7 & 10g/L, while average yields are being reported at 70% (Rader & Langer, 2015). This is due to advances in culture media and its optimization, in expression systems, genetic engineering and in cell line development. The advances in process development have come in the genetic engineering and modification of cell lines, rather than the equipment. Therefore for a bioreactor or fermenter that were originally designed with a fix capacity of 10 to 15 thousand litres, containing a cell density of 0.5 to 3 g/l of product per batch, is being replaced by titres of 3 to 7 g/l. Thus these new regularly achievable titres and yields will challenge new manufacturing facilities to embrace emerging technologies, while forcing existing legacy facilities producing blockbuster drugs, to adapt in order to remain competitive at commercial scale manufacturing. Increasing titre puts a strain on the ability of equipment downstream of bioreactors and fermenters, to process the same volume of fluid with an increased cell density (Gronemeyer, et al., 2014). Downstream equipment has been still designed with the intent of lower cell titres of the previously mentioned 0.5 to 3 g/l range, resulting in this equipment reaching is physical limits. Usually processing time, material consumption and costs associated with these have to increase. This results in downstream manufacturing becoming the "bottle neck" in the production train for some biopharmaceutical processes. However with increasing cell titre we will have an increased impurity spectrum associated with the higher levels of titre obtained from the cell culture. (Kelley, 2009).

2.2.2. Cell Culture Media Advancements:

A contributory factor to the improving titre has been the advancement and optimisation of media supplements for cell cultures. The growth in the biotechnology industry in recent years has driven efforts for improving product titre, as well as lowering the cost of media especially for commercial manufacturing of biologics. Media is essentially a nutrient rich fluid, used to supply cell cultures with the ingredients for growth such as a carbon source, nitrogen source, buffers, vitamins amongst other components (Gunter Jagschies, 2017). Different types of cultures (bacterial, mammalian, insect) will require different ingredients in the media supplements to promote cell growth (Fike, 2009).

Early media products were developed using blood serum products. These were efficacious as they provided all the components for many types of cell lines, relying on the serum, to provide the non-nutritive culture promoting potential (Keen & Rapson, 1995). Classic formulations would have included basal medium eagle (BME), ham's nutrient mixture and Leibovitz's medium (Gunter Jagschies, 2017). However these were of concern due to introducing contaminants to the process in the form of viruses and prions (Jerums & Yang, 2005). Therefore efforts were made to substitute these serum based media with chemically defined media formulations, to satisfy quality and regulatory concerns within the industry. However chemically defined media can be a laborious process for cell lines particularly Chinese hamster ovary cultures. However process developers have taken advantage of design of experiment (DoE) methods, in which a minimum number of experiments are conducted. By varying the parameters within these experiments under strictly controlled conditions, the results of the experiments are used to an optimise media strategy for cell culture (Pacis, et al., 2010). At the early stage of drug development at lab and pilot scale, these screening techniques have been used to increase cell metabolism, contributing to the increases in cell titre as discussed previously (Singh, et al., 2016).

2.2.3. Embracing Single Use Technology:

Traditionally biopharmaceutical plants, normally relied on inflexible stainless steel piping systems and tanks in processing their systems for media, buffer, product and harvesting. However there is an increasing trend towards the adoption of single use technologies in industry (Abhinav A. Shukla, 2013). As stated in the previous sections, the demand for biologic products is forcing biopharma companies to adapt a lean approach to manufacturing, including engaging with disposable technology suppliers.

Disposable technologies offer many advantages over using conventional stainless steel systems. Since the manufacture of drugs, including biologics is so tightly controlled by GMP guidelines to minimise the risk of bioburden, a key component of this is the cleaning requirements required between batches. The use of disposable technology removes any requirements for Clean in Place (CIP) or Steam in Place (SIP) requirements, which cause much downtime problems (Abhinav A. Shukla, 2013) as well as cross contamination concerns (T. Kapp, 2010). Also a tremendous qualification effort is required when dealing with cleaning and sterilisation validation of a single or multi product facility. Engaging with single use technology will remove the commissioning and qualification effort for CIP and SIP (Schmidt, 2016). Furthermore the removal of cleaning and sterilisation requirements, also remove the engineering and utility requirements typically associated with these systems. The footprint of the facility becomes much smaller, thus the capital that is required to be generated is not as significant as a stainless processing plant (Jacquemart, et al., 2016). Another benefit is that the start-up of a plant, incorporating disposable technology becomes shorter, making it an attractive proposition for companies (Langer, 2015).

The main beneficiaries in technology have been in the upstream areas of manufacturing. Many large multinational life science companies such as GE health care, Pall Corporation, Thermo Scientific, Sartorius Stedim and Merck Millipore, have pioneered studies detailing the advantages of single use technology. As a result the outputs of these studies have contributed to new developments and have ended up in a diverse range of disposable bioreactor types with different design, instrumentation, power inputs and scale of the cultivation containers (Eibl R, 2010). The fundamental principles for reaction engineering still remain and disposable technology must replicate the scale up methodologies, from lab scale to production scale, including mass and heat transfer as well as excellent mixing capacity.

The WAVE bioreactor was the first unit to break ground on the application of single use technology. A WAVE bioreactor contains a bag with cell culture mounted on a tray that can be continuously rocked creating a wave motion in the liquid which provides mass transfer and mixing. The rate of rocking and fill ratio of the bag with media and air can influence efficiency (Shukla & Gottschalk, 2013). They are suitable for a variety of cell lines and are usually used as a seed expansion unit in a seed train of bioreactors (Haldankar, 2006) up to 500 litres. GE healthcare are a prominent supplier of wave bioreactors with a vast portfolio of different types with a maximum volume of 1000 litres (GE Healthcare Life Sciences, 2015).

As stated above, the most common operation for cell culturing in large scale biopharmaceutical plants is the use of stirred tank bioreactors. For disposable technology to be taken forward in this area a method had to be conceptualised to allow a disposable item to be incorporated into a larger scale application. Thus plastic bags were designed that could be mounted on a cylindrical frame to support the bag. Initial challenges came in the form of oxygen transfer, mixing and obtaining worthy "kLa" (mass transfer) values (J. Smelko, 2011). However these have now been addressed and appear to match those obtained in stainless steel systems (A. Shukla, 2012). A number of companies offer these standalone stirred tank bioreactors each of which have slight operational differences on the other.

GE Lifesciences offer single-use bioreactor systems from 50 litres up to 2000 litres. These can be operated in batch, fed batch and perfusion mode with the addition of filtration recovery pumps within GE automation unicorn platform. (GE Lifesciences, 2017). Merck Millipore offer a "Mobius" stand alone single use stirred bioreactor. These are extremely flexible and modern with features such as magnetic stirrers, flexware bag films which hold the cell culture and an ability to connect with the facility's automation platform. A drawer is incorporated for easy installation and removal of single use assemblies. They range from 3 litres to 2000 litres. (Merck Millipore, 2017). Sartorius Stedim offer single use bioreactors with working volumes from 50L to 2000L and is based on conventional stirred-tank design. It is incorporates a platform around the vessel which allows easy installation of the bag. (Sartorius Stedim, 2017). ABEC Technologies offer "custom single run" (CSR) systems. They are similar in design to the other bioreactors on the market however with one clear difference. ABEC now offer a working volume of up to 4000 litres, which is twice the size of any of the competitor brands on the market at the time of writing (ABEC Technologies, 2017).

Never the less there is an appeal to moving to single use technology due to the perceived cost savings made in building and operating plants with such technology. To ascertain whether single use equipment has an overall cost benefit compared to the conventional methods, of using stainless steel equipment or a hybrid of disposables and stainless, an analysis comparing the use of these two technologies should be undertaken. Such a comparison has been completed by where they compare the use of disposables and stainless steel systems, in the production of an antibody fragment, using a recombinant E. coli fermentation process (Novais, et al., 2001). Both operational costs and capital costs were analysed, using a net present value (NPV) analysis. A positive NPV indicates that the

projected earnings generated by a project or investment exceed the anticipated costs. Generally, an investment with a positive NPV will be a profitable one and one with a negative NPV will result in a net loss. The analysis showed a positive NPV for the use of disposables with a 25% difference compared to a conventional option. The values were determined to be €76 million for disposables and $103 million for the conventional option (Novais, et al., 2001). However it was noted that in some instances it is not possible to predict the direct difference in the performance of some unit operations (for example the yield obtained in a conventional fermenter vs a disposable bioreactor) and the effect that this may have on NPV, which could be significant in deciding on choosing a disposable or conventional processing facility (Novais, et al., 2001).

Levine et al. also present an economic model, in the analysis of a typical monoclonal antibody production process, comparing conventional reusable stainless steel equipment and deposable single use technology (Levine, et al., 2013). A cost of goods (CoG) model was used which analyses the direct costs attributable to the production of the goods sold in a company. This amount includes the cost of the materials used in creating the goods, along with the direct labour costs used to produce the goods. Levine findings are similar to Novais in that there are noteworthy savings to be made in using single use systems, particularly in the upstream area of processing. There is a significant saving in material costs, due to the fact that there is no requirement for cleaning and sterilisation in single use equipment compared to the conventional equipment. The volumes of purified water required to make up cleaning solutions, as well as the cleaning detergents and acids than accompany these runs result in significant savings in costs. Also the high energy costs associated with generating clean steam using WFI as well as black utilities such as plant steam, contribute to the higher CoG percentage (Levine, et al., 2013). Furthermore Levine is in agreement with Novais in that the labour costs are significantly lower specifically in quality assurance and quality control departments, as the removal of cleaning and sterilisation activities afore mentioned, also removes the qualification and continuous monitoring of these (Levine, et al., 2013). Both processes were analysed assuming the same number of batches per year, with an operating cost in favour of disposables with a net savings of $5.32 million. However Levine argues due to the shorter turnaround time of using disposable bioreactors, due to not requiring cleaning and sterilisation, the amount of batches can be increased, thus lowering the CoG to $170/g of Mab compared to $225/g for the conventional options (Levine, et al., 2013).

Nevertheless there is still a reluctance, to convert fully to disposable technology. There has been more engagement embracing upstream single use technologies compared to the downstream counterparts. There is still some issues to overcome for disposables such as their limited scale, lack of diversity to expand beyond current achievements due to a lack of competition, the lack of standardisation and performance issues (Langer, 2012). There needs to be more diversification and competition in the market to reduce the costs of single use equipment and this making them a more attractive technology. There are hundreds of suppliers of conventional bioprocessing equipment, compared to only a few dozen disposable technology vendors (Langer, 2012). Furthermore as noted above only one manufacturer (ABEC Technologies) offer custom designed systems, for specific working volumes and this working volume is capped at 4000 litres. Single use bioreactor manufacturers still haven't discovered technology, that can provide the same stability requirements in welding, that will allow them to break the 4000 L ceiling and offer volumes similar to stainless steel bioreactor systems, that have recorded working volumes greater than 60,000 litres (Lopes, 2015).

Finally, the lack of diversity in supplier and working volume barriers are not the only concern for turning to single use technology, but the possibility for leachables and extractables to be introduced into the process. Biopharmaceutical processing materials must be evaluated to determine whether they impact the final drug product with regard to safety and efficacy (Bestwick & Colton, 2009). The definition of leachables and extractables are as follows;

"Extractables: Chemical compounds that migrate from any product contact material when exposed to an appropriate solvent under exaggerated conditions of time and temperature."

"Leachables: Chemical compounds, typically a subset of extractables, that migrate into the drug formulation from any product contact material, — including elastomeric, plastic, glass, stainless steel or coating components — as a result of direct contact with the drug formulation under normal process conditions or accelerated storage conditions and are found in the final drug product."

Leachables and extractables have the potential to alter the chemical composition of a final drug thus affecting its purity and efficacy as well as causing serious safety concerns for the patients who use the drug. Therefore as required by the regulatory bodies (FDA, EMA etc.) a significant body of data is required to justify that no risk is posed by the use of disposables for the production of biopharmaceutical products. The US Pharmacopeia

Convention (USP) has adopted a General Information chapter, <1663>, addressing the testing to consider when plastic containers are used in pharmaceutical processing. The Bio-Process Systems Alliance (BPSCA) has also created a risk based approach model as seen below in figure 5, to determine if there exist risks posed by extractables and leachables from a disposable system.

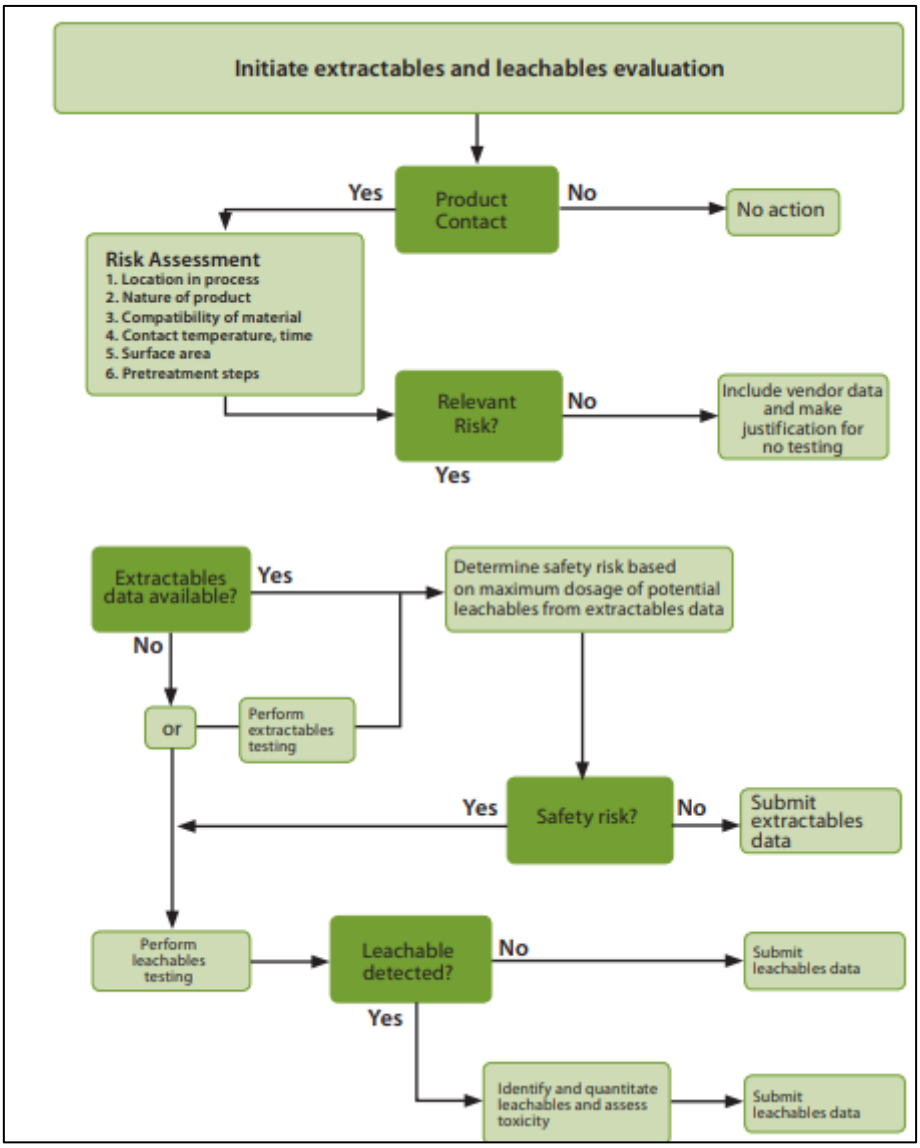

Figure 5 Risk based approach to an extractables/leachables evaluation (BPSCA).

The cost of extractables and leachables testing should be considered when deciding whether to use a disposables manufacturing platform and the risk assessment process, associated with this should also begin as early as possible, in the process development stage, as it is easiest to make changes at this stage (Bestwick & Colton, 2009).

2.3. Other Innovations:

There have been some other innovations made in biopharma industry in addition to the advancements in upstream developments discussed previously.

2.3.1. Continuous Bioprocessing

The concept of continuous processing has existed in the manufacturing industry for more than 60 years. Originally oil and the fine chemicals industry ran in a batch mode, where raw materials were charged into a vessel and reacted to produce their product of interest. Nevertheless the fine chemicals industry managed to move to a continuous processing model through by being ambitious and engaging with technology, for increased business gains (Malhotra, 2015). However there is hesitancy from biopharma companies, to change from a batch model to a continuous processing model. The regulatory submissions make this difficult as once a process is submitted for approval and successfully qualified, it is very difficult to justify moving away from this process, both to regulators and company management (Munk, 2015).

A continuous process by definition is one which operates twenty four hours per day, seven days per week and 50 weeks of the year, with two weeks of downtime for staff vacation. Therefore the facility would be operating for 8,400 hours, per year. Industry generally takes 15% downtime for planned maintenance and unscheduled shutdowns. This means 7,140 hours being available to produce a single product per year. These are the minimum operational hours required by a facility to be classed as a continuous process (Malhotra, 2015).

A small number of biopharma companies are slowly beginning to engage with the continuous processing model, as a result of a number of influencing factors as discussed earlier in the introduction section. These include an increase globally in market demand, pressures in delivery of product to market, competition from expiring patents and pressure to reduce manufacturing costs (Munk, 2015). Biopharma companies at an early stage in their pipeline for drug candidates, have the best opportunity to determine whether a continuous bioprocessing model may suit their process. If a process is suitable for continuous processing model then many advantages exist in availing of this model including operational flexibility, product quality and cost (Konstantinov & Cooney, 2015). A continuous bioprocess would include a continuous (perfusion) bioreactor (and cell recycle) a clarifying device, initial product capture, product polishing, and final formulation (Zydney, 2015) as per figure 6.

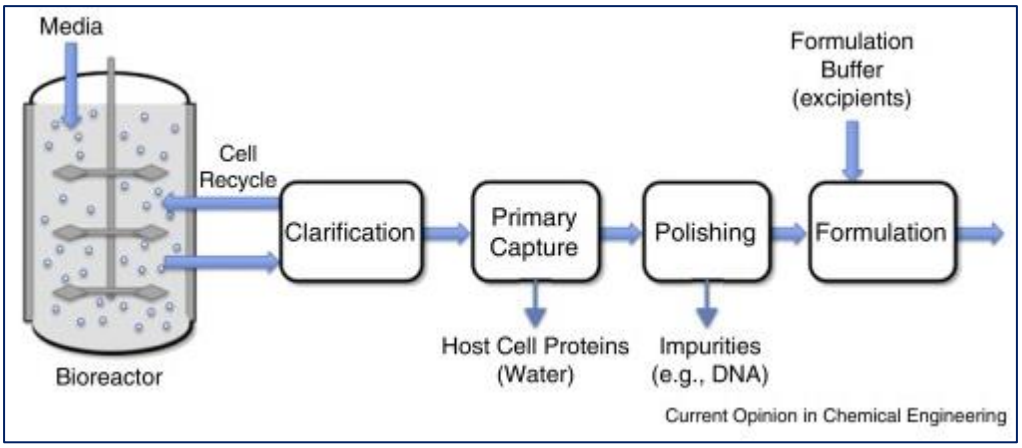

Figure 6 Schematic representation of a generic bioprocess. (Zydney, 2015)

Operational flexibility is achieved as both small volumes (<10kg/year) and large volumes (>100 Kg/year) of products even if stable molecules need to be accommodated. A continuous process designed around an intensified and simplified bioprocess requires smaller equipment that can be scaled based on time and parallelization, rather than volumetric expansion (Konstantinov & Cooney, 2015). Furthermore the size of the equipment will be dramatically reduced, resulting in a reduction in the overall footprint of a facility. Therefore small reactors of the single use variety could be used to produce batches in the various stages of clinical trials through to commercial manufacturing. This also removes a technical transfer requirement from a facility and the risks associated during a transfer (Daszkowski, 2013).

Perfusion bioreactors are becoming a popular mode to operate cell culture in. Typically there are 3 modes of operation for a bioreactor, those being batch, fed batch (where media is added to a culture once it is spent) and perfusion mode where cells are continuously removed from the bioreactor while fresh inoculum is added. Perfusion bioreactors have been implemented in the production of monoclonal antibodies, labile enzymes and cytokines (Warnock & Al-Rubeai, 2006). The perfusion bioreactor itself will be of the same cylindrical design as the other modes of bioreactors; however a device is required to remove cells that have reached optimum growth. Cell retention devices such as various spin filters, cross-flow filters, hollow fibre, vortex flow, centrifuges and acoustic settlers. Voisard in his analysis shows that solutions exist for the developments of large scale perfusion bioreactors as well as illustrates the advantages of using the various devices mentioned previously (Voisard, et al., 2003). The bioreactor residence time of the product is short, usually less than twenty four hours, which enable the production of unstable proteins with minimal degradation. The harvest is collected in large tanks and processed downstream in a traditional batch fashion,

starting with clarification for cell removal and a capture step. The utilization of filtration based cell retention devices enables integration of bioreactor operation and clarification into a single unit process. A schematic of various cell retention devices is shown in the illustration below;

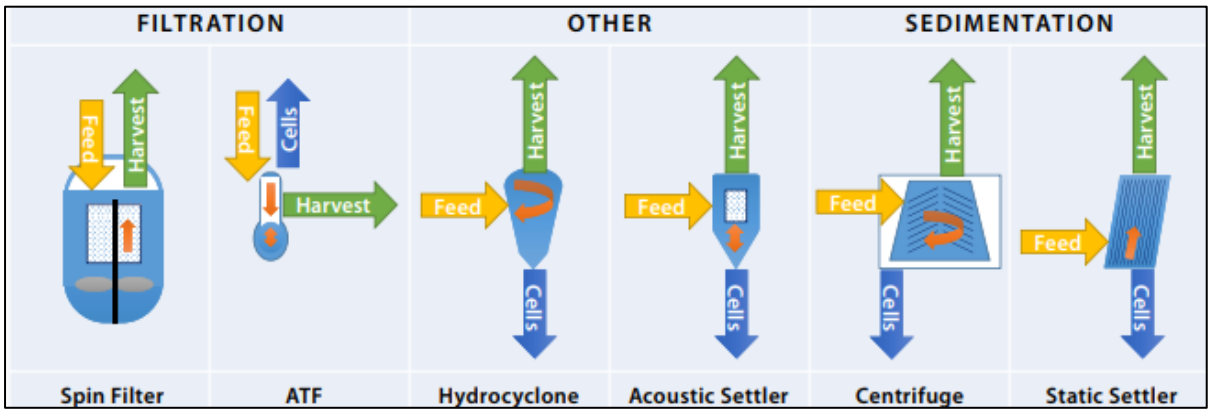

Figure 7 Overview of cell-retention devices used with Perfusion Bioreactors (Schmidt, 2017).

The table below gives an overview of the type of products, made using perfusion reactors. What is interesting to note is despite some companies using the technology since the early 1990's (Janssen, Baxter, Biogen), the complete conversion to continuous processing however has not been realised in industry. Less than 10% of biopharma drugs are manufactured through perfusion or continuous downstream processing (Hernandez, 2015).

Table 2 FDA approved biologics using perfusion processes (Schmidt, 2016).

Brand Name	Type	Approved	Company
Advate	Factor VIII	2011	Baxter International
Aldurazyme	Enzyme	2003	Genzyme (Sanofi)
Avonex	IFN β-1a	1996	Biogen
Campath	MAb	2001	Genzyme (Sanofi)
Cerezyme	Enzyme	1994	Genzyme (Sanofi)
Fabrazyme	Enzyme	2003	Genzyme (Sanofi)
Gonal-F	Hormone	1997	Merck Serono
Kogenate	Factor VIII	2000	Bayer Pharmaceuticals
Lumizyme	Enzyme	2006	Genzyme (Sanofi)
Myozyme	Enzyme	2006	Genzyme (Sanofi)
Naglazyme	Enzyme	2006	BioMarin Pharmaceutical
Rebif	IFN β-1a	1998	Merck Serono
Recombinate	Factor VIII	1992	Baxter International
ReFactor	Factor VIII	2008	Wyeth
Remicade	MAb	1998	Janssen Biotech
Reopro	MAb	1994	Janssen Biotech
Simponi	MAb	2009	Janssen Biotech
Simulect	MAb	2009	Novartis
Stelara	MAb	2009	Janssen Biotech
VPRIV	Enzyme	2010	Shire
Xigris	Protein C	2001	Eli Lilly and Company
Xyntha	Factor VIII	2008	Wyeth

What has been adopted, is a hybrid model which is a combination of continuous and batch processing as shown in figure 8 (Konstantinov & Cooney, 2015).

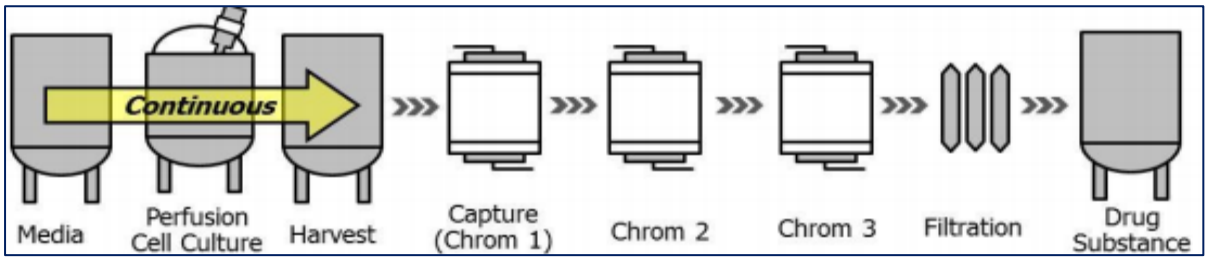

Figure 8 Hybrid Processing Model (Konstantinov & Cooney, 2015)

This process configuration allows utilization of small and even mobile bioreactors in the upstream production suite. However, the batch downstream operation still requires large equipment, which minimizes some of the benefit accrued in the continuous upstream design. Nevertheless, perfusion cell culture has been of tremendous use to the biotechnology community because it has enabled the production of unstable proteins, which is difficult to accomplish with traditional fed batch technology.

Continuous processing not only offers improvements in operational flexibility but also quality. Proteins that are secreted into a cultures supernatant can become labile due to instability from various factors such as, shearing and heat denaturation effects (Godawat, et al., 2015). However due to the lower residence time within a perfusion bioreactor and the reduction in exposure to potentially toxic side products within a culture, the stability of the protein can be maintained (Pacis, et al., 2011). Furthermore the Food and Drug Administration agency (FDA) are becoming more open to the adaption of continuous processing and in recent years have constantly made reference to supporting manufacturing companies that implement such a model. The agency's "strategic plan" from 2011 specifically quotes;

"1. Enable development and evaluation of novel and improved manufacturing methods:

a. Investigate the effects of continuous manufacturing (manufacturing using a continuous process, rather than a batch approach) on product quality.

b. Examine specific novel manufacturing technologies to determine how they impact product failure rates.

d. Promote two state-of-the-art manufacturing strategies – Process Analytical Technology, and Quality-By-Design approaches – for impact on manufacturers' ability to maintain consistent quality." (FDA, 2011).

Finally there seems to be a clear cost benefit in engaging with continuous processing compared to batch processes. The decreased footprint will result in much less capital investment and well as running costs of a facility (Walther, et al., 2015). Furthermore with less raw materials and a greater process efficacy from having a more controlled process, profitability should be maximised close to its potential (Konstantinov & Cooney, 2015) (Schmidt, 2017).

2.3.2. Modular Solutions:

As mentioned previously biopharmaceutical companies are under constant pressure to reduce both the costs of manufacturing and the time to reach market. One of the challenges that face biopharma companies is to know when to invest significant capital into increasing capacity, considering performance of potential drug candidates in clinical trials. This is especially true of small to medium sized companies, who might be targeting a smaller population whilst not having the financial backing and asset utilisation of some the lager multinationals. With the surprisingly high failure rate of candidates in late stage clinical trials, the cost of trials, as well as the relatively short time allowed between candidate approval and commercial manufacturing, a lot of complicated elements must be considered before engaging in facility procurement and design. Figure 9 illustrates the conundrum that biopharma companies face.

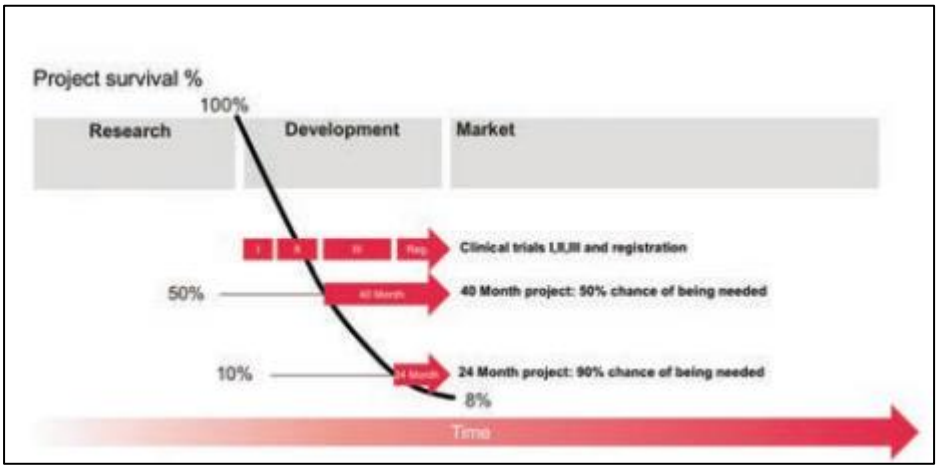

Figure 9 Time to Market vs Project survival (Munk & Moelgaard, 2016)

The longer into clinical trials, that a biopharma company holds off on a decision to pursue a potential drug candidate, the probability of the market requiring the drug increases, while the turnaround time for a project to supply the drug to market decreases (Munk & Moelgaard, 2016).

One concept that is an option for mammalian cell cultures is to engage in "modular solutions" for manufacture of biologic drug products, as show in in figure 10. A modular facility is one in which a factory or a cluster of multiple modules, are pre-fabricated off site and "dropped in" and installed at the location without much disturbance. A module (also known within the industry as a POD) is a prefabricated cleanroom box designed and built off site. This infrastructure is unlike stick-built cleanrooms, which are interconnected via ductwork that is often as large as the building structure itself. Instead of building a shell around the process, the process is being integrated into the shell. The modular concept capitalises on the emergence of single use technology as a manufacturing platforms for mammalian cell cultures. Despite the fact that there are many providers of SUT, in the biopharma market, only GE healthcare have taken advantage of the potential opportunities in the area (Shukla, et al., 2017).

GE's KUBio facilities are based on housing single-use technology for rapid switching between processes and includes all necessary infrastructure components such as clean rooms, piping and HVAC systems, designed to meet the various regulatory body regulations (GE Healthcare Life Sciences, 2018).

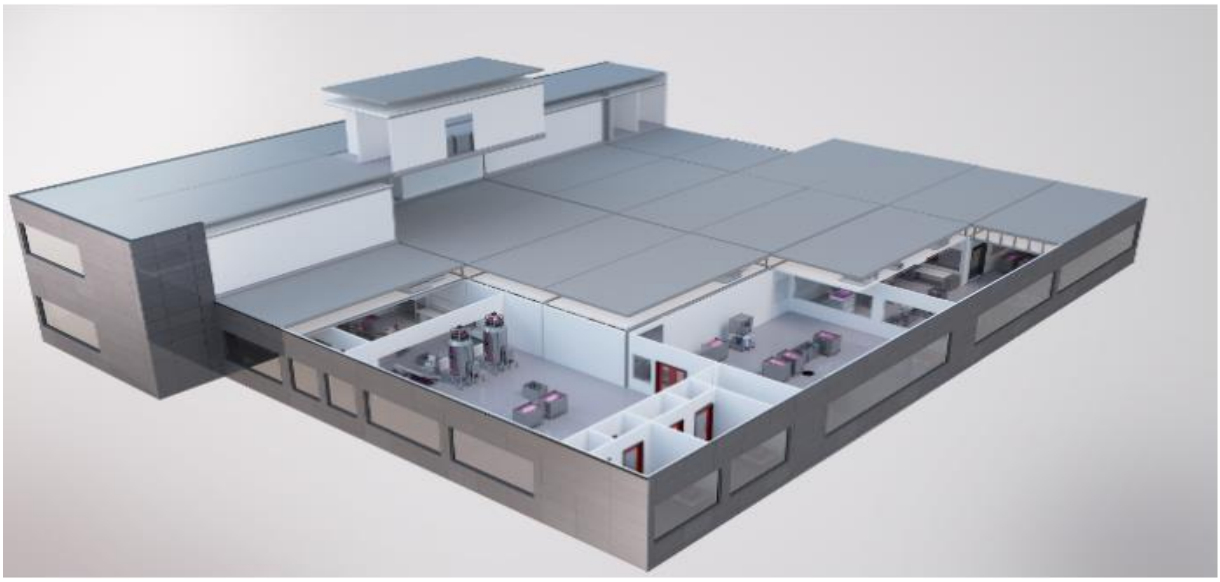

Figure 10 Example of KUBio Facility (credit GE Healthcare life sciences)

Ultimately the attraction of implementing modular bio manufacturing facilities is financial gain and speed of project delivery. Traditional biopharmaceutical manufacturing facilities typically cost $300 million to $700 million, while the time it takes to go through contraction and commissioning is from 3-5 years. A modular facility will be in the region of $30 to $100 million for a similar-scale taking a minimum of 18 months to construct and commission (Levine, et al., 2013). By maximising the use of single use technology, as the production platform and as a result the decrease in footprint and operational costs, significant savings can be made in using a modular facility for the production of monoclonal antibodies and vaccines (Levine, et al., 2013) (Munk & Moelgaard, 2016). The disadvantage to these facilities however as previously discussed, is that the facility can only accommodate a certain volume of product as well as certain types of cell lines, as single use technologies still have not broken the 4000L volume threshold as well as some heat removal requirements that would be requisite in fermentation processes (Lopes, 2015)(Langer, 2012).

As of the first quarter of 2018, modular facilities that are or will be using modular faculties include, JHL Biotech in Wuhan China, Pfizer Global Biotechnology Center in Hangzhou Province China, as well as a GE BioPark Cork Ireland, which will be a single campus hosting four KUBios owned and operated by independent biopharma companies manufacturing proprietary medicines.

2.3.3. PAT

Not only have the FDA encouraged manufacturers to engage with continuous processing technology, but also to embrace their risk based approach to designing a process. In 2002 the FDA launched a new initiative "Pharmaceutical cGMPS for the 21st Century: A Risk-Based Approach" (US Food and Drug Administration, 2002). As part of this initiative, Process Analytical Technology (PAT) is discussed and a sub team within the FDA was setup to collaborate with process development groups, to ensure quality was designed into a process, through identification of a products "quality target profiles" and therefore controlling there "critical quality attributes". This outlined in their PAT guidance document, "PAT Guidance for Industry—A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance" (US Food and Drug Administration, 2004).

The purpose of any manufacturing process is to produce a consistent high quality, safe and effective product. This is most pertinent to the Biopharmaceutical and Pharmaceutical companies who deliver a product that must be manufactured without error and to its

specification, to meet the needs of medicines for patients worldwide. However due to the complexity in making these products there is potential for error in many formulation steps of drug manufacturing. It was considered around the year 2000, that the pharmaceutical industry lacked well behind other advanced manufacturing industries such as the food, semiconductor and automotive industry. Pharmaceutical companies were slow to implement new technologies due to the perceived unknown reaction of the regulatory bodies. Furthermore there was little concern with the amount of waste being produced by processes and equipment due to mistakes in manufacturing techniques and a lack of lean methodologies being incorporated. The purity of product was being achieved by the waste generated as a result and response time to get product to market was not acceptable due to an increase in global demand.

Thus the FDA initiated guidance to address these deficiencies of product quality, demanding the pharmaceutical (and later Biologics) industry to modernise their approach to manufacturing (US Food and Drug Administration, 2002). This outlined in their PAT guidance document, "PAT Guidance for Industry—A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance" (US Food and Drug Administration, 2004). In addition to this, guidance documents were also released by the International Conference on Harmonization (ICH) with the goal of harmonising the route to market for new drug applications in the EU, USA and Japan.

QbD is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.

Traditionally biopharmaceutical products were developed using narrow process parameters and a set of operating ranges optimized to a single point. The product is defined and if it meets the quality expectation then the process is locked down and continually monitored for deviations. However if there is any variability to process parameters including raw materials, it will not be discovered until the final batch testing at batch release stage. QC testing post production was standard pre the QbD and PAT initiatives. If the product didn't meet specification then the process is redesigned. This inflexibility creates the waste and time consuming regulatory procedures to return the product to market as stated above. Historically this has been the method Biopharmaceutical companies have followed as shown in figure 11.

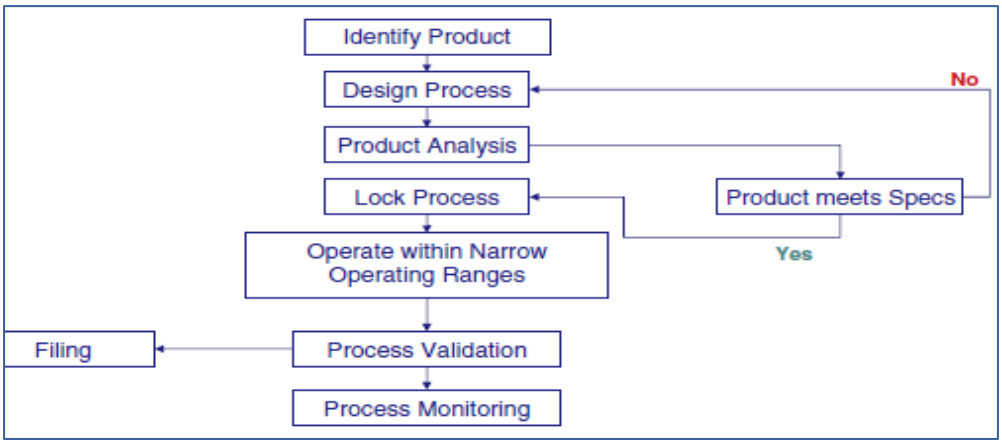

Figure 11 Traditional approach to Pharmaceutical Product development (Rathore, 2009)

A QbD system is designed to be more flexible than the traditional approach to process development. It requires the system designer to have a complete understanding of all raw materials and process parameters and what affect these will have on product quality. This should be repeated over the lifetime of the drug and updated continuously with any deviation and variations to allow a control strategy to be implemented that result in a consistent quality product.

Once a Biologic has been approved based on a given process any deviation in protocol may result in further clinical trials to test the safety of the resulting product. These trials are expensive and increase the time required to get the product to an already demanding market; thus there will be constraints on the tests resulting in the process not working at its maximum potential (Sommerfeld S, 2005). These barriers and constraints are forcing Biopharmacetical companies to lean towards a QbD approach and embraces tools to achieve this such as PAT and the "Design Space".

As stated in a recent guidance from FDA "Quality by design means designing and developing manufacturing processes during the product development stage to consistently ensure a predefined quality at the end of the manufacturing process" (US Food and Drug Administration., 2006). The QbD approach is illustrated in figure 12. These will be discussed in detail below.

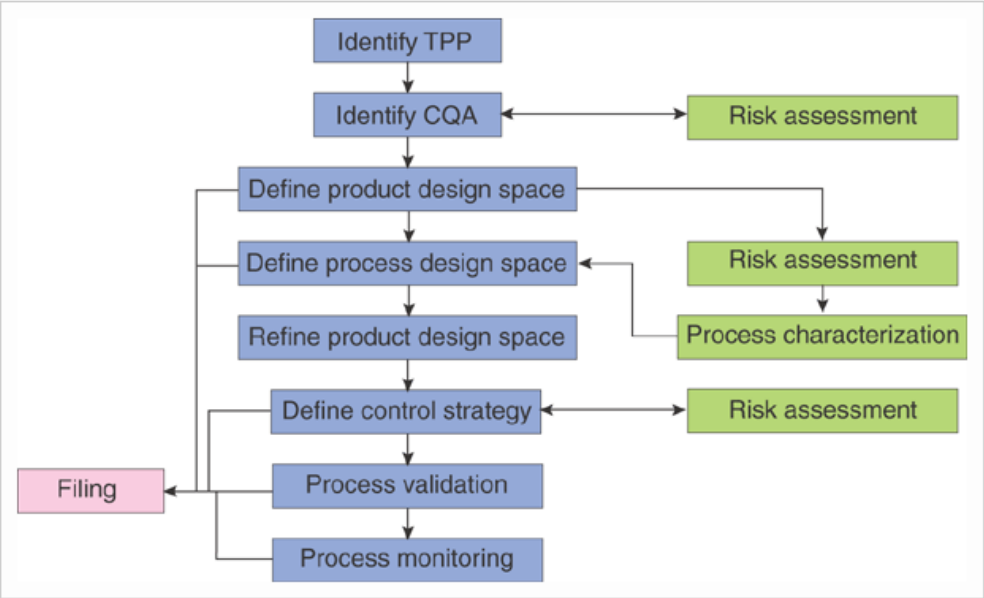

Figure 12 QbD Approach to Biopharmaceutical Product formulation (Rathore, 2009)

QTTP's

The first step in implementing QbD is to define the quality target product profile (QTPP). This is essentially the portfolio of the drug. It is not a regulatory requirement to have this document however it gives a clear understanding to the designer of the direction in which the process and variables should go. There is an end point. The QTPP should list all characteristics of the drug including its safety and efficacy (ICH Q8 R2, 2008). Details of the pharmokinetics, the dosage strength, the route for administration as well as the target population and market should as be detailed as part of the QTTP. To continually improve the portfolio the QTTP should be re-examined throughout the subsequent steps of QbD and updated appropriately with approval from the relevant regulatory authorities.

CQA's

Following identification of the QTPP's the critical quality attributes (CQAs) can be identified from these parameters. The CQAs are the properties and characteristics from a physical, chemical, biological and microbial specification that the drug is developed from. Limits and ranges for these should be declared and the drug should stay within these so as to meet the QTTP and thus the product quality of the drug (ICH Q8 R2, 2008). Due to the complexity of biotechnology products, compared to other manufacturing industry, such as the food and beverage or even the pharmaceutical (all excipients and raw materials may be quantified from their properties and stoichiometry), biologics will usually have a broad range

of QTTPs that can potentially affect safety and efficacy. Thus CQA are identified using risk assessment and analysis conforming to the international conference on harmonization guidance Q9 (ICH Q9, 2005). Guidance is given in this document on conducting risk analysis studies and tools to facilitate this. Tools such as using failure mode effect and analysis (IEC 60812, 2006) which can give an analysis on the failure that may occur in a manufacturing process, their severity and consequence.

Fault tree analysis (FTA) can be used to establish the pathway to the root cause of the failure by assuming the product has failed and evaluates all subsystems from this (IEC 61025, 2006). FTA can envisage potential completes or deviation from the product quality of the product thus giving clear incidences how a fault may occur in the how Biologic formulation pathway.

Hazard Analysis and Critical Control points (HACCP) is a methodology that was created specifically for risk analysis in food safety, however is also recommended in ICH Q9 to use this approach. Again the same principles apply, of conducting an analysis on all steps of food manufacturing from raw materials and handling through to production, distribution and consumption of finished product. This methodology can also be applied to Biologics and once a thorough understanding of the QTTP's is compiled, it can be used in conjunction with HACCP to give a thorough of the critical stages of manufacturing. HACCP works by defining critical control points, establishing limits within these and setting up a system to monitor these critical points. If the controls are not in place a control is established a corrective action to prevent the limits being exceeded. This has been adopted by the WHO and FDA as methodologies that may be used in risk analysis.

Other analysis include hazard & operability studies (HAZOP) and process hazard analysis (PHA) however these have traditionally been used more in safety studies.

Regardless of the Risk Management tool implemented, senior management must be fully committed to driving a model such as in figure 13; using scientific data and ensuring the documentation and astuteness of the studies are in proportion to the level of risk.

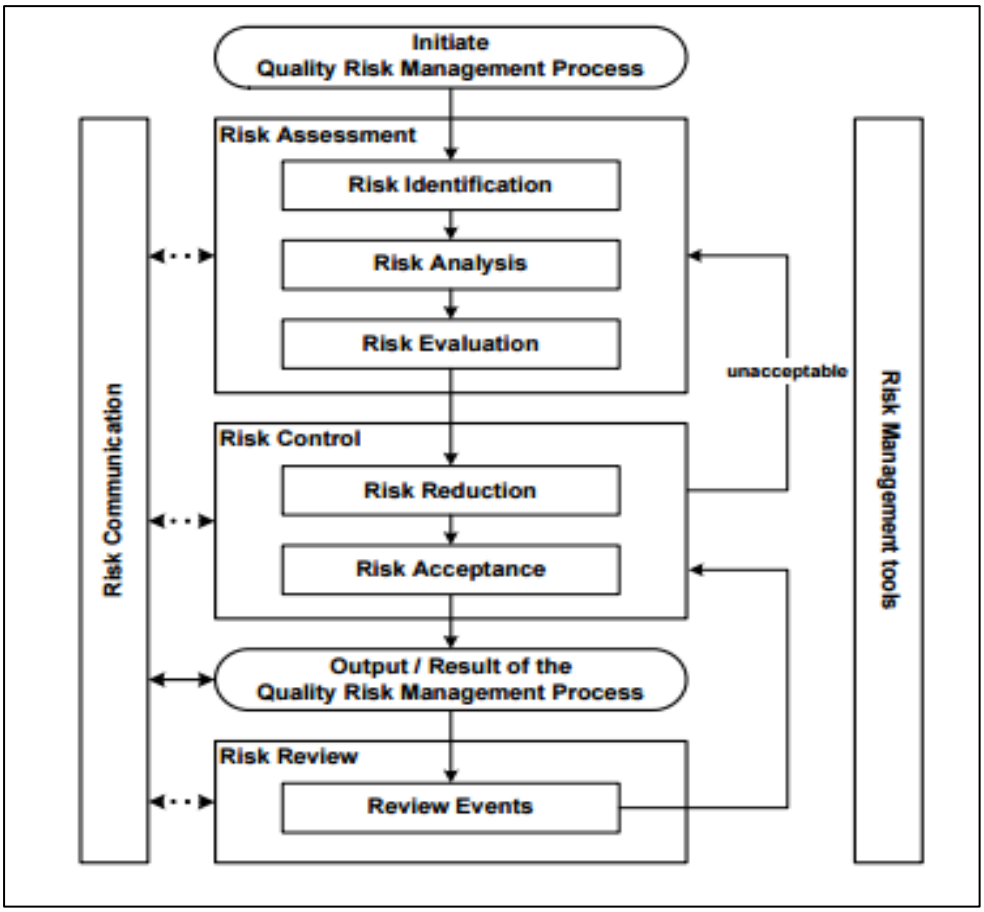

Figure 13 Typical Quality Risk Management Process (ICH Q9, 2005)

The conclusions from the risk assessment studies should be listed and ranked in order of criticality allowing a list of CQA's to be created which is the goal of step two. However this would have to be continually updated and revise at all stages of product development.

Product & Process design Space

In effective QbD systems the "Product & Process design Space" should be explored early before committing to any licencing applications. The design space is designed in ICH 8 as "The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality" (ICH Q8(R2), 2009). The relationship between the process inputs (parameters and properties of the input) and the CQA's are illustrated in the design space. On completion of the Risk analysis step, as in figure 13, the CQA's can be assessed and identified with each CQA serving as a branch or dimension of the design space, giving a multidimensional area in which to work (A.S. Rathore, 2009). Depending on the type of biologic product a variety of CQA's will exist and can have more than on quality parameter associated with it (S. Kozlowski, 2006). Parameters such as temperature, pressure and time as a function of each

other can be used to define the design space, and the rationale to why these are included should be illustrated in the submittal. The design space can be determined principally by three main methodologies or a combination of methods. Using traditional first principles, combining experimental data, reaction engineering models, stoichiometry and biochemistry to model and predict performance. Using statistically designed experiments (DOE) which will consider all impacts on the multiple parameters and how they interact with one another as well as multivariate analysis. Finally scale up correlation can be used such as computational fluid dynamics. This semi empirical approach allows the applicant to translate operating conditions between the different scales or pieces of equipment.

The final size of the design space will depend on the amount of data gathered from the various studies such as clinical data, non-clinical data, stability studies and all information gathered in pharmokinetics studies, microbial studies and process robustness (J. Harms, 2008). These should all be gathered using statistical methods thus allowing them to be easily derived and interpreted against the specifications laid out in the drug submittal (Apostol, 2008) as well as allowing the model to be updated and expand throughout the life time of the product. Once variability as much as foreseeably possible is established and captured in the product design, process design space can be created (J. Lepore, 2008). Rathore proposes a model for Process design space as illustrated in figure 14.

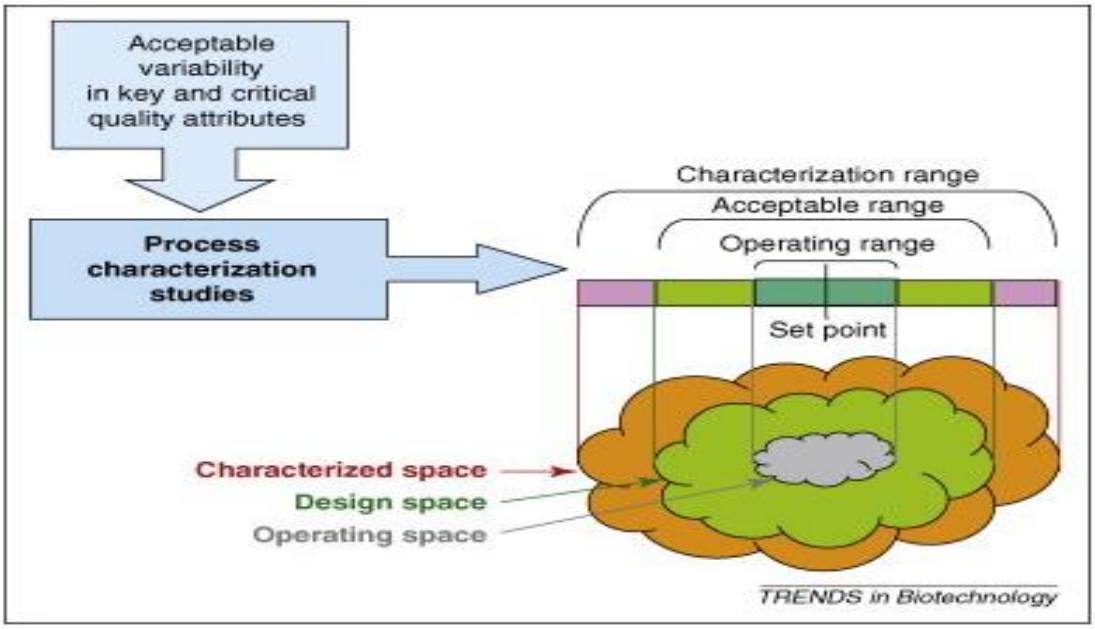

Figure 14 Design Space created from Process Characterization Studies (Rathore, 2009)

The “set point” is the specification of the product with a deviation either side of the set point giving us the operating range. The design space is set by the acceptable range. The characterization range is when the design space is taken to the edge of failure which can be helpful when deciding on CQA’s, however it is not recommended to operate near the failure range as any variation in process may take us outside the design space.

A design space is not a regulatory requirement. However the usefulness of proposing and receiving regulatory approval for a design space, for example if a parameter is changed within the operating or acceptable range in the design space, then re-approval is not required as this is not considered a “change”. It should also mean by operating in the design space, by default, will give the quality assurance in the final product, as well as gives manufacturing a complete understanding of the variables and parameters within the process thus giving flexibility across the entire manufacturing process. If desired the applicant may create a design space for the various operational units or more critical unit operations which is simpler than creating a design space for the whole process. This is more than likely dependent on resources and time dedicated to the application driven by desire to get market approval. Finally the applicant should consider scale up factors when describing the design space. As mentioned previously, the CPP’s define the CQA’s thus when CPP’s at production scale needs to be accounted for by the applicant. For example the type of media (liquid vs powdered requiring addition of WFI) or variability in media components required for mammalian cell culture, or sensitivity of cell culture to aeration bubbling and shear forces created mixing from aggressive mixing (Marks, 2003). These should allow an extension to the design space.

Control Strategy

A design space developed with an effective control strategy will ensure the manufacturing process produces a product that meets the QTPP and the CQA’s and based on all data gathering up to this point. According to the ICH Q10 guideline a control strategy is defined as “a planned set of controls, derived from current product and process understanding that assures process performance and product quality” (ICH Q10, 2008), the product will be produced consistently. In a traditional control model the processes are operated with fixed controls with defined set points and operational limits. This is an intransigent approach as for example, any variables in raw materials will result in a variation in product quality. There is no flexibility within the control strategy.

However a QbD approach will use a dynamic strategy with reactionary set points and operating ranges to compensate for any variability in process inputs such as feed material and raw materials (S. Kozlowski, 2009). There is much more complexity in Biologics manufacturing compared to traditional active pharmaceutical processing as discussed so a QbD control model should be used for Biologics manufacturing. QbD will use Process Analytical Technology (PAT) tools to accomplish this. PAT has been defined by the FDA as "a system for designing, analysing, and controlling manufacturing through timely measurements of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality" (US Food and Drug Administration, 2004). PAT technologies use inline measurements of process parameters with automation to enable real time release testing which should reduce or eliminate a need for end product testing (ICH Q8 R2, 2008). A QbD approach, will again as mentioned previously, encourage trending of the processes to promote feedback for unremitting improvement efforts post initiation of manufacturing as well as allowing the product and process to be continually understood. Also as a function of being required to implement modern day analysers via PAT, an increased understanding of the process and control of this is being obtained as a result (S Mercier, 2014).

Process Validation and Filing

Validation is the process of demonstrating that the precursor steps mentioned above will produce a product to an acceptable quality. The parameters defined in the design space and the methods for control strategy implemented are validated with documented evidence (A.S. Rathore, 2009). Confirmation of the lab scale or pilot scale systems, methods and utilities used to define the boundary of the design space is obtained and justified at production scale. The regulatory filing is then created which includes all the key documented parameters, compiled data, acceptable ranges that are established in the design space studies. The filing will also include all of the above sections discussed such as the product design space, the control strategy implemented, the results and justification of the validation exercises and a plan for continuous improvement via process monitoring.

Process monitoring

Once the Biologic product has been approved by the relevant authorities, the critical quality attributes must be monitored continuously, to confirm that the process is kept within the parameters of the design space (Rathore, 2009). This must be completed by statistical analysis to with the same level of effort and application as applied, in creating a risk

management process for CPPs and CQAs as well as control strategy development. If a validation system is implemented it will support the development of achieving consistency in the product quality and efficacy in the manufacturing process while also realising the benefits of implementing QbD into continuous improvement (Konold, 2009).

PAT Applications

As mentioned above for successful implementation of QbD, the biopharmaceutical characteristics must be well understood and the control of these must be achievable at manufacturing scale. Quality by Design is the overarching paradigm that uses a design space and PAT tools to achieve this. PAT tools facilitate implementation of QbD, as encouraged in the ICH guidelines, on line real time analysers are used to give an output of the parameters allowing dynamic control of the process to produce a consistent product. The ideal technologies (sensors/instruments) for biopharmaceutical processes, should operate in situ, be non-invasive and generate on-line information about multiple key bioprocess variables (A.P. Teixeira, 2009).

In bioprocessing instruments can be classified with respect to their level of invasiveness in the process stream. Sensors can be categorised into online monitoring, at-line and off-line depending, on the configurations available and the parameters to be measured. Figure 15 illustrates the various configurations of analysers.

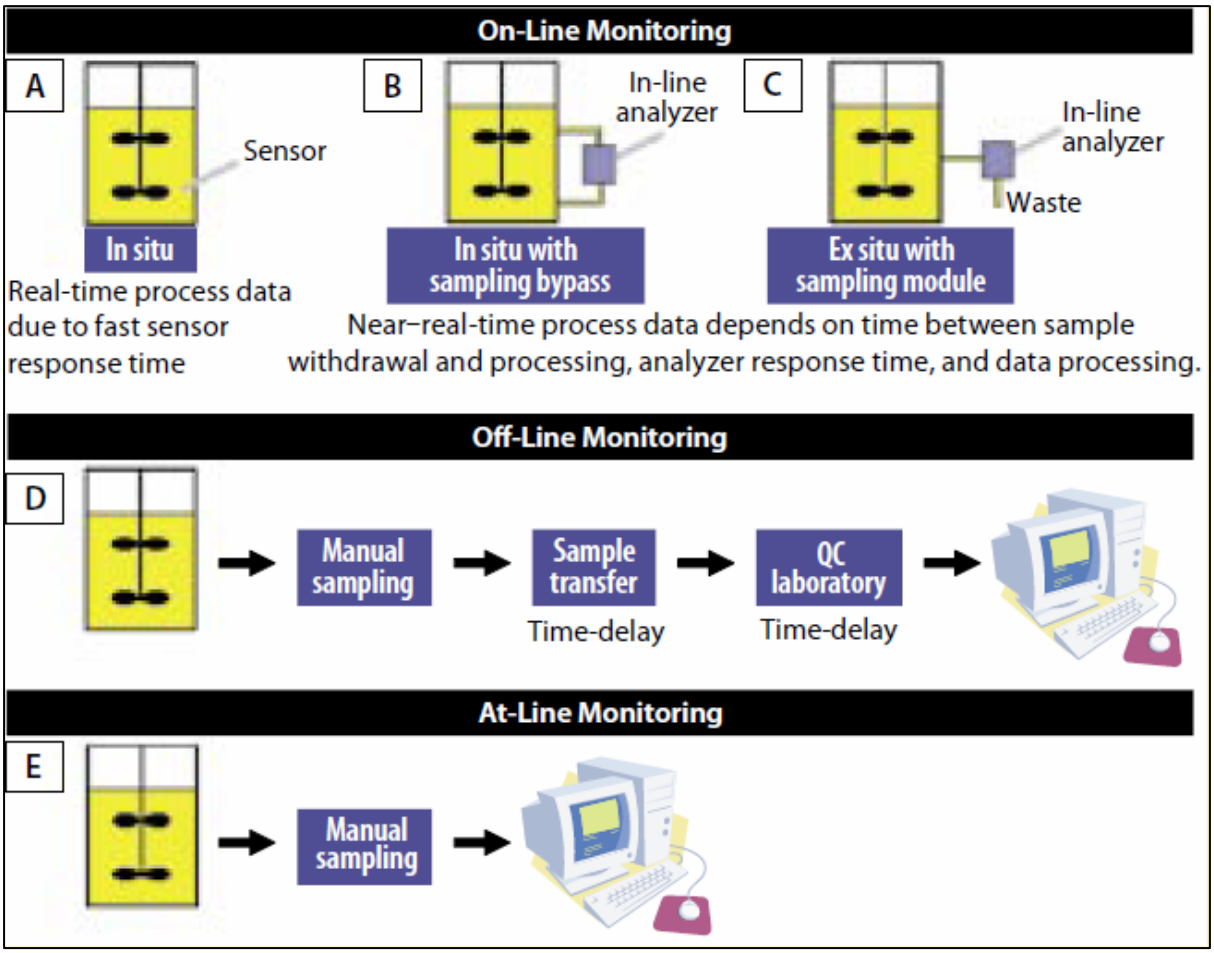

Figure 15 Classification of bioprocess monitoring, sensors and analysis configurations (Whitford & Julien, 2007)

Historically typical parameters that have been measured with in situ monitoring include dissolved oxygen, dissolved carbon dioxide and pH. These are electrochemical sensors that work, on line providing feedback of process parameters associated with a typical unit operation similar to configuration A in figure 15 (Mark, 2003). Gnotha demonstrates that a combination of multivariate analysis proposed in QbD and PAT, as well as an excellent understanding of process control (feed forward, backward, and PID control loops) allows reproducibility of quality accepted product concentrations with little variation. When the control system parameters go out of limits, the control loops must react immediately (automatically) to the response, to allow the system to return to steady state. If manual intervention is required it is too late as the system will have gone outside the design space and produced a variance in the expected result, a deviation has occurred in the process. This is without using specific PAT analysers only basic engineering control looping in its simplest sense, but a complete and thorough understanding of the process (E.Coli fermentation process) was known (S. Gnotha, 2007).

Texixeira discusses many analytical techniques using detailed and complex spectroscopic techniques for in-situ monitoring for the specific process parameters that exist within bioprocessing. Hybrid models are also illustrated to bridge the gap between theory and implementation into practices of mammalian cell cultures control. However in the authors experience this still not has been applied at manufacturing scale but is inevitable in the future once the control models are clearly understood (A.P. Teixeira, 2009). Techniques such as near infrared, mass spectroscopy and microscopy are now being used to give real-time feedback of a cell cultures constituents giving read outs on parameters such as glucose, glutamine, lactate, total and viable cell count, amino acids amongst other things (Whitford & Julien, 2007).

Mercier also argues that due to the vast volumes of data sets that are produced and the complexity of these to analyse in biochemical processing, the highest level of QbD and PAT implementation will never be reached. However a thorough understanding of the process will be obtained and useful models can be created from these, to better understand a process (S Mercier, 2014).

However there is much debate in literature, amongst the viability of implementing PAT analysers as swiftly as the regulatory authorities might like. Progress in PAT control has mainly been made at the cell culture and fermentation stage but not as rapidly in downstream applications, due to higher variability in processing (Rathore, 2014). Different cell lines and metabolism expressions are sophisticated and vary; a one type model will not work in all applications.

2.4. Lack of progress in downstream technology

As discussed in the previous sections, the advancements in technology for the manufacturing of biopharmaceutical products, has mainly come in the upstream sectors of processing. A great deal of progress has been made, involving improvements in cell titres resulting from advancements in cell line engineering, improving media supplements and bioreactor design. However this has displaced the bottleneck into downstream manufacturing with up to 80% of production costs being generated in the purification of cell culture mediums (Hunt, et al., 2001). A common protein concentration of 1 gram of an active protein per vial is now expected in therapeutic proteins (Aldington & Bonnerjea, 2007). This results in manufacturing models requiring large scale production facilities to house, purification

suites, that can handle the large batches required to meet the dosage requirements, thus resulting in higher capital and operating expenditure (Girard, et al., 2015).

A standard purification process has been used in the industry, developed from process knowledge acquired in the early 1990's for biologics processing. It generally involves, clarification steps (harvesting) by using centrifugation or filtration methods, a number of chromatography operations as well as viral clearance (if applicable) and concentration and diafiltration achieved by "UF/DF" skid (Liu, et al., 2010) (Abhinav & Jörg, 2010) (Chon & Zarbis-Papastoitsis, 2011). A typical monoclonal antibody purification process is shown in figure 16, the approach used by Amgen (Shukla, et al., 2017).

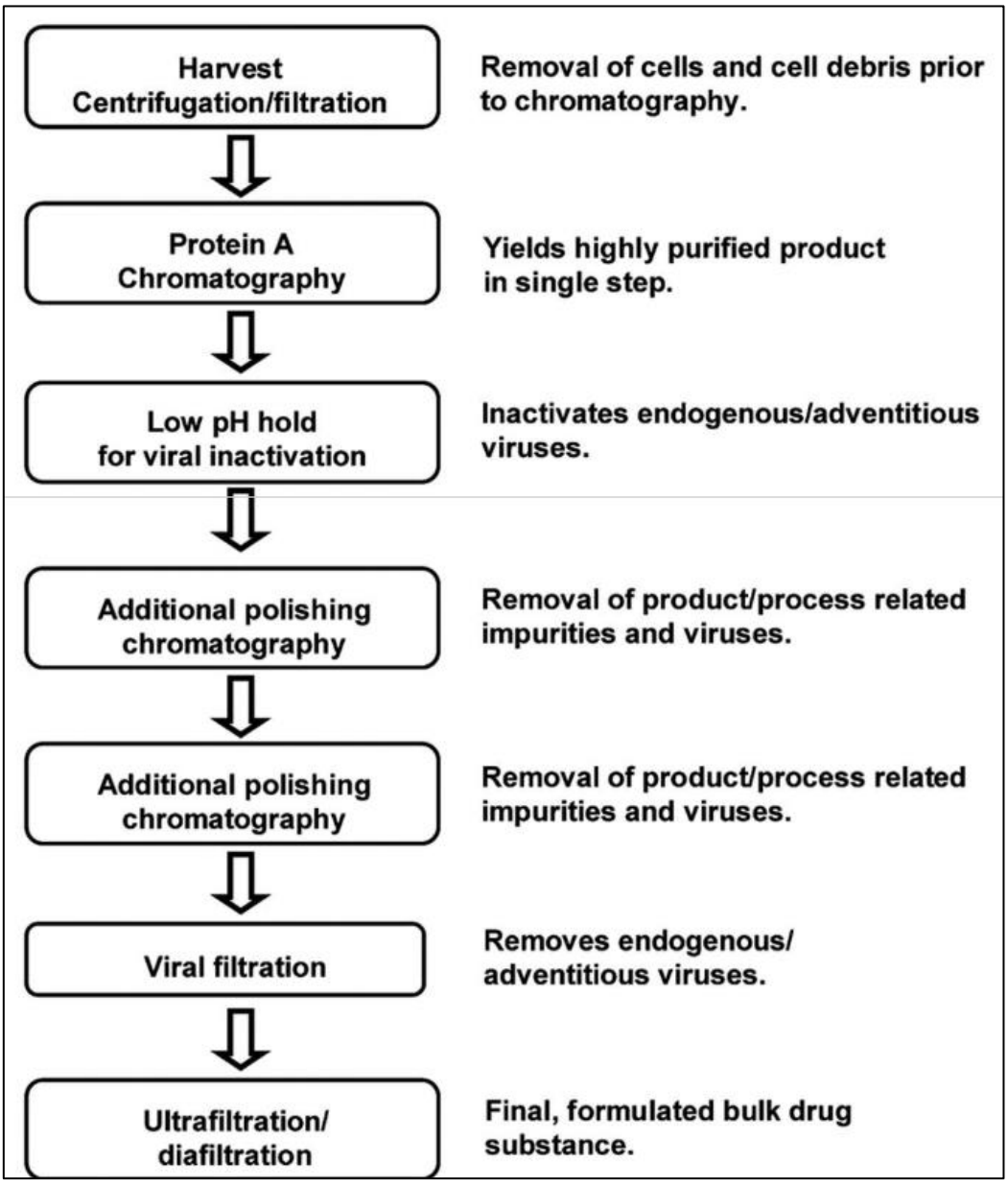

Figure 16 Typical Mab Purification Process (Liu, et al., 2010)

The variations in the process will be in the difference in the target binding sites of the desired molecule, as well as their ability to remove impurities associated with the target as produced at cell culture stage. The purification of a target protein consisting of capture, intermediate and polishing mostly involves chromatography (Jungbauer, 2005). This means that various types of chromatography modes & resins are used to overcome this, such as affinity, ion-exchange, size exclusion, hydrophobic interaction as well as other not so common resins such as "dual mode", which manipulates the hydrophobic and hydrophilic areas of a protein, to promote binding to that resin (Liu, et al., 2010).

The problem with commercial scale manufacturing is predominantly chromatography processes are run in a batch mode, with intermittent steps. For example a column using various buffers of salt concentration and varying pH, will requires specific processes to be run; equilibration, sample loading, elution and regeneration in the separation of a protein from undesired impurities (Chon & Zarbis-Papastoitsis, 2011). The eluate is usually collected in a vessel or bag and the next chromatography column is prepared for loading, which also requires the sample to be loaded under certain conditions. The flow-rate of the solution through a chromatography column is a critical factor as well as the back pressure of the system itself. While it may be possible to gravity feed solutions through a column it is more typical to pump the solution through, to maintain controlled operating conditions such as flow-rate and back-pressure. In the process of scaling up from lab to commercial scale, process designers will take advantage of the linear flow rate (or superficial velocity) measured in centimetre an hour (cm/hr). This is independent of column scale. Therefore it is possible to retain comparable conditions between columns of different volumetric capacity, by maintaining the linear velocity at a constant and by increasing the diameter of the column being used in the process. However the largest column diameter available on the market, at the time of writing is 1.8 meters. Also a column will only be able to provide a certain bed height of resin before the design pressure of the column is exceeded (typically from 20-40cm in height) therefore the column will only allow a certain capacity of a sample onto it to achieve the separation desired (Shukla, et al., 2017). There is a trade-off between optimum selectivity and overall flowrate and velocity performance, which inherently limits the amount of product that can be processed per cycle (Perez-Almodovar & Carta, 2009). Herein lays the bottle neck in bio manufacturing presently. The key to overcoming this will be the industry embracing novel technologies to alleviate the capacity issues of current batch chromatography systems (Girard, et al., 2015).

2.4.1. Novel Tech 1- Periodic counter current chromatography

As mentioned in the previous sections discussing continuous processing, the key to alleviating bottle necks within the downstream purification process maybe to embrace fully or partly a continuous processing. The debate still remains around the requirement for a full continuous process from an economic stand point as well as if the technology to support such a model will be available, particularly in the viral and ultrafiltration/diafiltration steps (Godawat, et al., 2015). However what is clearly required is a continuous or semi continuous operation to in the capture of Mabs. One mode of operation which may have the potential to unlock this bottleneck, is employing the use of periodic counter current chromatography (PCC) in purification.

PCC is currently offered as a custom package from GE Healthcare. PCC employs the use of three or more chromatography columns to create a continuous purification step. A column used for capturing Mabs, such as Protein A, is usually loaded up to 90% of 1% breakthrough (BT) capacity of the resin in batch mode. This is in an attempt to recover as much product as possible, not losing any to waste, while adding in safety factors into calculation as overage (Pollock, et al., 2013). As the sample is loaded onto the top of the column most binding occurs here, while the area towards the bottom of the column is underutilised, resulting in excess buffers used for the various bindings, washing and elution steps (Mahajan, et al., 2012).

However PCC is different in that it allows loading up to much higher percentages of breakthrough capacity of columns. A three column PCC system is shown in figure 17 used by Makahan to separate a Mab from a cell culture fluid (Mahajan, et al., 2012).

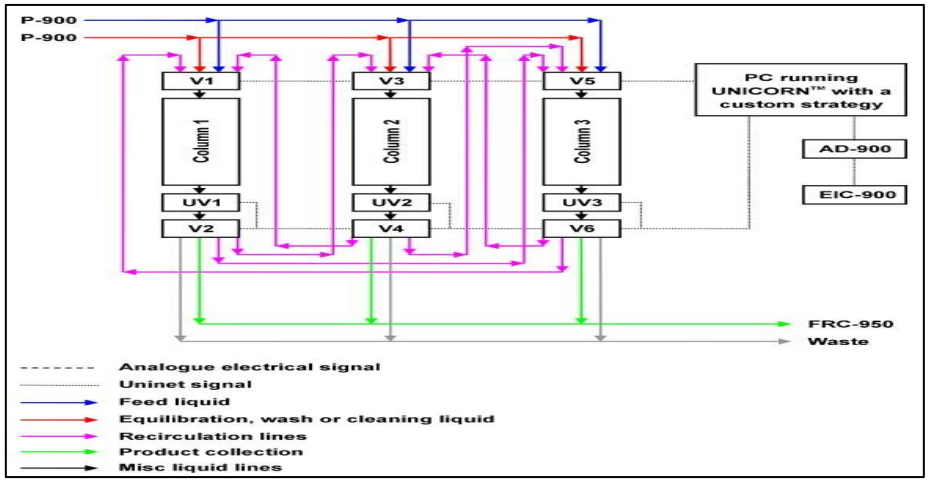

Figure 17 Three Column PCC system schematic (Mahajan, et al., 2012).

The columns are positioned in series, connected by block valves, with UV meters monitoring the process feed, as well as the flow out of the columns. The use of UV allows the system use a dynamic control function, which signals when the column has become saturated allowing the system, to switch to the next available column. Figure 18 details the flows of harvest cell culture fluid (HCCF), through the various columns.

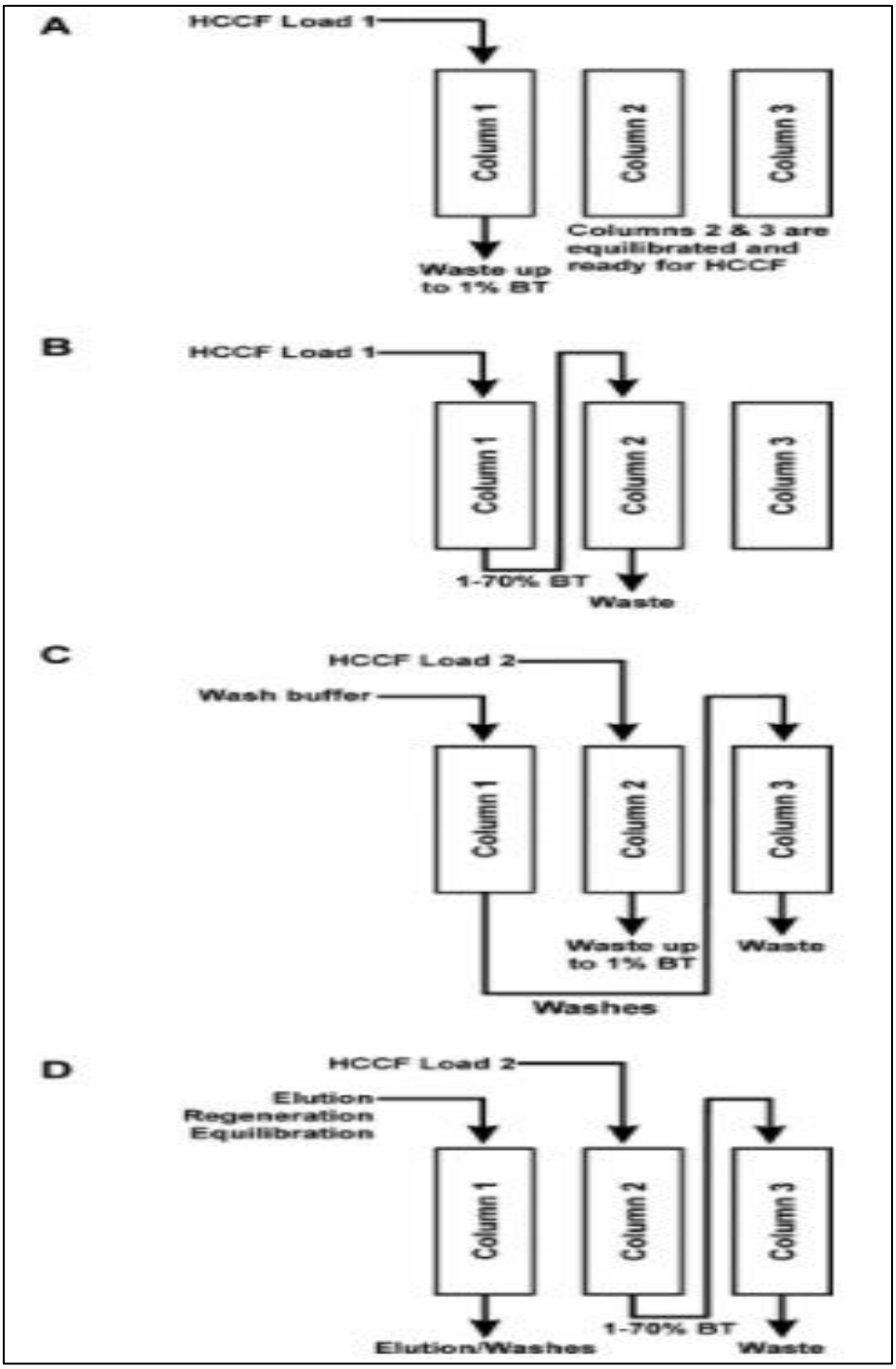

Figure 18. Three Column PCC process flow (Mahajan, et al., 2012).

The HCCF is loaded onto an equilibrated column (column 1). The other columns in series are also equilibrated and ready for use. When the BT capacity has been reached from column 1, the difference in UV absorbance will detect this and switch the flow to column 2 (step A). Column 1 washes are then sent to column 3 to capture any further unbound protein, while column 2 processes the main HCCF load. Column 1 then undergoes elution, regeneration and equilibration in preparation for the next cycle. The same process follows along through column 2 to 3 and back to 1. Break through (BT) capacity is the capacity of the column to bind the product with the affinity of the resin available. Figure 19 illustrates the differences in the utilisation of this resin in batch mode, compared to PCC.

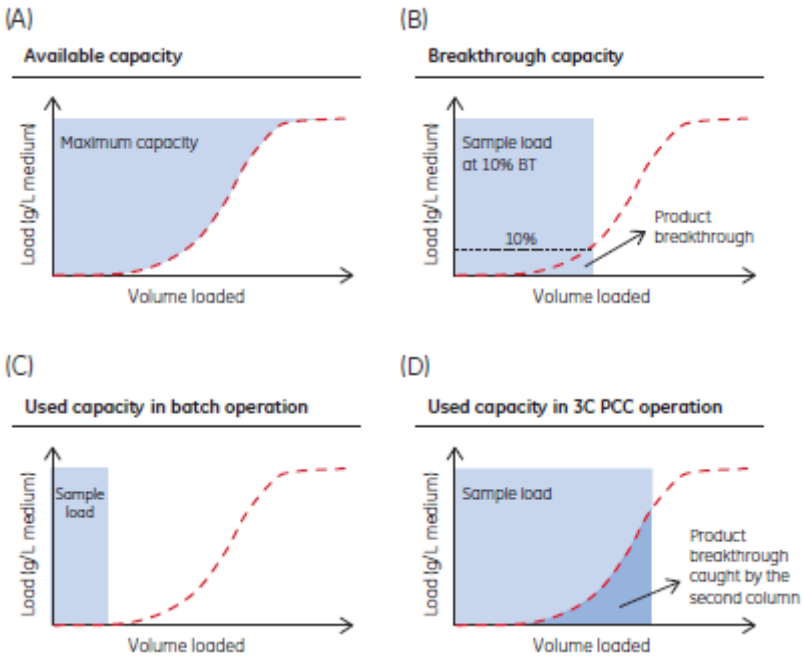

Figure 19. Capacity utilization for standard batch chromatography vs PCC (GE Healthcare Bio-Sciences, 2016)

We can see the difference in capacity utilisation between graph C and D, as well as the removal of any risk of losing product, as it is washed into an available column. The benefits of using PCC compared to batch chromatography are promising for this reason alone. By using PCC the amount of resin required for the process step can be reduced significantly, therefore column size will also reduce as well as the buffer consumptions required for a smaller column. Mahan and Pollock are in agreement that there is significant savings in capital and operational costs associated with using these modes (Mahajan, et al., 2012) (Pollock, et al., 2013). Furthermore this technology is also aligned and suited with the continuous processing model that the industry can begin to embrace to save costs as well as

that is encouraged by the FDA and other regulatory bodies, as discussed previously (Shukla, et al., 2017) (Girard, et al., 2015).

2.4.2. Novel Tech 2 – Expanded Bed Adsorption

In large scale commercial biologics manufacturing, before the crude product reaches the purification or initial capture step, the process stream must be sufficiently clarified, to remove as much cell debris as possible. Capacity of columns is one bottle neck, however the bottleneck can shift upstream of a capture (Affinity) column even before it reaches this point. The standard techniques used for removal of nonviable cells and cell debris are centrifugation, depth filtration and microfiltration for extracellular cell lines such as mammalian cells and yeasts where the protein is excreted into the supernatant. These processes at large however are inefficient and have a poor yield associated with their operations. The high shearing effects experienced in centrifugation from the spinning plates, causes protein denaturation, as well as actual losses to waste within the bowl, due to the design of the solids removal systems, resulting in low yields (Shekhawat, et al., 2018) . Depth and microfiltration also remove cell debris with the function of protecting columns from fouling, becoming blocked and exceeding the pressure of a packed bed as well as preventing inadequate separation. However depth and micro filters are prone to becoming fouled themselves, as a result of a gel layer building up on the surface and the filtrate flux decreasing (Yamada, et al., 2017). Filters should be sized appropriately to avoid this happening, however variations in batch composition or a piece of equipment performing inefficiently upstream of the filters, may cause fouling and blockages (Li, 2017). This can result in long processing times or equipment down time leading to losses in operational delays. One possible solution to remove this bottle neck is by using expanded bed adsorption (EBA).

The main advantage of EBA is being able to carry out both clarification steps and product capture in one single step. “DSM Biologics” who are a contracting manufacturing organisation (CMO), demonstrated using a single use version of EBA, to capture a monoclonal antibody, directly from a bioreactor harvest without prior clarification (Chon & Zarbis-Papastoitsis, 2011). A schematic of an EBA system is illustrated in figure 20 (Kennedy, 2005).

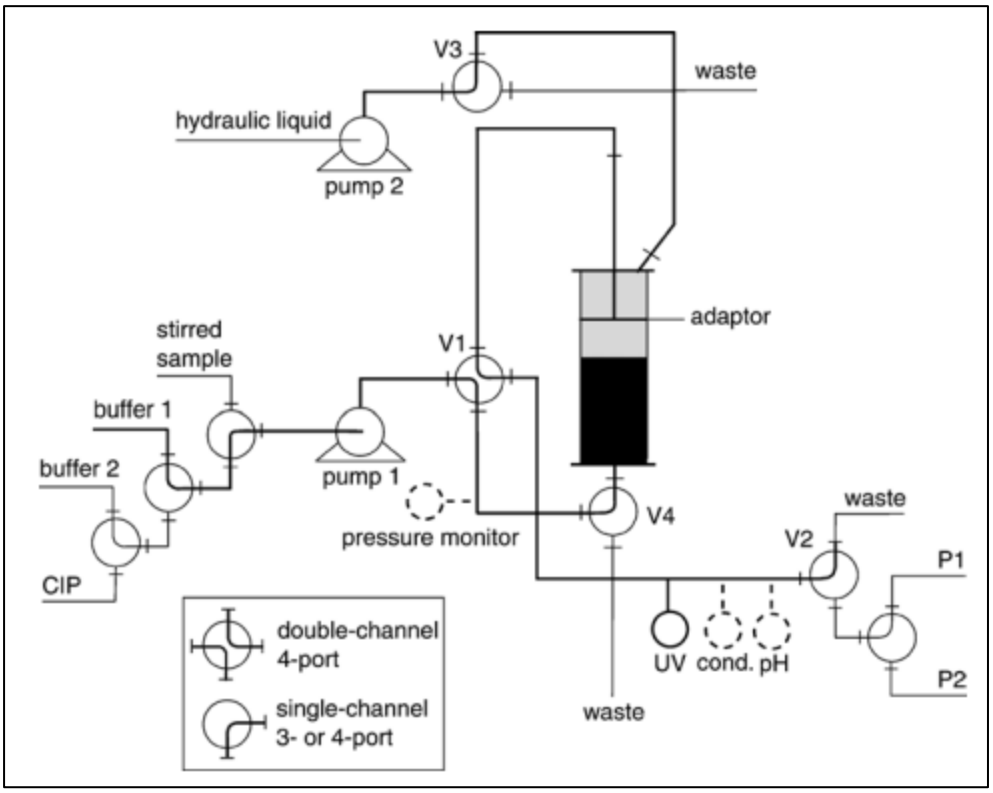

Figure 20 Schematic of EBA system (Kennedy, 2005)

The concept is similar to capture chromatography and the resin types are comparable in there affinity for the Fc region of the monoclonal antibodies. However it is not affected by the same fouling scenarios that are seen on these types of columns. Unlike traditional resins, in which the beads tend to be relatively uniform in size, the beads of EBA resins are variable, typically ranging from 50 to 400 mm in diameter and do not require to be packed like traditional columns. Furthermore where in traditional chromatography column operation, the top adapter plate moves down and compress the packed bed, EBA columns work by the top adaptor pulling up creating a fluidised bed. Equilibrating buffer is passed over the fluidised bed and the crude process can be pumped up through the column. Waste is removed and subsequent washes elute the product. A simplified version of this process is shown in figure 21 (Kennedy, 2005).

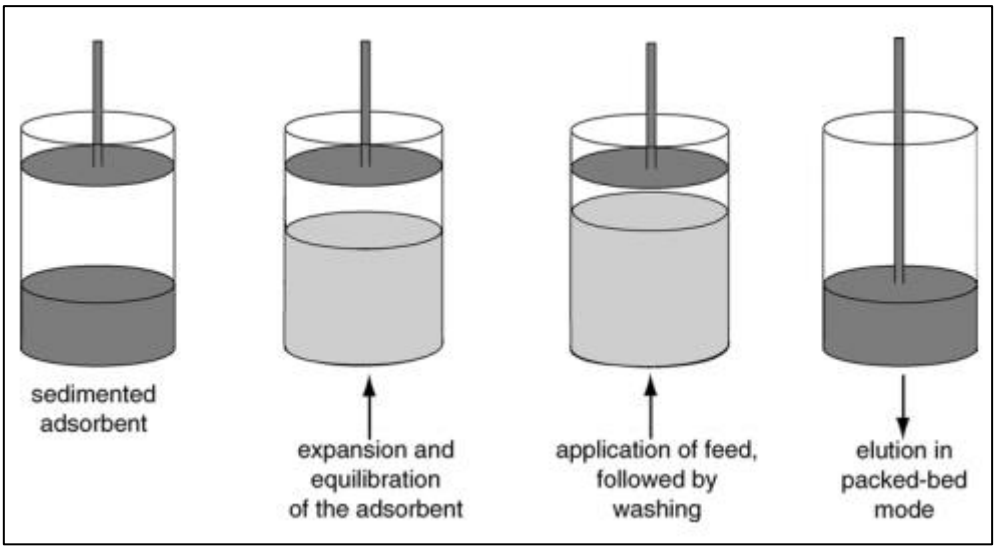

Figure 21 EBA Operation Simplified (Kennedy, 2005)

EBA has been reported in literature to be suitable both for both animal (mammalian) and microbial cell lines of high densities, with a high order magnitude of separation, as well as an increase in the level of removal of cell debris, host cell DNA and protein (Anspach, et al., 1999). The advantages to this technology are obvious in that it reduces capital and operational costs with the removal of a number of centrifugation and filtration operations (Cunha, et al., 2016). Arguably a third type of operation is also removed in the need for packing the column with resin and the difficulty's this introduces in traditional columns. It also is capable of handling most cell lines and is not affected by variations in feed stock concentration or the introduction of air (Anspach, et al., 1999) (Frej & Hjorth, 2018).

However similar to batch chromatography systems they can only accept a maximum volume of feed which is dependent on the capacity of the resin. EBA also require more column washes to release product (up to 20), than traditional columns (Anspach, et al., 1999). Nonetheless with the combination of a buffer recycling system or buffer conditioning system, the potential for EBA to replace familiar clarification techniques is evident (Anspach, et al., 1999) (Kennedy, 2005). With the reduction in batch sizes due to increasing titres, EBA should be investigated as an alternative to centrifugation and filtration processes for clarification (Frej & Hjorth, 2018). (Junior, et al., 2016) (Cunha, et al., 2016)

2.4.3. Novel Tech 3 – Membrane Chromatography

Continuing on the discussion of the bottle neck being at the initial chromatography step, another potential technology to reduce or relieve this is by incorporating the use of membranes in chromatography systems. The use of single use membranes, in downstream processing has been applied to viral clearance steps successfully, with an increase in the log clearance of viruses as well as a reduction in costs, compared to other batch chromatography methods (Frau, et al., 2010). However due to the superior mass transfer capabilities of membranes compared to resin beads, membrane chromatography is now being assessed for other areas of downstream processing, such as in the capture and polishing steps (Orr, et al., 2013).

The main difference between conventional resin bead chromatography and membrane chromatography lies in the difference in structure between the matrices of the respective purification techniques. Figure 22 illustrates the difference between the two matrices.

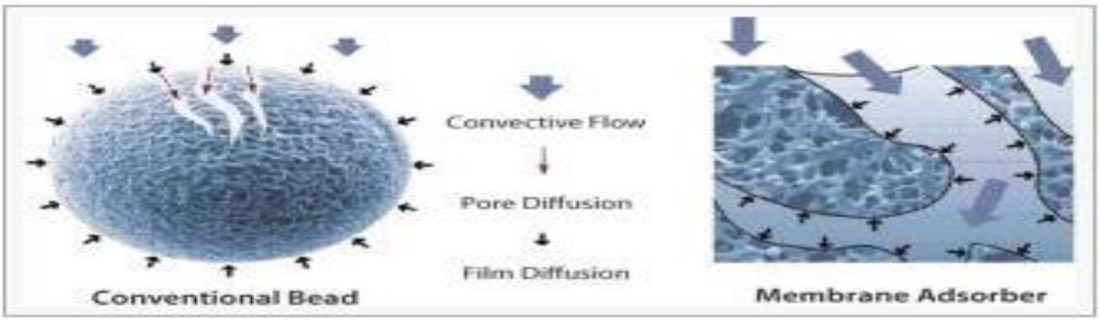

Figure 22 Conventional Resin Bead versus Membrane Adsorber Structure (Fraud, et al., 2009)

A significant advantage of membranes compared to beads, is that they don't rely on pore diffusion to transport molecules to the respective binging site. Transport is mainly accomplished by convectional flow (Fraud, et al., 2009). Therefore much higher flowrates and lower pressure drops are allowed, in the operation of membrane chromatography (Orr, et al., 2013). There is also an advantage in that due to the lower void space within the resins compared to membranes; less wash buffer is required for the various chromatographic separation steps. Furthermore there is no requirement for packing as membrane absorbers are pre packed. Membrane chromatography is used for a wide variety of protein separations with a number of different types of modules available including, stacked discs, flat sheet, hollow fiber and radial flow (Ghosh, 2002). An illustration of these types of modules is displayed in figure 23 below (Suen, et al., 2003).

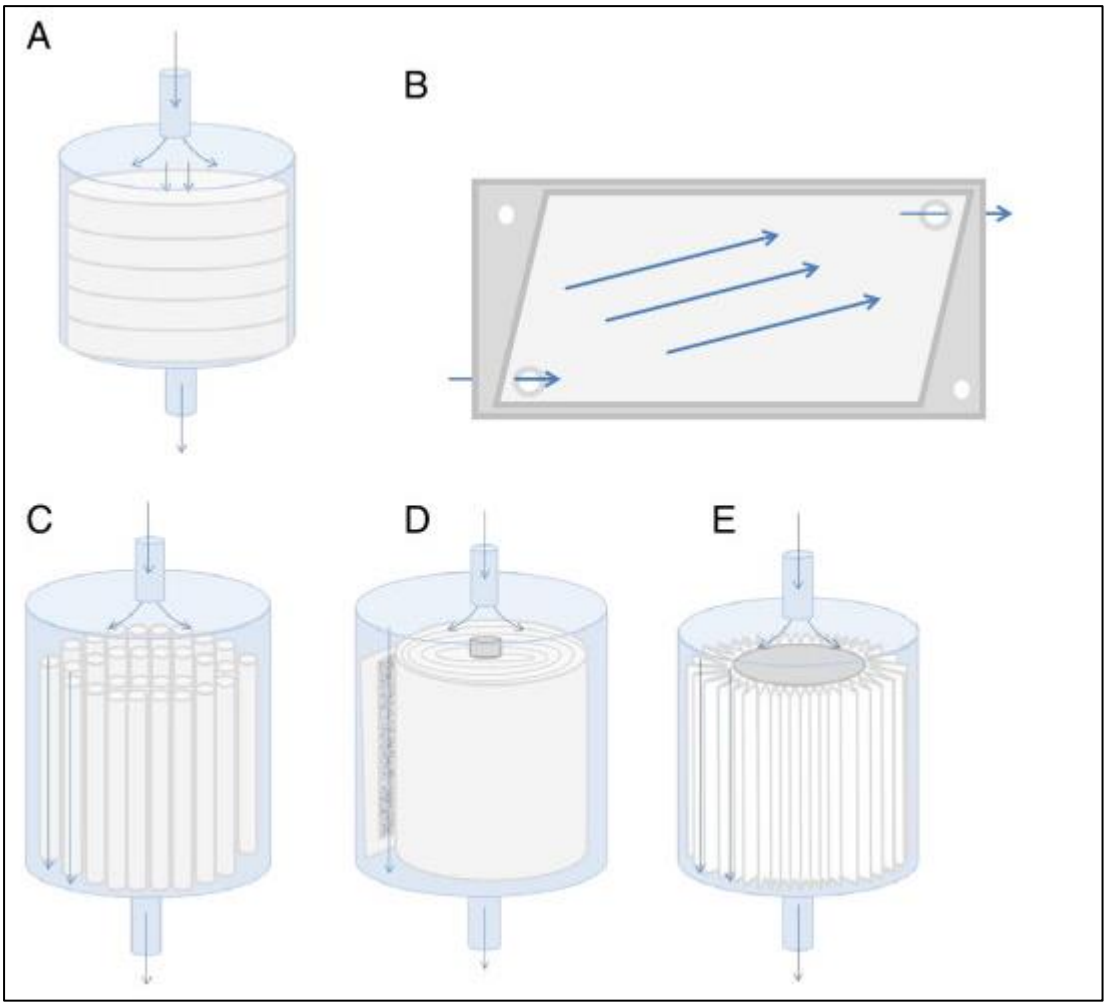

Figure 23 Summary membrane chromatography modules (Suen, et al., 2003)

However there has been a reluctance to engage with membrane chromatography as a capture or polishing step, as the binding capacity and resolution performance is considered to be moderate, compared with resin chromatography types (Bhut, et al., 2010). As there is a reduced surface area, due to the larger membrane channels, there is less binding capacity associated with membrane chromatography (Orr, et al., 2013). Therefore attempts are being made to improve the surface chemistry of absorbers, as binding capacity is the limiting factor at present, if overcome that will shift the paradigm in the direction of membrane chromatography. Several studies exist in the literature demonstrating this. The performance of a new anion exchange membrane (AEX) adsorber, a commercially available membrane adsorber from Sartorius Sedium, as well as a resin column (from GE) under preparative scale conditions is compared by Bhut (Bhut, et al., 2010). Using breakthrough protein analysis, the study found that the AEX membrane performed better in terms of capacity, binding and separation compared to the existing commercially available membrane (Sarto Bind D membrane) and the resin column (HiTrap DEAE FF). The improved adsorptive functionality

was accomplished by using surface grafting, a form of atom transfer radical polymerization, to yield a tertiary amine functional AEX membrane (Bhut, et al., 2010).

Similar findings are also shown by Wang, which describe the application of multimodal membrane adsorber in the use of a chromatographic stationary phase in a bioprocess (Wang, et al., 2015). Compared with commercial cationic multimodal adsorbers, this multimodal membrane adsorbers have superior static, dynamic and load capacity as well as allow a higher ionic strength salt to be used in its application, due to the incorporation of functional groups that provide orthogonal modes of interactions (Wang, et al., 2015). Flowrate again was not limited by the membrane therefore; a high linear velocity could be used, indicating that adsorption rate not mass transfer is the rale limiting step of the bio-separation application (Wang, et al., 2015). Again it would suggest that, a multimodal membrane adsorber would outperform a similar HIC chromatography process.

2.4.4. Novel Tech 4 – Pre Packed Columns

The requirement for more options in downstream processing is growing, particularly in the use of single use disposable systems. The predominant chromatography mode used in monoclonal antibody processing, is the use of Protein A in affinity chromatography. However a number of issues exist around the use of this matrix. The costs for per litre of protein are an average $12,000, its binding capacity and life span is being reduced due to the increasing titres seem in upstream processing, as well as the problematic issues seen in column packing of these resins forcing process designers to look at other options (Langer, 2014).

Pre packed chromatography columns are now available as an alternative to the in house method of self-packing. Most resin suppliers (GE Healthcare, Millipore, Pall, and Sartorius) now offer columns that are pre packed at the site of the resin manufacturer, where it is packed into a disposable container and shipped to the end user. These columns as they are essentially an “off the shelf package”, can be pre-qualified and immediately released for GMP manufacturing. The largest available commercial pre packed column is available from Repligen at a diameter of 80cm. The pre packed columns are known as OPUS (Open Platform User Specified) columns and can be filled with any resin as required by the process. A prepacked column is similar to a disposable column as it has an upper and lower adaptor as well as a mesh plate to hold in resin. However all these components as well as the column housing is a disposable plastic such as polypropylene or platinum cured silicone which are all USP (United States Pharmacopeia) VI certified. The flow distributor is a proprietary design

to Repligen and this promotes unique flow distribution for resin and product flow within the column. An image of the column is shown below in figure 24.

Figure 24 OPUS Pre Packed Column (Repligen)

Once concern with columns is when they are moved the resin bed may shift and the asymmetry may shift out of specification. This would be of particular concern for pre packed columns as the packed could be disturbed during shipping to site, requiring re packing at the vendor facility. However the shipping validation of these columns, specifically asymmetry and HETP (Height Equivalent Theoretical Plate) has been tested and is in accordance with the medical device standard for shipping, ISTA Standard 1A (Grier & Yakubu, 2016).

In summary pre packed columns could be an alternative solution to in house packed columns. This option could be affectively used in scaling up processes as well for contract manufacturing organisations, who require quick changeovers in the manufacturing of different products. However the costs of materials and labour for in house packing, versus using pre packed columns, must be analysed individually by the end user to ensure the economics are favourable. Also the current diameter is limited to 80cm compared to 1.8m in a stainless steel or glass column.

3. Chapter 3: Removing the Buffer Preparation Bottle Neck using In-Line Conditioning Technology.

The previous sections have discussed the bottle neck witnessed in manufacturing as a result of increasing titres in upstream processing. As a result of the commonly used "batch model", in commercial manufacturing, the bottle neck has now shifted towards the capture and purification areas in downstream processing (Sterling, 2011). This primary cause of bottle necks has also created secondary follow on bottle necks, in the services supplied to support downstream unit operations. These secondary bottle necks result in an overall increase in lead time and costs in the production of a batch for a particular biologic product. These services include purified water (in the form of WFI) and buffers, required for the various stages of chromatography and filtration in downstream purification processing (Sterling, 2011).

3.1. Conventional Method for large Scale Buffer preparation and Storage

The most common method for preparation of buffers at commercial scale manufacturing is to procure powders of the specification required for the process and then hydrate these powders using a purified water source, for example WFI. This is usually accomplished by using a suite of tanks, including buffer preparation and storage tanks. At commercial scale due to the large columns being used (which can be anything up to 1.8 meters in diameter) and the number of different types of buffers that can be used in the various modes of chromatography, as well as the three chromatography steps which is typical in a biologics process, all these factors result in requiring very large suites containing many vessels. Furthermore if a facility is designed with a defined range of buffers with a known range of specification, buffer preparation can be completed in liquid form with the concentrate stored on site in the plant and sent to the buffer make up tank to be diluted to a defined concentration by WFI. If making buffers from powder stock or from liquid concentrates, each have to be sterile filtered into a buffer hold vessel, accomplished by a set of 0.22 micron filters, where it is stored until called by the user skid. Typically buffer volumes greater than 100,000L could be required per production batch. A typical buffer preparation suite is illustrated in the images below.

Figure 25 Conventional Buffer Preparation & Hold Systems

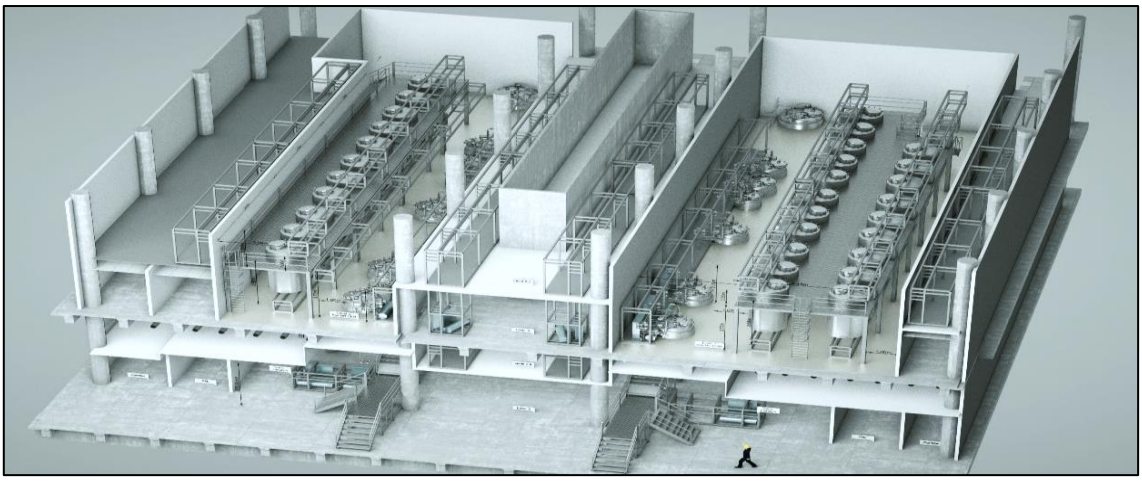

Figure 26 Facility model of a Buffer Prep & Hold Suites – (Biologics Plant Singapore)

Tank volumes can range from 500 to 15,000 litres, requiring multiples of these. This allows buffers to be pre made and be delivered to the chromatography columns "just in time", resulting in a major footprint of a facility being allocated to buffer preparation and storage activities, as is clearly illustrated in figure 25 & 26 above. Adding to this, the interconnecting pipe work required between the WFI source to the preparation and hold vessels, as well as the pipework required to be delivered to the point of use, the "clean" and "black" utilities to support processing including for SIP and the HVAC to support the graded area of the suite, adds up to significant capital investment required for a Biologics

facility. The buffer preparation and hold suite above amounted to an initial capital investment of €15 million. This does not include the commissioning and qualification effort required for such a large suite and the continuous performance monitoring (PQ - Cleaning & Sterilisation Validation) of the systems associated with it large scale buffer preparation.

Langer gives us details of a survey conducted by "BioPlan associates Inc." of a number of biologics facility's, that illustrate the costs for buffer preparation can reach close to €10million per year (Langer, 2016). This included both stainless steel and single use facility's using in house buffer preparation operations, as per figure 27.

Facility Type	Costs
All facilities	$19.72/L
<100,000 L/year	$29.17/L
100,000–500,000 L/year	$18.30/L
>500,000–1,000,000 L/year	$17.93/L
>1,000,000 L/year	$11.13/L

Figure 27 Average annual costs for in-house buffer preparation by liter of production.

The study compared the costs of in house production versus potentially outsourcing all buffer make up activates. However the costs to outsource were close to in house production with the gains in the removal of labour and utilities, negated by a potential risk in loosing supplier of QA/QC problems arising with the use of an external vendor (Langer, 2016). For larger commercial manufacturing platforms, outsourcing is not a greater advantageous option.

The operation of buffer suites is also a labour intensive process and time consuming to run. It requires accurate weighing and dispensing either at the point of use where the buffer is hydrated or else in a GMP warehouse. It then has to be staged and brought into the buffer suite, which is usually situated in a graded clean room. The powder is then loaded into a mixing tank, while measuring the weight added and verifying this against a batch record for the process to ensure correct buffer concentration is obtained. After mixing with WFI online or offline tests for conductivity and pH are conducted before it is transferred to either a buffer hold tank or directly to the point of use while being sterile filtered to reduce possibility of cross contamination. The above steps require many

interactions with a batch record, some which may require second person verification. This interaction with documentation is one of our “MUDA” wastes.

Buffers can also be charged into single use bags, where they can be stored at the point of use. Following on from this, the vessels in which the buffers have been prepared are cleaned in place (CIP) or flushed with WFI and Steamed in place (SIP) before the systems can be released back into manufacturing of further buffers. Not only operational but the continuous maintenance costs, of these suites can add total operational expenditure of the facility. The larger the suite, the more engineering supplies required for the various elastomers and seals embedded in the sterile boundary containing the system.

The production scheduling of this is extremely complicated as a number of various systems are required and rely on the other to be released for buffer preparation. Toumi, using batch simulation software (Super Pro Batch Simulator) creates an operational model for a multiproduct facility producing monoclonal antibodies in two different production trains (Toumi, et al., 2010). As part of this project, they attempt to analyse the bottle necks that exist in a current process with two production trains. They attempt to remove the bottle neck by rearranging or removing unnecessary operational steps. Not only in the creation of the model, but the output of the exercise itself, determines that buffer preparation is the most complicated of all the production areas to model accurately. In the study an assumption is made that 40 different buffers are required for use in both production trains, which require 40 different production recipes (i.e. a combination of specific set out of batch record instructions and a specific control system recipe). Figure 28 illustrates the Gantt chart of the systems occupancy during production (Toumi, et al., 2010).

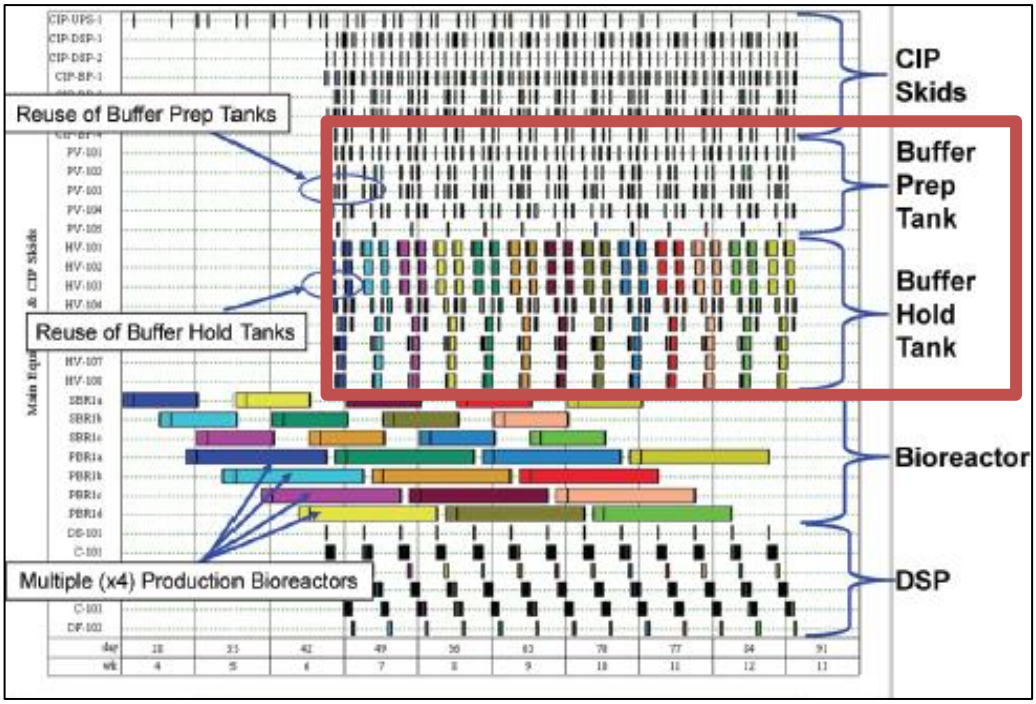

Figure 28 Equipment Occupancy (Toumi, et al., 2010)

We can see within the red square, the usage of the buffer preparation and hold suites are intense, frequent and are required to be prepared for downstream processing, well in advance of the unit operation beginning, to satisfy a "just in time" model. Figure 29 illustrates the amount of potential constraints that may occur in buffer prep due to the reliance on other systems within the facility to allow processing.

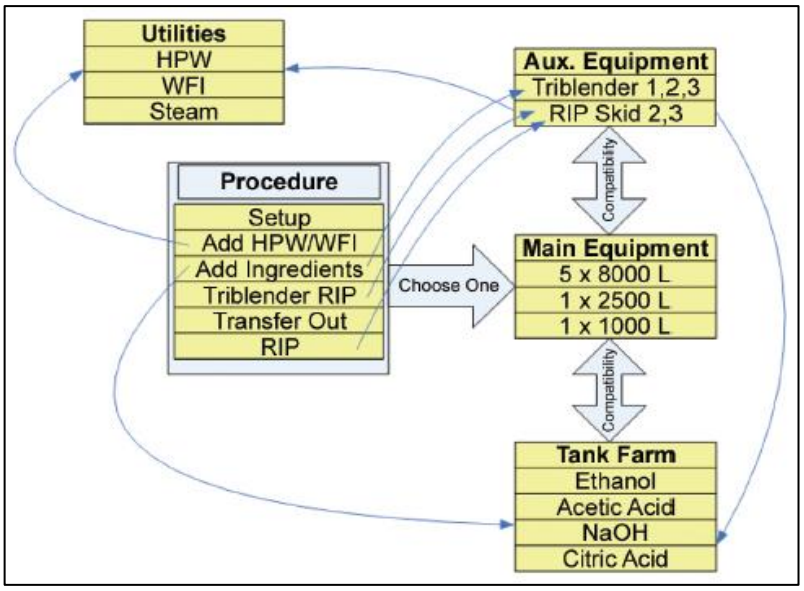

Figure 29 Buffer Preparation Constraints (Toumi, et al., 2010)

The analysis determined that a number of bottle necks existed within buffer prep operation itself specifically, manifesting as a result of cleaning of shared equipment for buffers. An example being if a 1000L vessel, is required for mixing of two different tank farm concentrate stocks there would be a delay due to cleaning of the vessel and its shared delivery lines, between different buffers. The WFI system at peak demand was also unable to supply the required volume of WFI, for the various buffer prep activities. This is typical of what is seen in buffer preparation suites. Cycles times of individual operations are extended as a result the overall lead time of the production process. The root cause being a constraint in one or more, associated sections of shared auxiliary equipment, raw material or personnel to run the operation.

3.2. Buffer Specifications

Buffers are essential in downstream processing to ensure process robustness, maintain the quality of product and ensure the expected yield is achieved. Buffers typically come in liquid form and are made up of a mixture of acid, base and salt, usually a weak acid and its conjugate base (Linderholm, et al., 2017). The key specification for a process buffer is to maintain the pH of the solution, as it is being varied by the introduction of trace amounts of a strong acid or base, required by the specific purification process (Jungbauer & Walch, 2015). To determine this calculations are conducted to determine the amount of ingredients to mix together to produce a buffer of a specific pH and ionic strength. The specification of the buffers must also allow for any slight variations, by the introduction of more or less buffer components or contaminants into a system. This is known as buffering capacity and is defined as “the calculated number of moles, of strong acid or bases that give rise to a change in one pH unit per unit volume of solution” (Carredano, et al., 2018). If the process deviates slightly outside its operating range, a buffer with poor buffering capacity maybe unable to respond to this resulting in a low process robustness and undesirable yield. It is a measure of the protection a buffer offers against changes in pH. Buffering capacity is dependent on two primary elements, the pKa value and the concentration of the buffer. Changing buffer concentration can cause the pKa to within a buffer concentration range (upper and lower) therefore the pH which is directly correlated with pKa can also change in proportion to this (Carredano, et al., 2018). The Henderson-Hasselbach equation can be used to determine pH. The formula is given below;

$$pH = pKa + \log\left(\frac{[A^-]}{[HA]}\right)$$

Changing pH and ionic strength can be used, in the manipulation of a proteins isoelectric point (pI), for a particular mode of chromatography or filtration. The pI is the pH at which any given protein has an equal number of positive and negative charges. Drifting above or below this pH, either towards the acidic or basic regions, the proteins will be charged either positively (acidic) or negatively (basic) depending on the direction of the drift. This property has important biochemical implications in protein purification. An example of which is hydrophobic interaction chromatography (HIC), which uses a changing salt gradient to bind and elute proteins to the columns matrix. Buffers used in biologics should have a pKa residing between 6 to 8 (Mohan, 2006). There is also a number of other criteria that they should meet including, having a high solubility in water as well as low in organic solvents, they should not permeate, interfere or create any toxic effects within the cell and the Ionic composition of the medium and temperature, should have minimal effects on buffering capacity. Buffers usually fall within four buffering systems, these being acetate, phosphate, citrate and Tris along with sodium chloride (Mohan, 2006). An important aspect to note is that temperature shift and dilution effects have a noticeable effect on pH, due to the common ion effect of adding two buffers together of similar ions. This can lead to deviations in processing and a poor yield due to the effect of pH on pKa and therefore pI (Carredano, et al., 2018) (Mohan, 2006). This is an important factor when considering the makeup of buffers at manufacturing scale.

3.3. Inline Dilution vs Conditioning

The previous section discusses the challenges that are experienced in the capital costs associated with buffer preparation suites, as well as their operation in a typical biologics purification process. Due to pressure of time to market as discussed in chapter 2, as well as the uncertainty of when to commit to facility construction, little attention has been given to process optimisation of buffers and the focus of developers still lies in product purity, consistency and potency (Jungbauer & Walch, 2015). In an attempt to address the rising capital and operational costs of buffer preparation, alternatives are available to the traditional buffer preparation methods. One of these alternatives is the use of In-line Conditioning (ILC) technology, a system offered by GE Healthcare.

With ILC, buffers are prepared from concentrated stock solutions of salt and a purified water source (e.g. WFI), plus an acid and a base component. A similar concept has been experimented in industry previously in the form of "In-line Dilution" (ILD) systems. This involves preparing a buffer of a known concentration, diluting this buffer with WFI outputting the buffer of desired concentration. A number of publications have been written demonstrating the benefits of using this technology (Matthews, et al., 2009) (Patterson, 2009) (Malone & Li, 2010) and a number of companies offer the platform as a skid package, for integration into an existing or new facility including Lewa Bioprocess Group, AsahiKasei and TECHNIKROM. A simplified drawing of an ILD system is shown in figure 30 below.

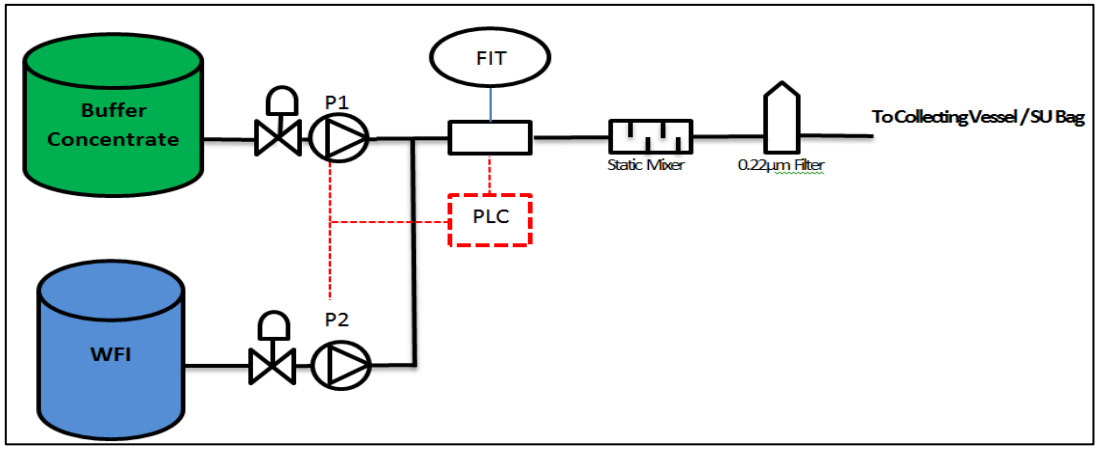

Figure 30 ILD Process Flow Drawing

The process works by attaching a concentrate buffer to the pump shown as P1. A WFI supply is connected to pump P2. Given the known concentration factor Buffer and WFI are mixed together inline in a static mixer, with a flow meter monitoring the total flow of the process stream. Using basic process control, the two pumps can be automated to give the specified diluted buffer which after passing through a sterilising filter, can be sent to the point of use of the buffer or into a SU bag for storage. Benefits include reduced facility foot print as large buffer tanks are substituted for smaller single use bags containing the buffer concentrate and also the elimination of utilities to service and clean the tanks.

However there is one significant draw back with the use of ILD systems. As mentioned in the previous section dilution of buffers has an effect on changing pH and the

common ion effect will limit the concentration factor when preparing a stock solution as illustrated in figure 31.

Dilution Effect on Buffer	pH Before Dilution	pH After Dilution
NaH_2PO_4—Na_2HPO_4	6.50	6.84
Citric acid—Na_3 citrate	5.00	5.35
Tris HCl—Tris base	8.00	7.92
HAc—NaAc	5.00	5.05

Salt Effect on Buffer	Infintely Dilute	50 mM Buffer	50 mM Buffer, 0.5 M NaCl
NaH_2PO_4—Na_2HPO_4	7.21	6.84	6.42

Figure 31 Effect of Dilution and Addition of Non-buffering Salt on the pH of a Concentrated Buffer (Carredano, et al., 2018)

Using ILD only one buffer concentrate, can make one “process buffer” as required by the dilution factor (e.g. 10x). However the pH and conductivity of the buffer may have drifted during dilution, resulting in additional manipulation of the buffer required with subsequent titration or dilutions to bring it back within spec. As buffer specification is a critical process parameter, a small change in pH (typically +/- 0.15 pH) will result in a deviation in the production batch. The majority of chromatography processes operate by producing a buffer gradient, either pH or conductivity shift, to activate or deactivate a particular mode for separation. ILD systems cannot achieve this by only using the concentrate and the diluent process streams. It does not allow for dynamic control where mass balance and stoichiometry is taken into account. Therefore ILD systems are limited in the buffers that they can produce, which for a facility that may require multiple or large volumes of buffers, this solution may not be a flexible enough to deviate from the conventional approach. Furthermore for ILD systems to supply buffer on demand, for downstream purification processes, the concentrated buffer needs to be prepared accurately, either by making up in house or procuring a stock concentrate from a buffer vendor. Dilution requires accurate pre adjustment at the concentrated buffer level, since post dilution titration can fix the accuracy of one buffer property, only at the cost of other parameters of the buffer.

However if a system can add more pumps for delivery, as well as create a control system that monitors and adjusts for the other critical parameters of a buffer, such as pH and conductivity, then the effects seen in in-line dilution can be counteracted. This is the principle of ILC technology which is illustrated in figure 32.

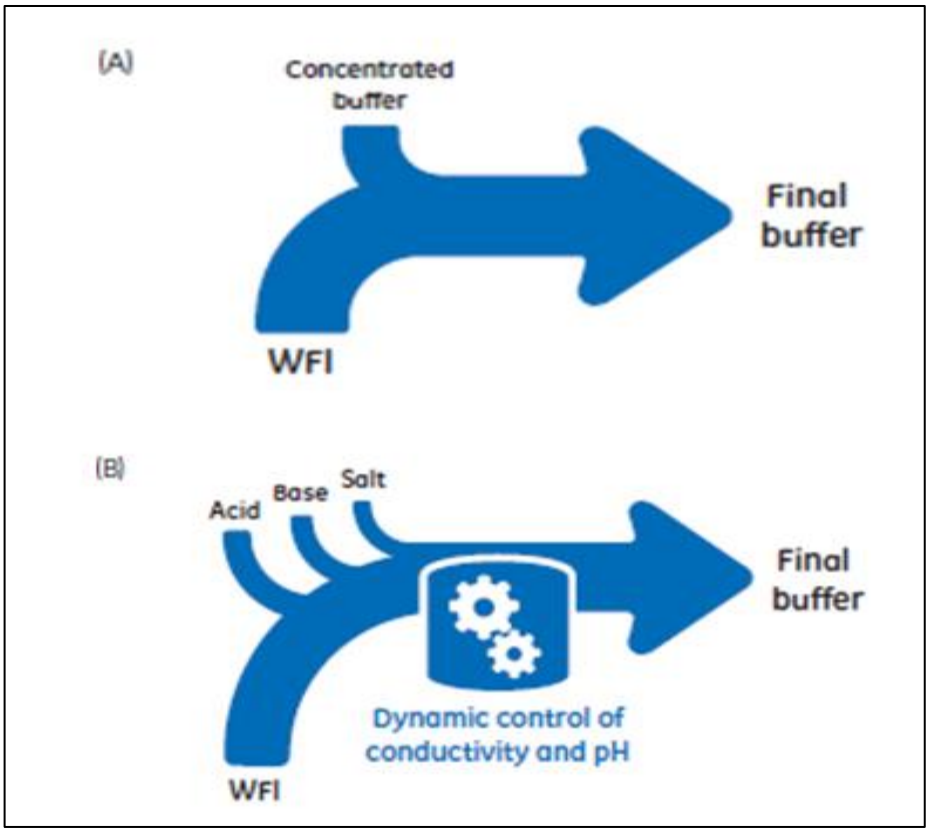

Figure 32 (A) ILD vs (B) ILC

Similarly to ILD, ILC is a system for in-line buffer formulation at large scale, using stock solutions and a purified water source (WFI), in combination with a purification step such as chromatography or filtration. With ILC significant floor space and tank volume reductions are possible even more than ILD, compared to conventional buffer preparation methods, as the need for additional tanks or buffer bags to adjust. Stock solutions contain only one buffer component and therefore can usually be much more concentrated than a prepared concentrated buffer. Using ILC, it is possible to prepare buffers of different strength, pH and salt concentration from the component stock solutions, independent on effects seen in in-line dilution. Therefore no rework of a buffer is required, streamlining the process.

3.4. ILC system overview

A process flow diagram for an ILC system can be seen in figure 33.

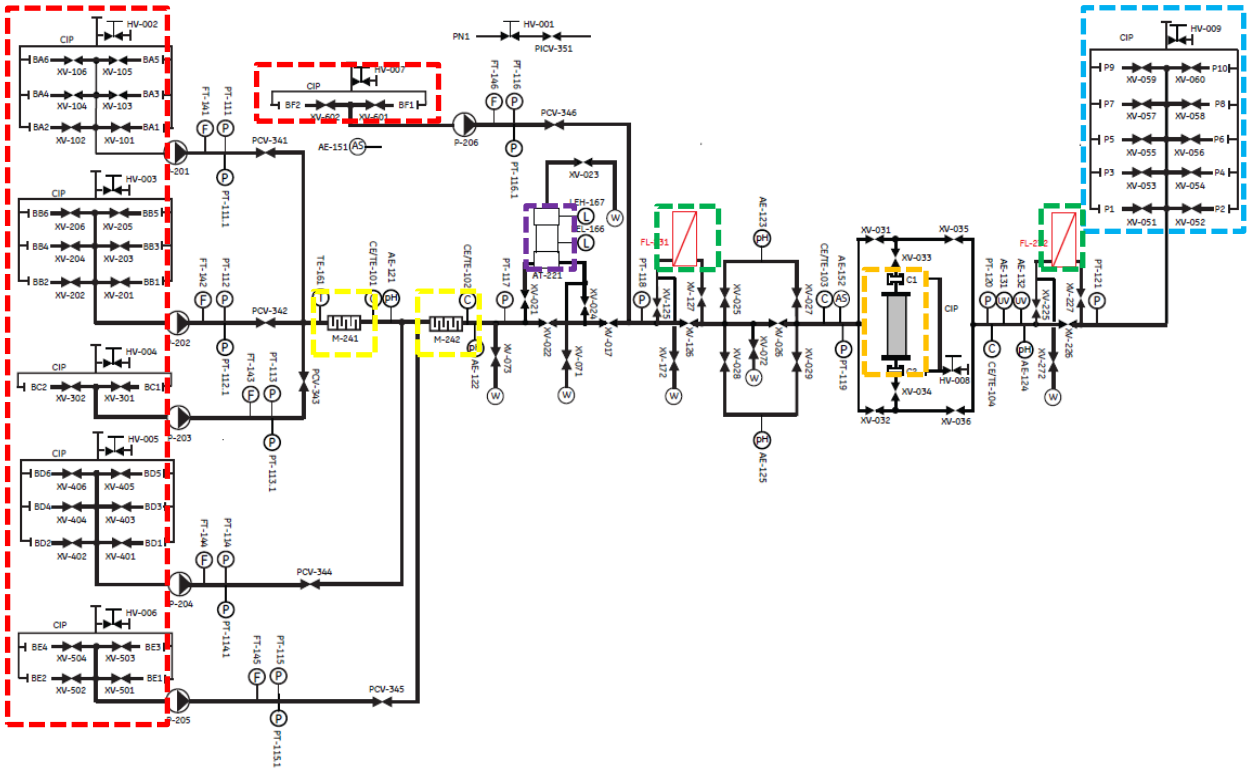

Figure 33 ILC Process Flow Diagram (GE Health Care)

The ILC can be connected directly to a chromatography column, to supply downward or upward flow as shown in the orange dotted square. A flexi hose connected the ILC skid to a column therefore any sized column can be accommodated.

The inlets to the skid are shown in the red dotted squares. These are composed of a number of block manifolds, each with a defined number of connections to attach the various types of buffer as well as the sample being used in the process. This system allows; an acid, a base, WFI, a low salt concentration buffer and high salt concentration buffer, to be connected to the system simultaneously. The pipe specification for the various buffers is polypropylene to accommodate the use of corrosive acids, bases and buffers that might not be suitable for stainless steel.

The outlet ports are shown in the blue dotted square. Ten outlet connections are available for use for various functions, such as collecting pre made buffers for storage, collecting chromatography fractions, collecting the bulk drug substance from a chromatography step and for sampling, via a nova aseptum assembly, in supporting various quality control release tests. The skid is fitter out with “jumper spools” on both the inlets,

column connections and outlets, to allow for CIP on the skid with a cleaning solution such as sodium hydroxide.

Uniformity in the buffer solution is a key requirement of the system. If the buffers are not blended correctly the required specification will not be achieved, therefore wasting buffers. To aid in the mixing the various buffer solutions, two static mixers are placed at the junctions where the acid, base and WFI lines meet as well as where the salt buffers are introduced to the system. These are shown in the yellow dashed lines on the PFD in figure 33. An image of the type of mixer is shown in figure 34.

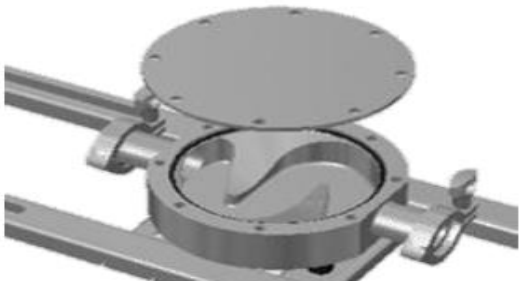

Figure 34 Inline Static Mixer (GE Health Care)

The ninety degree bends as well as the increased velocity seen at the periphery of the mixer walls, increases turbulence therefore supporting blending of the buffer liquids. By coincidence two more sets of components on the skids help in the mixing process however this is not their primary design intent.

An air trap (bubble trap) is installed on the common process line of the ILC system, shown in the purple dashed lines in figure 33. The function of the air trap is to trap air and remove it from the system before reaching the chromatography column. Air bubbles can damage chromatography matrices therefore removal of air is critical in this system. A vortex is created in the air trap and the liquid in the air trap is pressed downwards and outwards by the centrifugal force generated while air is separated in the centre of the chamber. The rotation eliminates pockets of stagnant liquid, which prevents unwanted build-up of solids and simplifies the cleaning of the air trap. Liquid levels in the system air trap are continuously monitored by switches that use ultrasound to monitor the liquid levels in the air trap and send this information to the control system. The ultrasounds are transmitted through the air trap and allow liquid levels to be monitored without the need for contact with the process buffers. Therefore despite its main design intent the centrifugal rotation created in the bubble traps, aids in the mixing process of the buffers.

Additionally two inline sterilising filters (0.2µm) are available for use on the skid shown in the green dotted line in figure 33. The first filter position is located in the flow path between the air trap and the column to reduce the potential for bioburden in the system, as well as preventing foreign objects from contaminating the column. The second filter position is located in the flow path between the column and the outlets with the same intent of reducing product contamination. The filter can be single use opticap type that can be disposed of after each batch or else a cartridge type that are inserted into a stainless steel housing. Again despite the design intent of reducing bioburden the back pressure created within these components aid in blending the buffers together.

A number of instruments are integrated into the system to provide the various control functions that are capable with ILC. Each manifold block has a dedicated pump and flowmeter and well as pressure transmitters to monitor for pressure spikes. pH meters as well as combined conductivity and temperature probes are positioned at the junctions of the system for process control. UV meters are installed after to column to monitor for protein and capture the bulk drug substance as well as fractions if required.

3.5. In-line Conditioning Functionality

ILC systems are controlled by a software package from GE known as Unicorn. The difference between ILC and traditional chromatography systems, is the capability of ILC to formulate buffers with good accuracy utilising the instruments as mentioned above. This leaves us with an ability to produce buffers using three different types of control modes, “recipe and flow”, “pH-Flow” and “pH-Conductivity” feedback. Only one control mode can be used at a time. The control philosophy is displayed in figure 35.

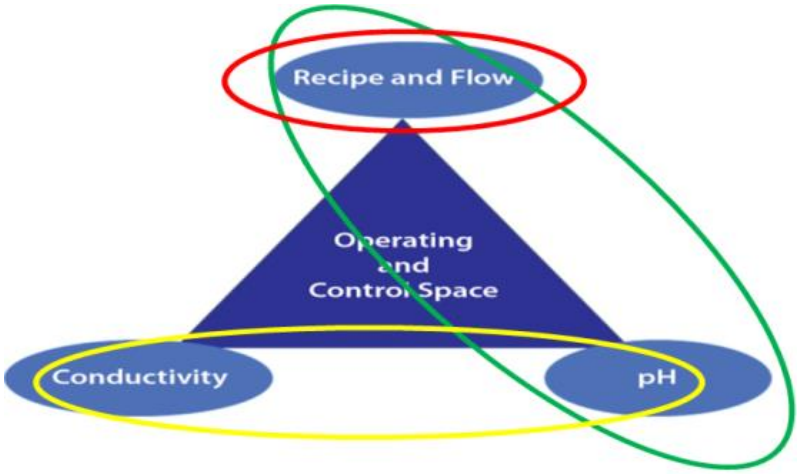

Figure 35 ILC Control Mode options (GE Health Care)

Each control mode has advantages and disadvantages associated with it. Choosing the particular mode that is used will depend on the scenario in the process.

In flow and recipe mode the system uses pre-defined recipes to determine the flow set point for each pump. Flow control mode ensures the correct flowrate from the stock concentrates are obtained including WFI. The four different pumps on the system are set to a percentage of flow, the sum being 100%, with a defined flowrate in litres per hour (L/hr). The pump percentages have to initially be determined by calculation, using GE proprietary algorithms. The percentages can be also adjusted in a step change or linearly to obtain a gradient. In this control mode pH and conductivity instruments, can be used to monitor and alarm if the wrong specification is seen by the system. This is a useful application if the wrong stock concentrate is used by an operator for a particular chromatography step. The disadvantage of this control mode is accurate stock solutions are required; therefore the system will not respond if pH or conductivity drifts. Also if temperature is not controlled in the process, pH and conductivity may also change, which this control mode cannot compensate for.

In pH-Flow feedback control, the pH probes on the skid are used to control the pumps to achieve the target value. The flow feedback element of this mode ensures that the buffer concentration is kept constant. In instances that a salt buffer is being used, it will maintain the salt concentration at the desired level including over a gradient. In this control mode, within the unicorn recipe, the buffer and stock solutions concentration needs to be specified for the acid and base buffer. The drawback of using this control mode is the potential for bias to be seen in relation to the pH meter. If this is incorrectly calibrated or drifts during processing, the incorrect buffer may be produced. In the previous control mode algorithms are required to determine the pump percentages. This control mode can also be used to determine the pump percentage to be inputted into the recipe. The control mode can be used as an analogue machine solving the equations of the buffer equilibrium.

In pH and conductivity feedback, both the pH and conductivity probes on the skid are used to control the pumps to achieve the target values. If a salt buffer is being used, the specification of the acid, base and buffer concentration must be included in the recipe. If no salt is required then the stock solution can vary. This mode of control is suitable for buffers where there is a set point for conductivity, allowing the pumps to easily adjust to meet the set point. Similarly to the last pH-Flow control mode the drawbacks of using pH-

conductivity lay in the potential for both monitors to drift during processing or to be setup or calibrated incorrectly. The table below illustrates the specific control mode and the advantages and disadvantages of using a specific mode

Table 3 Advantages and Drawbacks of ILC Control Modes

Control Mode	Benefits	Drawbacks
Recipe & Flow	1. Robust if temperature is constant 2. Recipe can be determined by algorithm or pH-Flow 3. Insensitive to bias in pH or conductivity meter.	1. If temperature varies may lead to variations in buffer 2. Accurate Stock Solutions required 3. No dynamic control used.
pH-Flow	1.Delivers Correct pH regardless of temperature shift 2.Generate recipes to be used in Recipe & Flow	1.Sensitive to bias in pH meter 2.Requires accurate stock solutions
pH-Cond	1.Delivers correct pH regardless of temperature shift 2.Delivers correct conductivity regardless of temperature shift	1.Sensitive to bias in pH meter 2.Sensitive to bias in conductivity meter

In determining which control mode to use, it is necessary to understand the three different types of acid and base mixing;

1. Weak Acid and Weak Base (Weak Weak)
2. Strong Acid and Weak Base (Strong Weak)
3. Weak Acid and Strong Base (Weak Strong)

In other words the target pH may be obtained by appropriate trade-offs between corresponding acid and base flows (weak acid and weak base) or by adjusting the flow of a

strong component (strong acid or strong base) while keeping the flow of a weak component (weak base or weak acid respectively) constant. Using a strong component for titration locks the target flow rate of the weak component because this gives the buffer concentration which should be constant. Combining these three different types, with the possibility to include conductivity feedback or not and to include salt or not, results in the total of seven sub-modes of control when using pH/conductivity feedback. The seven sub modes are the following:

1. pH-Cond Salt Weak Weak
2. pH-Flow Weak Weak
3. pH-Cond No Salt Weak Weak
4. pH-Cond Salt Strong Weak
5. pH-Flow Strong Weak
6. pH-Cond Salt Weak Strong
7. pH-Flow Weak Strong

Figure 36 illustrates what control mode should be used in which the buffer type collapses to. Note it can be seen how some combinations collapse to the same sub-mode of control.

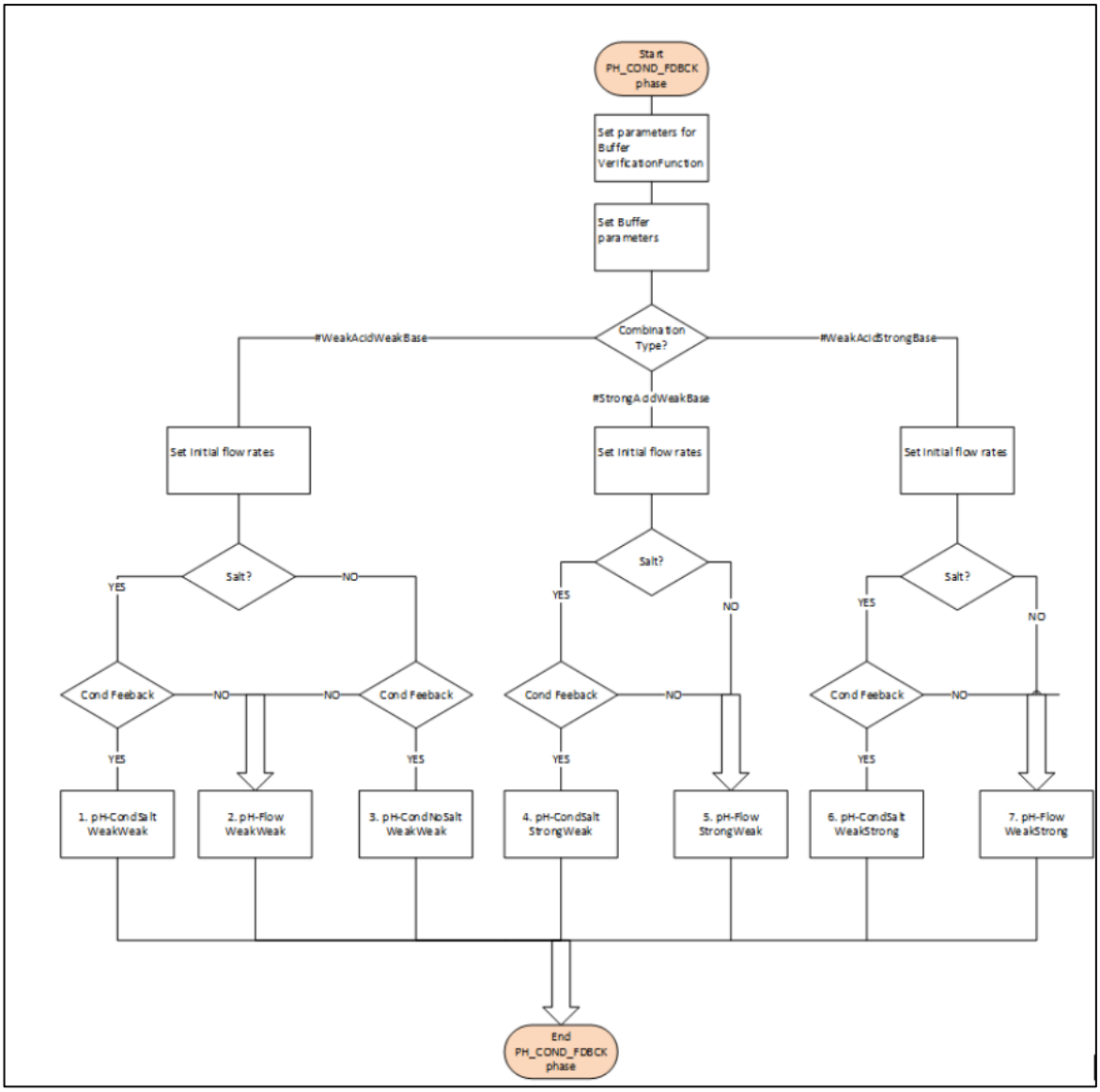

Figure 36 Control Mode Decision Matrix.

The dynamic control capability within the system is shown in this matrix as well as the system flexibility in its ability to produce a range of buffers. The robustness of the ILC control system to achieve the above control modes is illustrated in figure 37.

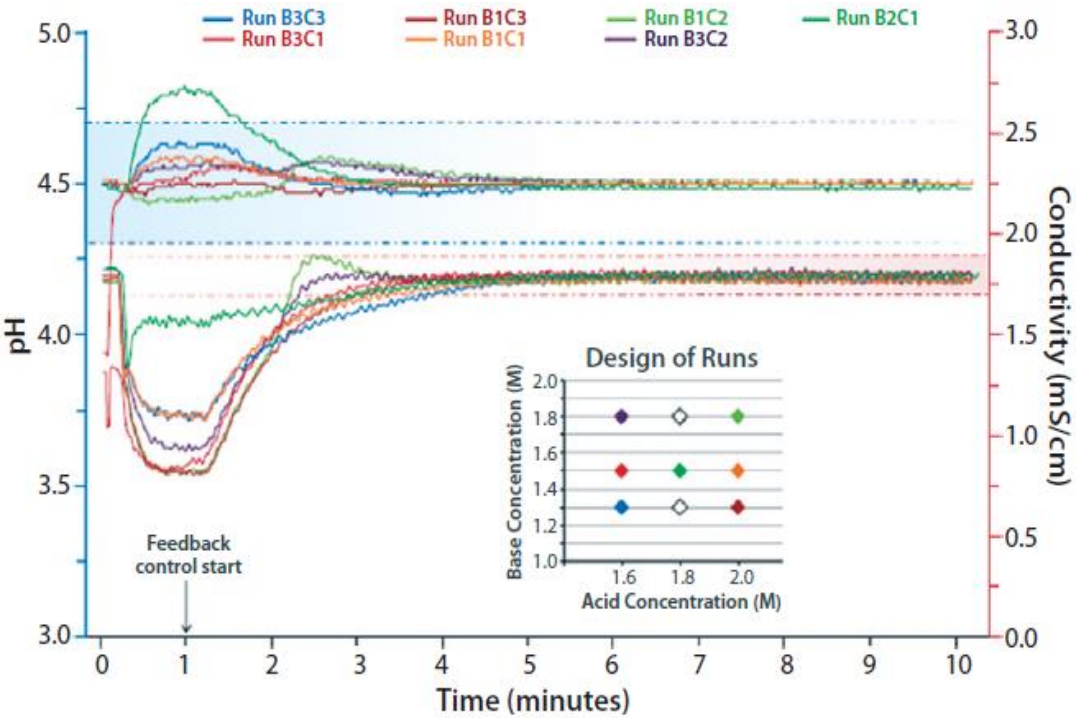

Figure 37 Conductivity and pH curves from 7 runs in a robustness challenge study using pH and conductivity feedback control (Carredano, et al., 2018).

The system was challenged with three different concentrations, of individual component stock solutions, compromising three acidic solutions and three basic solutions, of the same buffer system (Carredano, et al., 2018). The range of concentrations was mixed in different combinations for each run, while applying the same unicorn method. As the data reveals, all combinations resulted in the same buffer, with the same critical quality attributes. The specifications required a pH of 4.5, shown in the blue dotted range and a conductivity of 1.8 mS/cm shown in the red dotted range. Therefore the system demonstrates its robustness that does not depend on starting concentrations of the acid and base solutions. It is noted that the curves look to be out of control at the beginning of the run, however this is due to the pumps not being optimised. With adjustment of proportional, integral and derivative parameters (PID) within the control system, the pumps can be setup to respond quicker to allow the system to hit set point faster. Therefore the time to reach specification is reduced allowing buffer volumes to be saved (Carredano, et al., 2018).

3.6. Facility Layout Options

Due to the capability of the ILC skid to produce buffers from concentrated stocks, the dynamic of the downstream area of the facility can be changed to suit the buffer requirements of the process. An image of a typical sized ILC skid is shown in figure 38.

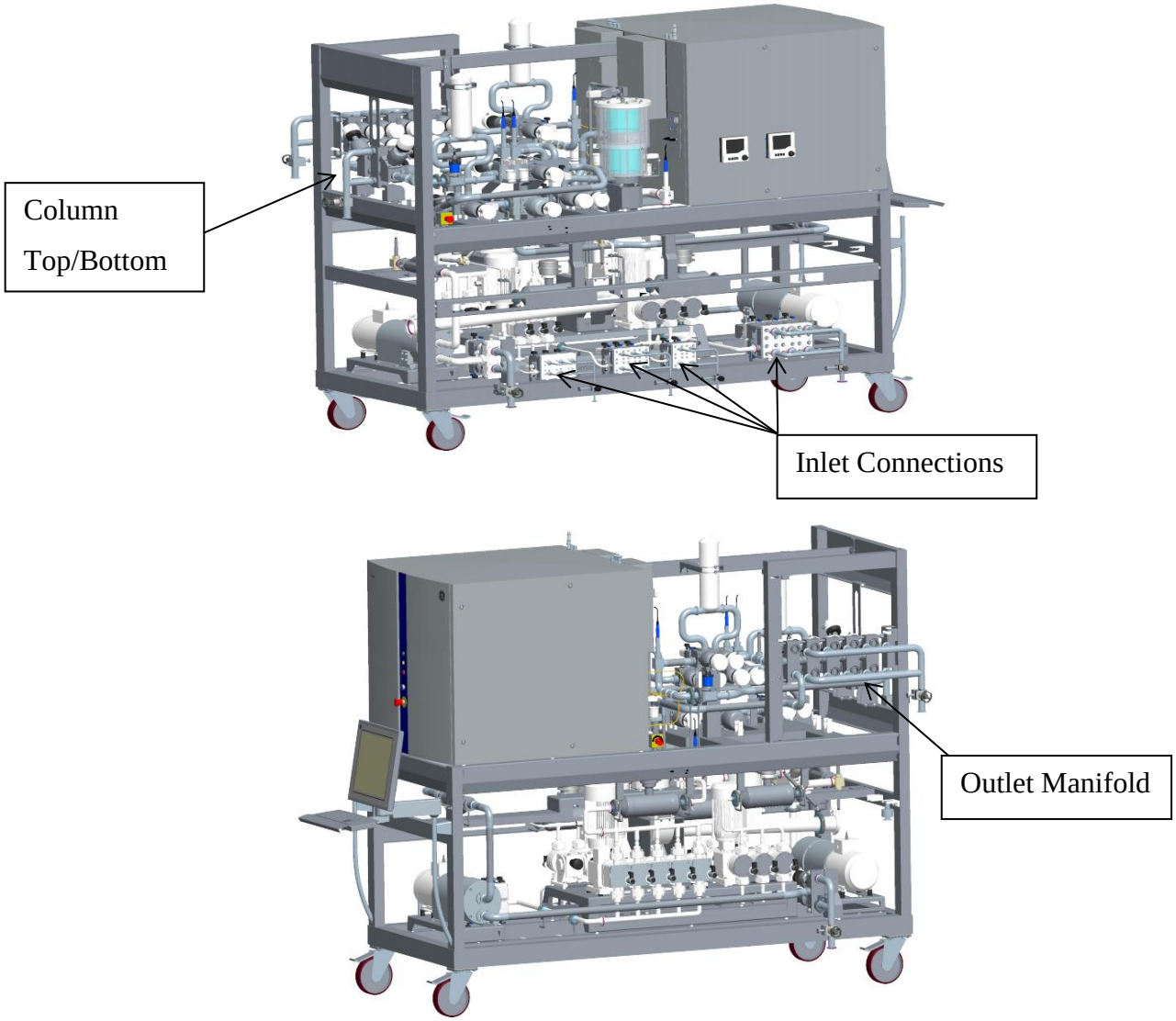

Figure 38 Typical ILC Skid Model (GE Health Care)

The skid itself will occupy approximately 3-5m^2 of floor space within the facility. Based on the type of process (mammalian vs E.coli), the volume of stock concentrates required and whether the facility is multi product or dedicated a single "block buster" drug, the layout can be adjusted to suit the operation of the facility. A number of layout concepts are detailed in the following sections, below each with much less foot print requirements compared to conventional buffer preparation suites, as discussed previously. All concepts are heavily influenced by single use technology and make as assumption that the

manufacturing areas are operated as a clean room of EU Grade D or C. The first layout presented below in figure 39 is the “ballroom” concept.

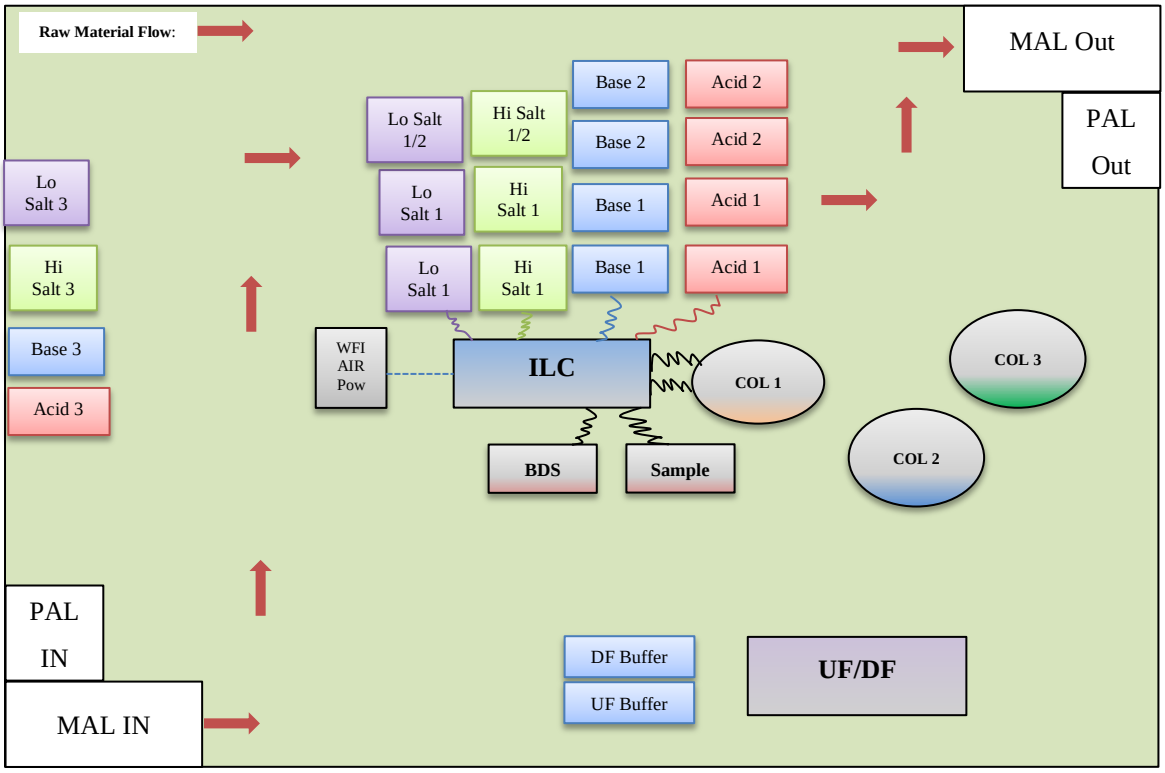

Figure 39 Ball Room Concept

The ILC is positioned at the centre of the cleanroom and supports each purification process within the room. A support column supplies services to the ILC consisting of WFI, air and power. The concept is based on using single use (SU) bags, within a rigid “tote” as a means to store buffer concentrates in a clean room. An example of which is the Meissner flex station tote shown in figure 40.

Figure 40 Meissner Flex Station Rigid Outer Container (Meissner)

A buffer vendor can be used to supply the concentrates at the required specifications, in the single use (SU) bags and tote. These totes can be moved in and out of the clean room via the material airlocks (MAL) and personnel airlocks (PAL). The flow of raw material is show by the red directional arrows. Once in the room they are connected to the ILC inlets via disposable tubing that is already incorporated on the bag. These connections can be made with "sterile connectors" supporting sterile processing. The sample product which will be typically coming from a harvesting suite can also be filled into a bag, transferred into the purification room and connected to the ILC. Typically there are three chromatography capture and polishing steps in purification. These are represented by COL 1, 2 and 3. The room can accommodate all processes. COL 1 is connected to the ILC first and the sample is run onto the column, supported by buffers 1 made on demand for that process. On completion of the run the bulk drug substance (BDS) is collected in another tote and sampled for the various required QC release tests. After a CIP cycle is complete on the skid while waiting for the QC release tests (if required) COL 2 is connected to the skid and the second purification step is run as supported by buffers 2. The process is repeated again for COL 3.

The reduced foot print also allows a UF/DF skid to be located in the room. On completion of polishing it is necessary to switch buffers, (diafilter) that will allow the bulk substance to be formulated and for potential storage in a blast freezer. The ILC can "pre make" the buffers for the diafiltration step ahead of time. These buffers are stored in the room and connected to the UF/DF skid when required to process the column eluate.

The ballroom concept has many advantages. Even though the cleanroom itself is small, all purification processing can occur in the one room. Once all raw materials (buffers) are moved into the area there is very little labour associated with processing, as all interaction with the equipment has been localised. The room encloses all the requirements for the process to be run. Furthermore once the buffers have been consumed the totes can be "collapsed" inward and removed from the area, allowing room sanitisations to be easily complete due to the available space. Furthermore as the totes are flexible and the necessity for "fixed piping" has been removed, any buffer concentrate and product can be processed in the room. This setup is desirable especially for CMOs (Contract Manufacturing Organisation) who require quick turnaround for different products. The disadvantage of this concept lies in the reliance on the operator to setup the equipment correctly, specifically making the right hose connection, moving all raw

materials in and out of the room, selecting the correct buffer and also by being local for the manual interventions required within the process. With the use of SU consumables the requirement for operator interaction increases, for example making and breaking the correct SU tube connections. Therefore labour intensifies by using this approach, requiring an increase in production personnel, to move the batch through the process.

A second facility layout is presented in figure 41. This layout is conducive towards continuous processing, if for example periodic counter current chromatography is implemented and relies more on automation to process the batch. However for the purpose of this discussion, an assumption is made that the typical batch purification process requiring three chromatography steps is used.

The area is divided up into three segregated cleanrooms shown in green, which can be operated as a grade D or C classification. Each cleanroom has a dedicated ILC skid, which is supplied buffers via transfer panels (TP-1, 2, 3) from a "GMP tote staging area" shown in blue. The totes for the particular chromatography steps are brought into the staging area, via the MAL IN, ahead of the batch, "manifolded" together with SU tubing and connected to the transfer panels. Once processing is finished the totes are removed from the area via the MAL out. Applying a 5 S methodology, the totes can be further divided to "lean" out the staging area, making the connections to the transfer panels more streamlined and obvious to the operator. On the clean room side the totes are then connected to the ILC inlets with flexi hoses. A controlled non-classified (CNC) corridor borders the processing areas and allows movement of personnel between the staging area, chromatography rooms and suites upstream and downstream of these.

Each cleanroom would also contain a hold tank, for receiving of product from the previous processing area. Typically a harvest cell culture fluid (HCCF) would be transferred from a recovery suite, into the first tank (TK1). Once a satisfactory level is reached the product can be transferred onto the first purification step via the ILC 1. This would undergo the typical wash and elution steps, before being transferred into TK2 for the second stage of purification. The process is repeated in the same fashion for the subsequent steps and can then be transferred downstream of the last chromatography step for further processing, such as viral inactivation and UF/DF. The advantage of using this process incorporating intermediate hold tanks and fixed piping to transfer between skids is a straight through process model can be applied. As ILC can adjust for pH and

conductivity online, the eluent coming off the first chromatography step can be immediately adjusted to match the conditions required for loading onto the second chromatography column.

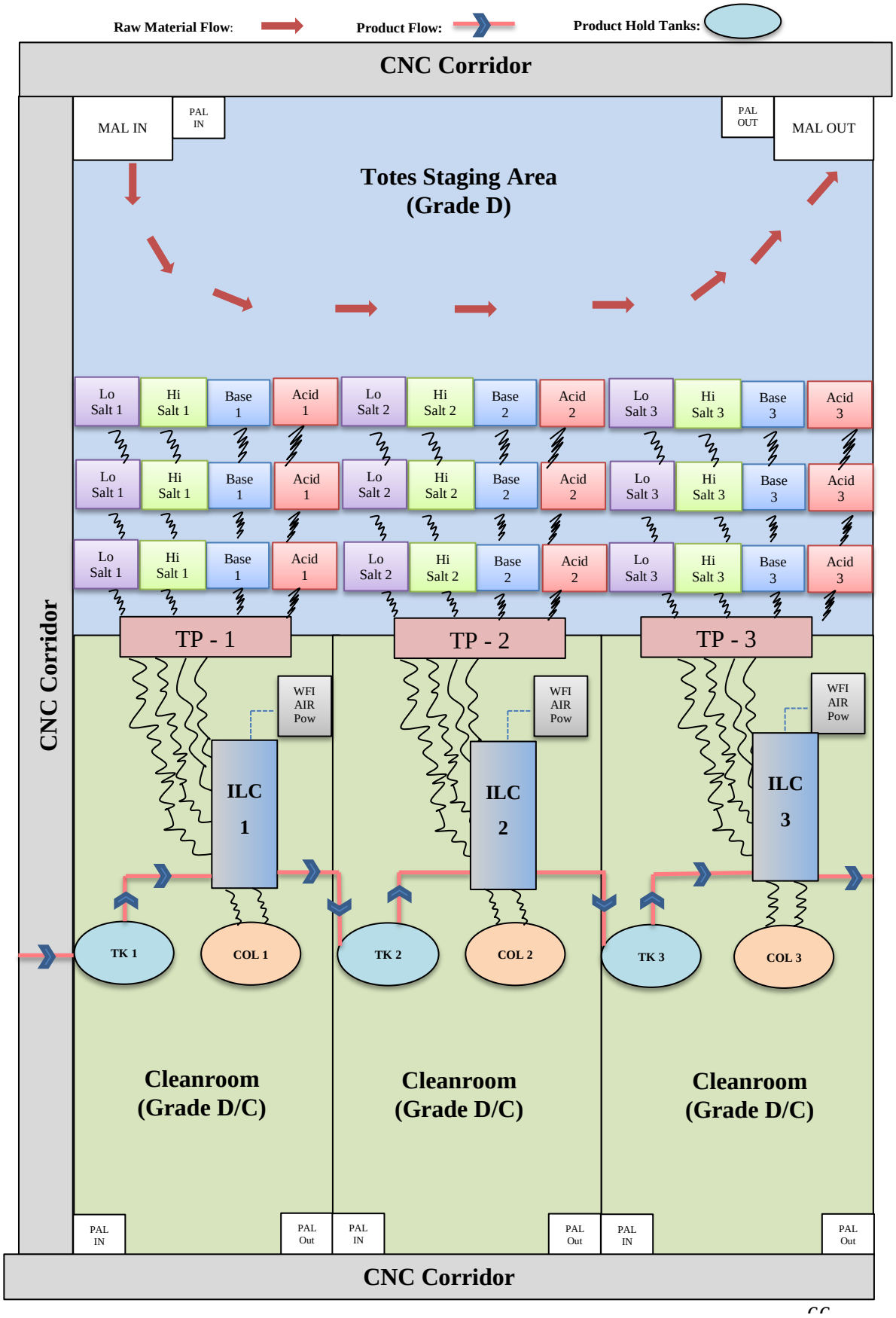

Figure 41 Automated Straight Through Processing using ILC Skids

The cost of labour, although similar to layout 1 due to the equivalent number of totes to be transferred into the staging are, is reduced as there is little manual intervention from an in process point of view. Also there is much less batch record steps as all data is recorded on the control system. Documentation interactions account for one of the "MUDA" wastes, which is greatly reduced in this setup.

The disadvantage of applying this layout and process design is the extra capital and operating costs associated with the additional tanks and piping, as opposed to layout 1. Both the tank and piping would have to undergo cleaning between batches. This would require bringing CIP delivery lines to the vessels, transfer lines and transfer panels. However this is typical of a conventional biologics facility and would not be insurmountable by any means. Furthermore there would be ongoing maintenance costs associated with the non-disposable stainless steel equipment, as well as repeated cleaning validation activities. None the less, the advantages of implementing this straight through processing model is could be a step towards embracing a commercial scale continuous processing model.

3.7. Case Study - Kedrion Biopharma

Kendrion Biopharma is a subsidiary company of Kendrion SpA. Kendrion engages in the production and distribution of various plasma-derived therapeutic products to treat haemophilia, immune deficiency, contagious diseases, and other illnesses. Their headquarters are based in Italy with supporting production and research facilities worldwide.

To expand its portfolio and to include immunoglobulin G fractionate of blood plasma (IgG) as an offering, Kendion commissioned the design and construction of a new biologics facility in Tuscany, Italy. In the production of IgG, large volumes of buffers are required. As part of the production process 26 different buffers are required to support both chromatography and filtration purification processes, with up to 70,000 litres of buffer required per batch. 200,000 litres are required over a three week period. (Fabbrini, et al., 2017). To achieve this buffer requirement per batch using conventional buffer methods, as discussed in previous sections, would require significant capital investment as well as significant footprint dedicated to buffer prep in their new facility. Furthermore Kendrion were concerned that their buffer prep using conventional methods would create a bottle neck and limit production capacity (Fabbrini, et al., 2017). Kendrion with the assistance of

GE Healthcare assessed ILC technology to see whether these bottle necks could be removed from their manufacturing model (Fabbrini, et al., 2017).

The IgG process uses two chromatography steps and a final ultra-filtration step in the purification of the molecule. It was determined that from the 26 buffers required over the various unit operations, only 11 buffer stock concentrates were required. Kendrion used 4 different formulations of acetate buffers. Therefore two highly concentrated stock solutions of the equivalent acid (HAc) and base components (NaAc), an illustration of which can be seen in figure 42.

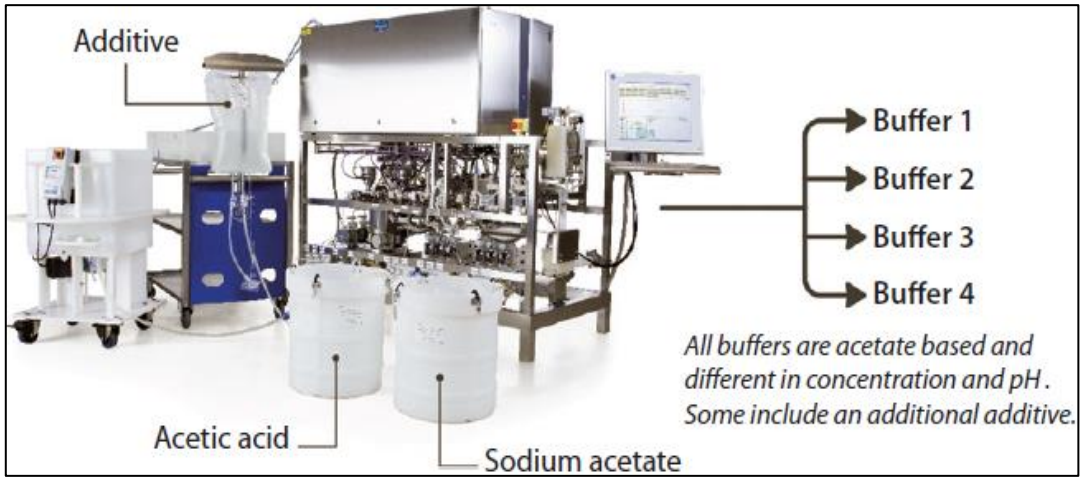

Figure 42 Kendrion Production of Buffers from Acetates (Fabbrini, et al., 2017).

The facility is setup with each of the chromatography steps and ultrafiltration step having dedicated ILC systems, as well as a further ILC used for centralised buffer make up. As the risk of using new novel technology for a critical part of processing was high compared to the conventional method, a proof of concept study was completed at the factory acceptance test (FAT) of the ILCs. As part of the FAT the 26 buffer formulations were successfully made using the ILC, with the 11 acetate stock solutions (Fabbrini, et al., 2017).

As discussed in the previous sections the advantages of using ILC compared to the conventional methods for buffer preparation are potentially significant, as seen by Kendrion.

	Reduction	Cost Savings
Tank size	84%	€700,000
Stainless steel tanks	Now all single-use	None
Number of stock solutions	58%	None
Preparation time	69%	€3,000/ batch
Number of operators	4–5	€90,000
Overall footprint	61%	€200,000

Figure 43 Space savings and operational (Fabbrini, et al., 2017).

As seen in figure 43 there is a significant saving in capital expenditure of €900,000 across the facility as well as the footprint being reduced by over 60% which itself will save on additional costs, including on HVAC and building maintenance. Also the move to SU equipment resulted in a sevenfold reduction in buffer-storage as Kendrion moved entirely to single-use buffer totes in the new facility. This had the additional advantage of eliminating our need for labour intensive, CIP and corresponding cleaning validation efforts. Automation enabled the process to be converted from one that is operator intensive to one that is process intensive (Fabbrini, et al., 2017).

4. Overall Conclusions and Recommendations

The aim of this thesis was to explore the reasons why bottlenecks exist within biologics manufacturing processes, what the bottle necks are and the potential technology's available to alleviate these. It is clear that Pharma companies are at a cross roads in terms of how to approach biologics manufacturing. When the fundamentals of what instigated the initial growth of the pharma (biopharma) industry are examined, many of these still apply. The biopharmaceutical industry is growing exponentially, with no forecasts of this slowing down. The world economy is expected to continue to grow in the coming years. Developed markets in USA, Canada and Western Europe, as well as the countries that are at a stage of newly advanced economic development including the BRIC countries (Brazil, Russia, India, China) are fuelling this growth. Another 3 billion consumers will enter the emerging markets. Furthermore adding to this, the demographics of society is rapidly changing with 16% of the global population being over 65 years if age in 2050, compared to just 8% in 2010. These factors will drive the demand further for access to all drugs, including biologics. Seven of the top ten bestselling medicines globally currently are biologics.

Therefore the onus is on biopharmaceutical companies to with intrinsic flexibility in facilities, to respond to the market need as quickly as possible. However products that are in high market demand might cause unintended consequences on the process design of the related facilities, which may introduce rate limiting steps and subsequently bottlenecks. It is clear from the discussion in the thesis, that "Research and Development" departments are under increasing pressure to produce drugs for submission, not only from a cost perspective but from a competiveness and speed to market point of view also. The costs of research and development studies, the competition between drugs targeting similar diseases, being first to receive regulatory approval, the emergence of biosmilars as well as the enormous costs and the complexity of manufacturing biologics, all contribute to the complications when planning the type of facilities to build and when to build them. The time therefore to experiment with novel technologies to streamline biologics processing is not being allowed within the above mentioned constraints. The dominant mode of manufacturing is still a batch processing model which seems to be in conflict with the requirement of the market, to be as flexible as possible.

It is clear from the research that the increasing titres and the suitability of upstream processing to single use technology, has swung the bottle neck to downstream unit operations. The levels of achievable titres achieved in commercial manufacturing of up to 10 g/l are now causing a requirement for large capacity purification equipment or multiples of these. Purification costs are much higher than cell culture and fermentation costs, therefore the return on investment in a facility for manufacturing of biologics, may be difficult to achieve.

Considering the above it is critical that with any new facility flexibility as much as possible is built into its systems. To build flexibility, the personnel involved with product development and particularly those with global focus and influence, must engage with innovative technologies such as the systems presented in this thesis. The facility of the future must be flexible and agile enough to add or subtract capacity and retool quickly to produce new or different products. The most appealing area to engage in is continuous bioprocessing;

- It is inevitable that for the biopharmaceutical industry to overcome this hurdle, particularly in purification processing, then single use technology will need to be incorporated into a significant amount of the equipment train.
- A continuous system will have to be as automated as possible
- online analysers are developed to allow the batch to flow through unperturbed and be released positively for continuous systems
- PAT instrumentation needs to be incorporated into all areas of the manufacturing process.

Continuous processing can also support the flexible concept as the scale for continuous processes would be much smaller, close to clinical scale. Therefore in theory, multiple continuous processing trains could be incorporated into a facility where one conventional batch process would fit. For example a facility containing a number of 20,000 litres batch reactors and supporting services versus a continuous processing suite housing 2 perfusion reactors of 200 litres. In summary for continuous processing to become a reality a combination of single use technology, as well as designing a process with a QbD framework embracing PAT. It is clear from the literature industry is still quite a number of years away from this mode of manufacturing being realised. This is because a number of issues.

Firstly the factors discussed in the previous section contribute in terms of the costs of R&D to explore these areas, as well as the pressures faced from speed to market and patient demand. Furthermore despite encouragement from the regulatory bodies to do so, biopharma companies are hesitant to move away from the tried and trusted batch technology model. The diversity in supply of single use equipment needs to be broadened. A select few suppliers are currently dominating the single use technology arena. Until further vendors enter the markets, which will increase competition and drive down costs, in some instances converting fully to a SUT continuous process may not be the most sensible option. Finally some products that are being developed are not conducive to continuous processing due to instability around the molecules, which may lead to aggregation issues which may have a negative effect on the efficacy and safety of the product.

If a continuous model cannot be applied, there are other options that lean away from the conventional methods, as well as incorporating the batch model but with flexibility. A hybrid model can be selected, where either the upstream or downstream unit operations, use a continuous model, with the other remaining in the conventional batch mode. However this does not remove the bottle neck but only reduces the manual interaction involved with that part of the process, which in turn will reduce costs.

Another concept discussed in the thesis is the “modular” approach to biologics manufacturing. The pre constructed modules provide an attractive solution incorporating flexible manufacturing. The advantages of the solution is the speed of deployment of the modules (POD’S), the relative ease to do this as well as the reduced costs compared to commissioning a traditional stainless steel facility. Again the modular solution relies on single use technology, to support its processing, as the relatively smaller size of the POD’S will not support large stainless steel systems. This option is appealing in many instances. If a company is a producer of orphan drugs and requires a less costly option to produce biologics, (as usually orphan drugs tend to have high manufacturing costs associated with them) then a modular facility could be the right option. The modular solution is also attractive for start-ups that may require a platform to support manufacturing for clinical trials. Also PODS should be considered in instances where a biopharma company may want to supply product into the emerging markets, where they can be easily deployed and started up. The complications of exporting drugs into these countries would be removed by having a facility local in the region. The disadvantage to

these systems is again the same as single use technology, vendor diversity, scale and costs.

It seems a full conversion to continuous processing, a hybrid model or converting fully to disposable equipment will not become a common platform and a “one size fits all” approach in biologics does not work due to the complications of manufacturing such products. Therefore examining the current batch model process, in the interim before technology bridges the gap to allow a full conversion to continuous processing, technologies should be strongly considered to remove the “secondary” bottle necks caused as a result of the “primary” downstream bottleneck being resolved. Figure 44 revisits the typical biologics process from a high level point of view.

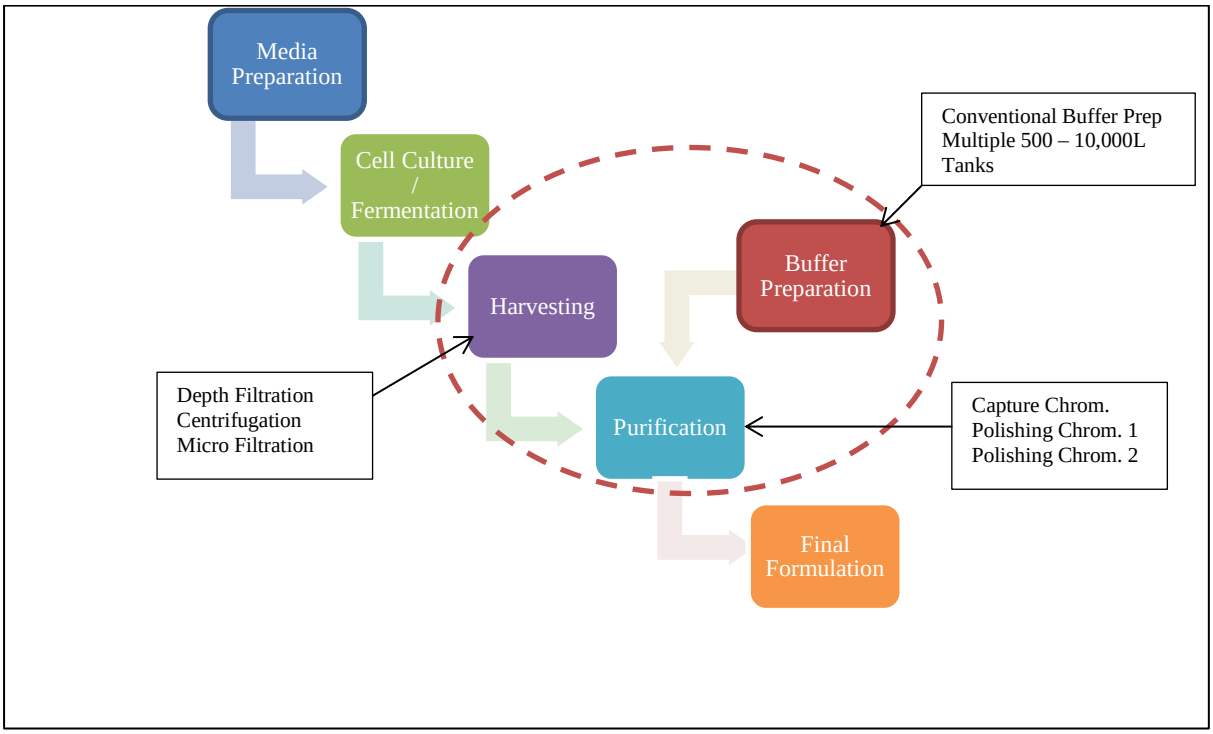

Figure 44 Biologics Manufacturing Process

The areas within the red dashed line are the unit operations in which the bottle neck exists within biologics manufacturing at present. After the cell culture (or Fermentation) step, the harvesting process will consist of clarification and capture methods to begin to initial stages of purification. It is clear from the literature, in that this is the specific equipment being used as part of the batch model that is being overwhelmed, by the higher titres created in upstream processing.

Specifically the bottle neck is seen in;

- Clarification equipment, for removal of cell debris prior to chromatography is primarily achieved by various modes of filtration and centrifugation. The problem brings membrane fouling, saturation and the relatively lower yields seen in centrifugation. Also some shear sensitive products may not be conducive to these methods.
- Capture chromatography step (Protein A or equivalent) the breakthrough capacity cannot accept the full clarified cell culture as the columns have become over saturated.

However the alternatives technologies, as discussed in this thesis is worthy of consideration by process development engineers, when designing their processes. Each novel technology is appealing for its own reasons.

Expanded bed adsorption (EBA) has the potential to combine both clarification (harvesting) steps and the initial capture chromatography step. If a matrix is developed with an affinity to match that of Protein A, then this technology, with its potential to combine multiple (Clarification and Capture) steps into one should be considered. Likewise membrane chromatography has the same potential as EBA. Once similar concerns over its binding capacity are resolved, with definitive data that the technology performs as well as conventional methods, then this may become another chromatography option.

Similarly periodic counter chromatography (PCC) is another capture chromatography step, with the potential to remove the bottle neck completely from downstream processing and could be integrated into a continuous processing train. As the breakthrough capacity of a resin is almost discounted as, extra columns can be added, once the technology has been proved at commercial scale, it could be a very powerful method in purification processing. As extra resin is required per batch the lifetime of these as well as the costs of procuring resin would have to be analysed to ensure a profit was made by the specific product in question, if this method of processing was chosen.

Also pre packed columns have the same potential as SU upstream equipment once the technology to offer larger diameters is discovered. The diversity in vendor supply is also

true of prepacked columns. Once more vendors enter the market costs will be driven down.

Finally as illustrated in chapter 3, regardless of what type of operational model used in-line conditioning technology can be integrated immediately into any biologics process and replace the traditional buffer preparation approach.

Any of the above techniques or a combination of the above, supported by buffer preparation by ILC, completely changes the landscape of purification processing even at large commercial scale volumes. Revisiting figure 44 and replacing the conventional method with the novel technologies discussed in this thesis, changes the illustration as seen in figure 45 below.

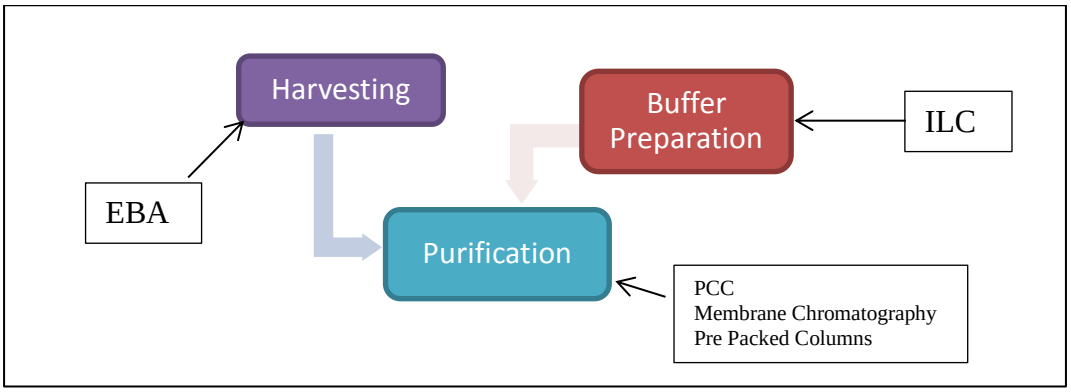

Figure 45 Integration of Novel Technologies into Harvesting & Purification Processing

The technology exists to remove partial or all bottle necks within biologics manufacturing as shown in figure 45. The larger pharma companies with significant capital to utilise must be encouraged from process and product development stage, to engage with such technologies and demonstrate innovation. Those who will engage with innovation will accelerate ahead of the pack. Acceptance and adaptation of novel technologies and solutions to support a more agile and flexible pharmaceutical manufacturing sector, is key in meeting future challenges. The new biopharma reality requires new innovative systems and for many companies this is more than an upgrade to existing plants. It requires careful analysis and planning as well as courage to embark with novelty. The next generation of biologics products must support faster product approvals, as well as investment decisions with high uncertainty in forecasting and high dependency on flexibility. Based on this approach, it should be possible for the biopharmaceutical industry to meet the challenges of the new biopharma reality.

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