A Review of Chromatography Methods for the Large Scale Downstream Protein Purification of Monoclonal Antibodies

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Abstract

This abstract gives the brief summary about this research project.

Researchers have continually proposed new non-chromatographic methods for manufacturing biopharmaceutical products. However, this report will show that chromatography remains the backbone of the downstream operation. Establishing a robust chromatographic manufacturing platform is a critical element that manufacturers of monoclonal antibodies (mAbs) must consider, covering activities from small scale to commercial processing. This report reviews a variety of methods utilised as part of the purification process within downstream biopharmaceutical processing of largescale commercial biologics. A protein's properties such as its composition, molecular size, shape, charge or hydrophobicity will determine the modes of chromatography applied to optimise the purification process. Chromatography steps tend to be specific to a protein or type of proteins. Typically, the downstream process will have multiple chromatography steps which require large columns to be pre-packed with resins that must pass an evaluation prior to use. In industry, the traditional practice of packing resin into columns has widely been replaced and this report will look at current trends. The different media / resin that are packed into the column will dictate the type of chromatography. Major advancements in resin matrix and ligand chemistry has benefited the industry in the choices of Chromatography, including but not limited to Affinity Chromatography (AC), Ion Exchange Chromatography (IEC), and Hydrophobic Interaction Chromatography (HIC) and Mixed Mode Chromatography (MMC). Traditionally, downstream manufacturing is a batch process, and this report will examine advancements with significant interest in continuous processing due to the high costs associated with certain resins, particularity protein A resin. It is no surprise that large scale chromatography is targeted for developments in relation to new platforms and innovative, more efficient approaches. Evidence will be discussed on the impact new single use technologies and continuous chromatography is making and changing to change the way some manufacturers deliver important medicines to patients.

***Keywords**: Chromatography, mAbs, Large scale, Platform Approach, Protein A, Column Packing, Batch Processing, Continuous Chromatography*

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To my children, Ciaran, Niall & Aoife, please remember; "The more you read, the more things you will know. The more you learn, the more places you'll go." Thanks to my caring wife Brigid who has been the one to burden more that her fair share of home life responsibilities; Thanks for allowing me the space and time to complete this course, without you this would not have been possible.

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List of Abbreviations

Definition	Acronym
Affinity Chromatography	AC
Alternating Tangential Flow	ATF
American Society of Mechanical Engineering	ASME
Anion Exchange Chromatography	AEC
Anion Exchange Chromatography	AEC
Aqueous two phase Separation	ATPS
BioPhorum Operators Group	BPOG
BioProcess System Alliance	BPSA
Boiler and Pressure Vessel Code	BPVC
Capture, intermediate Purification and Polishing	CiPP
Cation Exchange Chromatography	CEC
Cation Exchange Chromatography	CEC
Chinese Hamster Ovary Cells	CHO
Clean in place	CIP
Continuous Counter-current Tangential Chromatography	CCTC
Contract Manufacture Organizations	CMOs
Critical Quality Attributes	CQAs
Deoxyribonucleic acid	DNA
Diethylamino ethyl	DEAE
Dimethyl amino ethyl	DMAE
Dynamic Axial Compression	DAC
Dynamic binding capacity	DBC
Dynamic Binding Capacity	DBC
European Medical Agency	EMA
Expanded Bed Absorption	EBA
Extractables and leachables safety information exchange	ELSIE
Food and Drug Agency	FDA
Full Time Employees	FTEs
Gas Chromatography	GC
Gas Liquid Chromatography	GLC
Heating Ventilation and Air Conditioning	HVAC
Height Equivalent Theoretical Plate	HETP
High Molecular Weight	HMW
High Performance Liquid Chromatography	HPLC
Host Cell Proteins	HCP
Hydrophobic Charge Induction Chromatography	HCIC
Hydrophobic Interaction Chromatography	HIC
International community for harmonisation	ICH
Investigational New Drug	IND
Ion Exchange Chromatography	IEC
Ion Exchange Chromatography	IEC
Isoelectric Point	pI
Isopropyl Alcohol	IPA

Liquid Chromatography	LC
Log Reduction Value	LRV
Low Molecular Weight	LWM
Merck Sharp and Dohme	MSD
Mixed Mode Chromatography	MMC
Monoclonal Antibodies	mAbs
New Drug Application	NDA
Non-secreting murine myeloma	NSO
P-aminobenzoic	PABA
Paper Batch Record	PBR
Periodic Counter- current Chromatography	PCC
Polyamide	PA
Poly-ether Ether Ketone	PEEK
Polyethylene	PE
Polypropylene	PP
Polytetrafluoroethylene	PTFE
Process Analytical Technologies	PAT
Product Quality Research Institute	PQRI
Programmable Logic Controller	PLC
Quality by Design	QbD
Research and Development	R&D
Residual Protein A	rPA
Risk Assessments	RAs
Scanning Electron Microscope	SEM
Simulated Moving Bed	SMB
Single Use Technologies	SUTs
Size Exclusion Chromatography	SEC
Sodium chloride	NaCl
Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis	SDS-PAGE
Sodium hydroxide	NaOH
Standard Operating Procedures	SOPs
Static Binding Capacity	SBC
Thermal Responsive Protein A	TRPA
Thin Layer Chromatography	TLC
Total carbon compound	TOC
Ultra filtration/ Diafiltration	UF/DF
United States Pharmacopeia	USP

CHAPTER 1: Introduction

This introduction provides the aim of the report and how each section is broken down.

Patients have benefited from the development of novel biopharmaceuticals in the treatment of life altering disease, such as cancer and autoimmune disorders. In recent years, there has been a surge in the research and discovery of monoclonal antibody therapeutics. This report aims to review the current large scale chromatography techniques applied by manufacturers in the purification of monoclonal antibodies and examine trends and possible future applications. Section 2.1 provides a brief introduction to chromatography and some innovative advancements made to date, from its discovery by Mark Tswett in 1903. Section 2.2 reviews the influence of column materials and configuration, along with the impact of column packing methods on chromatography. Limitations in terms of column packing techniques are highlighted in terms of scalability and repeatability concerns, which has sparked an industry focus on automated solutions and pre packed columns to meet project schedules. The approach taken by manufacturers for the evaluation of column packs is also discussed with examples of chromatogram tracers from good and poor packs. The industry response to higher industrial titres is reviewed in section 2.5, with emphasis on improvements in media beads and ligand chemistry, which provide superior resins with higher Dynamic Binding Capacity (DBC). The advantages of these innovations to traditional chromatography platforms are examined. Section 2.6 details the chronological sequence of chromatographic steps that constitute a column cycle, along with the modes of chromatography that can be applied to establish a Manufacturer's platform. Section 2.7 compares platforms employed by a selection of large scale manufacturers, with industry examples. Cost and competition within the industry has culminated with vendors and manufacturers investigating newer, more efficient approaches. Section 2.8 will look at if this inefficiency is changing the way manufacturers are applying this to their manufacturing facilitates. Continuous chromatography processing is an option, perfusion systems with a capture step are being considered as a replacement to large packed columns due to the cost of protein A resin. These new methods will be examined for feasibility, and if there are industry examples of their adoption to replace expensive resins. Finally, Chapter 3 summarised the main conclusions arising from this study and future prospects for this research.

Monoclonal antibodies (mAbs) are produced via upstream and downstream processing. In upstream processing the mAbs are expressed in suspension cell culture in large bioreactors under controlled conditions. The purification process is initiated by harvesting the cells using a disk

stack centrifuge followed by clarification using depth and membrane filtration. The mAb then enters downstream processing were it is purified using a range of chromatographic, virus clearance and filtration techniques. This report will focus on the current chromatographic techniques involved in downstream processing for large scale manufacturers, taking into consideration new and future trends.

1.1 Monoclonal Antibodies (mAbs)

In 2018, Ireland's exports increased by 15% to €140 billion. Of this, medicinal and pharmaceuticals products represented a third of all exports (Taylor 2019). The biopharmaceutical sector directly employs 28,000 people and this is expected to rise to 36,000 by 2020 (Moran 2017). Ireland is one of the world's preferred locations for the manufacture of biopharmaceuticals and this has delivered €10 billion of investment to the country since 2008. (IDA Ireland, 2019).

Figure 1: Ireland is home to many of the top Biopharmaceutical companies (IDA 2019)

It is predicted that somewhere between 2021 and 2026 that 50% of all new drugs in development stage will be biologics (Jozala et al. 2016). Up until 2016, over 50 monoclonal antibodies (mAbs) had been approved between Europe and the United States of America (USA), with a further 50 in phase three clinical trials (Reichert, 2016). From 2008 to 2013, global sales of mAbs rapidly increased from $39 billion to ~ $75 billion which overtook any other recombinant protein therapeutic. This trend set looks to continue, and global sales are estimated to reach $125 billion by 2020 and $138.6 billion by 2024 (Yang et al. 2019).

MAbs are proteins that have an affinity to specific cellular antigens and have been proven to possess high selectivity for extracellular targets. MAbs are prescribed to patients to treat a wide

variety of conditions such as various cancers, multiple sclerosis and other autoimmune diseases (McMahon et al. 2018). Chromatographic separations are important to both the analysis of biological material as well as their manufacture (Scott 2012). Chromatography steps in the downstream process are crucial for the purification of biopharmaceutical products like mAbs. In relation to cost and time, chromatographic methods represent a significant portion of the overall downstream purification effort. Improvements have been made on several fronts, including the resin media, automation and Single Use Technologies (SUTs) to reduce the cost of large-scale chromatography (Challener 2016). Another area of improvement relates to column design and understanding what separation method to apply to ensure maximising the productivity of the downstream process (Hroncich 2016).

2.1 Purification Platforms

Over recent years, the downstream manufacturing process has delivered a standard framework across the biopharmaceutical industry. This framework is not a template that all processes can follow due to differences in the properties and behaviours of different mAbs during the manufacturing process. What this standard framework does deliver is consensus on the unit operations that should be included in the purification process (Shukla and Thömmes, 2010). The three schemes provided in figure 2 all rely on protein A chromatography as the initial capture step.

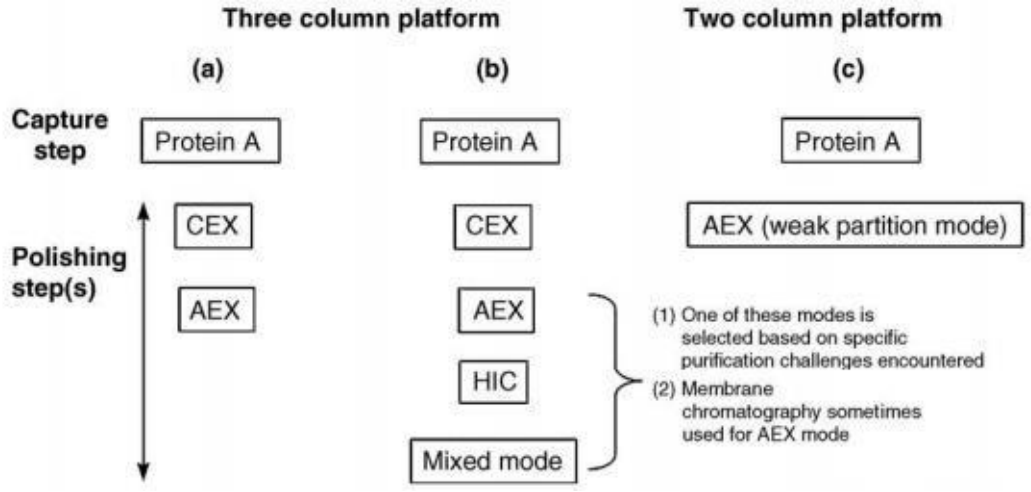

Figure 2: Potential Downstream Chromatography Framework (Shukla and Thömmes, 2010)

Protein A chromatography is an excellent initial capture step and can provide >98% purity in one purification step. This chromatography step affords the specific binding of mAbs at the Fc

region due to its affinity for the protein A ligand, immobilised on the resin. The reversal of this interaction can be completed by lowering the pH conditions within the column. To take advantage of this low pH pool, it's typical to see a low pH viral reduction step after a protein A step, one of two orthogonal viral removal steps. The further polishing steps serve to remove process related impurities such a Host Cell Protein (HCP), Deoxyribonucleic Acid (DNA) and reduce aggregated proteins (Shukla and Thömmes, 2010)Despite the effectiveness of current techniques, the biopharmaceutical industry has realised that a change in approach is necessary. Manufacturers face new concerns about the high cost of resins, coupled with competition from multiple treatments that are targeting the same disease. Many blockbuster drugs on the market face patent expiries which affords the manufacturers of biosimilars access to the market. This competition has resulted in a renewed focus on manufacturing cost and the potential that continuous processing can bring (Gjoka et al. 2015)

As per figure 3, Kelly (2007) estimated the cost for raw materials at Protein A, including resin and 0.2µm filters at over 50% the overall downstream cost and for a two column platform this was 60%

Dransart et al. (2018) estimates protein A resin today is still the largest percentage cost of the overall raw materials for the downstream mAb manufacturing process and this increases for every other chromatography step in the platform. The cost burden of these expensive resins can be reduced by replacing the raw material or using it more efficiently

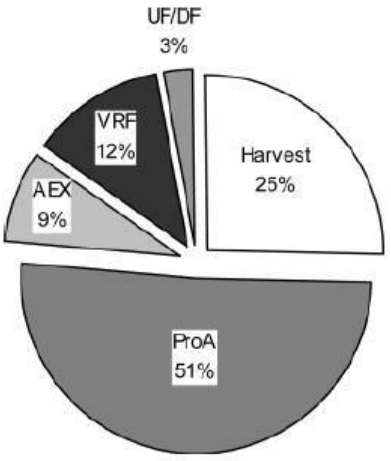

Figure 3: Raw material cost per Downstream Unit operation (Kelley. 2007)

Although manufacturers and vendors see the requirement for change, manufacturers have to balance the requirement to meet patient demand for their product and the impact that change will have on their supply chain. Issues with manufacturing sites, such as production delays, capacity issues, and loss of manufacturing site are responsible for two thirds of all shortages. Capital cost, retrofitting existing facilities, and the need for regulatory reviews in relation to process changes, all deter manufacturers from investing in new approaches and technologies. For new treatments, the propensity to stick with the well-defined, tried and trusted approach may succeed over a novel approach despite the longer-term advantages such as efficiency or reduced cost (Peters 2019).

CHAPTER 2: Literature Review

2.1 Introduction to Chromatography

This chapter reports the background information of how chromatography was developed and its applications.

Mikhail Tswett was hailed as the original inventor of chromatography. Born in 1872, this not so well-known scientist would leave a lasting legacy. Tswett was singularly aware of the importance of chromatography and its potential application. Working as a botanist in 1903, Tswett discovered that if a liquid mixture containing multiple solutes was passed through a vertical column containing calcium carbonate, the compounds could be separated into several layers and individual bands, which formed based on their reduced absorption affinity from top to bottom. His most notable demonstration was being able to show the existence of two native green pigments that came to be referred to as "Chlorophyll a and b" (Zechmeister 1956)

25 years after Tswett, there had been no real progress and several attempts had been made to discredit his finding. Richard Kuhn, Edgar Lederer Paul Karrer and Laszlo Zechmeister began the "Explosion of Chromatography" as described by Ettre (2007).

During the period of 1906 to 1952, there were some findings of importance. Plane chromatography was first developed using paper as a plane support. This was followed by utilising layers of silica gel, replacing paper, and this became the new technique known as Thin Layer Chromatography (TLC). Meanwhile, column chromatography would make a huge leap when A.J.P Martin and R.L.M Synge introduced partition chromatography known as the "plate concept". Recognised for their work, both men received the Nobel Prize in chemistry. Their article referenced the potential possibilities of gas chromatography (GC) and it was not until 10 years later that Martin refocused his energy on moving this forward. Around the same time, Dutch nationals van Deemter, Zuiderweg and Klingenberg introduced the "Rate Theory". In this theory, the chromatography process using packed columns was described in terms of kinetics. It explained the diffusion and mass transfer of steps in Gas Liquid Chromatography (GLC) in detail. At the time, GLC was found to be very easy to use and efficient at performing separations of volatile mixtures that could not be established by distillation. Having seen such success with GC, the older Liquid Chromatography (LC) method was revisited. In 1963, Gigging published a paper in which he applied some of the conditions used in GC to LC. The conditions included the application of high pressure to LC and the method was coined High Pressure Liquid Chromatography (HPLC) which delivered efficiencies equal to that of GC

(Miller 2005). 60 years after van Deemter application of rate theory equation to chromatography, Lapidus and Admundson defined the simplified expression for HETP (Height Equivalent to a Theoretical Plate) which still remains popular today.

The first biopharmaceutical treatment can be traced back to the late 19th century, when the first vaccine was developed. The early 20th century saw breakthroughs with the discovery and application of products such as insulin. This was originally derived from an animal source which posed inherent risks, due to contamination and potential adverse reaction. During the Second World War, penicillin was also produced at large scale. In 1973, a major breakthrough witnessed the discovery of recombinant DNA technology by Cohen and Boyer, but this was not fully realized until 1982. Regulatory approval was sought by Eli Lilly and approved for their recombinant human insulin, "HUMULIN® N", created from genetically engineered e-coli. The following years have seen a wide variety of treatments using this technology. In 2017, in what was described as another paradigm shift, the first gene therapy was approved. "LUXTURNA™", manufactured by Spark Therapeutics, is used to treat congenital blindness (Lalor et al. 2019).

The biopharmaceutical industry is wrestling with alternatives to batch processing and has done so for some time. Many continuous upstream processes are now available but downstream is lagging behind. Trends over the last 20 years are depicted in figure 4;

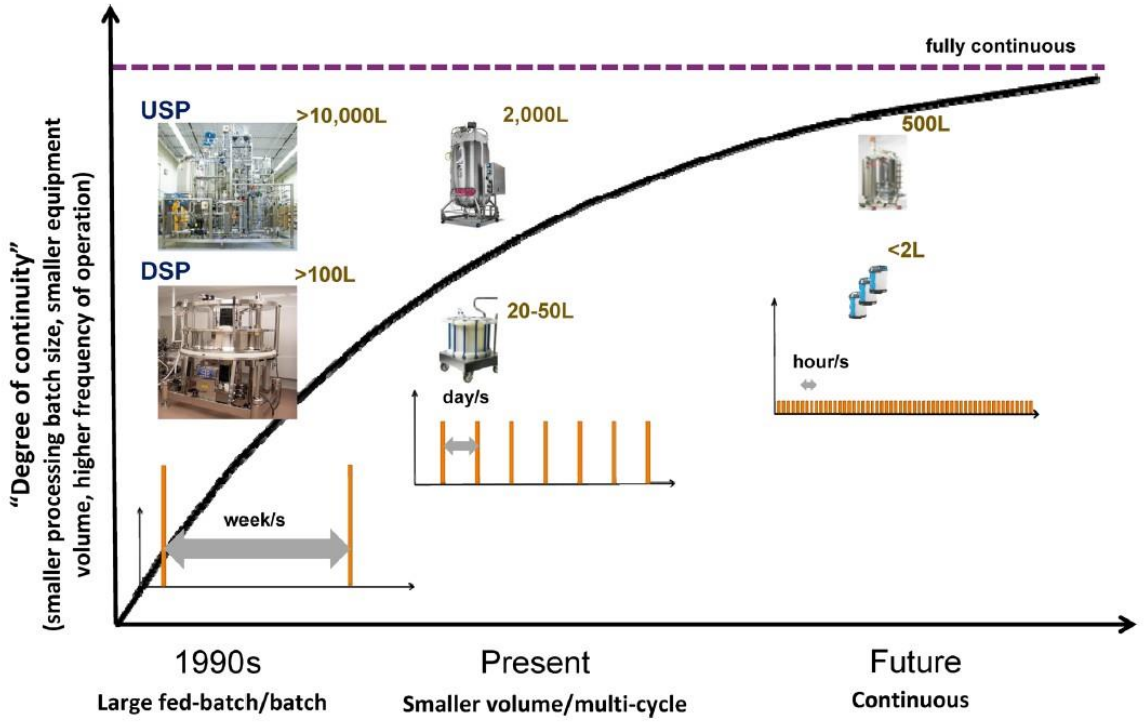

Figure 4: Trends in mAbs production platform (Croughan, Konstantinov and Cooney, 2015)

The dominant design through the 1990's was centred on large stainless steel bioreactors capable of holding 10,000L to 20,000L of cell culture product. Currently, while some operators still possess this capability, manufacturers have been moving away from a stainless steel approach to smaller, single use systems that can hold up to 2,000L, operating with smaller output at a higher frequency. The possible end position is envisioned to be a fully continuous, end to end process but this requires further development (Croughan, Konstantinov and Cooney, 2015).

2.1.1 Chromatography in other Industries

Chromatography plays an important role in the food industry. It can be applied to numerous stages and can be utilised to determine the nutritional quality of the food and the additives present. Vitamin C is a naturally occurring nutrient that is present in many foods, including fruits and vegetables. The vitamin is susceptible to depletion during food processing. As a result, the depletion of vitamin C has become a reliable indicator for the depletion of other important nutrients and has become a standard test throughout various food processing stages. Rapid test methods are commercially available which can deliver quantitative analysis. Food spoilage is another aspect chromatography can help deliver quantitative results – A general food spoilage indicator is pyruvic acid which is an indicator of lactic acid producing bacteria. This will negatively impact flavour and will continue to increase when refrigerated. This test can be coupled with a technique to measure sweetness and the organic profile of a solution. This can be displayed in a chromatogram in which each acid is represented graphically by different peaks. This test is much quicker than a plate method which could potentially take several days. It can also quickly identify an issue before it becomes a serious health risk to the public and/or prevent a bacterial outbreak issue for the manufacturer (Bio-Rad 2018). Recent studies have been conducted on vegetables that have grown in fields that were irrigated using waste water. Waste water treatment plants may fail to remove all household, industrial chemicals or the remnants of personal care products from wastewater streams. Understanding what pollutants are present in the soil and which pollutants have accumulated in the root of the vegetable are necessary to fully understand the risk posed to the general public. HPLC can provide qualitative and quantitative data (Aparicio et al. 2018). As of 2014, 60% of all testing with regard to chemical analysis is currently performed using chromatography (Kondeti et al. 2014).

The pharmaceutical industry also uses a variety of chromatography techniques, depending on the requirement. Chromatography data can be utilised for the submission of a New Drug Application (NDA), as regulatory bodies expect that qualitative and quantitative data has been generated. New analytical methods of separation have been developed that can effectively separate minute quantities of therapeutic drugs from contaminants and by-products. Chromatography can be used to support a variety of studies that are required in order for a drug to be considered for approval; including clinical, toxicology, metabolic, and diagnostic studies (Ahuja 1992). Continuous chromatography has existed since the 1990's and its introduction was observed in the form of Simulated Moving Bed (SMB). Due to complications that resulted from the racemic drug, "Thalidomide", which resulted in thousands of children being born with disabilities across Europe, regulatory authorities imposed limitations within the industry in relation to racemic mixtures. As a consequence, racemic pharmaceuticals must be separated into the corresponding enantiomeric compounds (Vogg et al. 2018). SMB was industrially applied to separate these enantiomers effectively. Other chromatography applications include forensic science, animal derived nutrition tests and post mortem toxicology (Ahuja 1992).

2.1.2 Application of Chromatography to Purification of Biologics

Chromatography can be referred to as the "work horse" in terms of the purification processes used for manufacturing biopharmaceuticals. Chromatography is an extremely effective purification method that can be utilised in a vast range of industrial applications. It's the optimal separation method selected by manufacturers due to its high resolution. A critical part in the manufacturing process, it involves the efficient recovery of the desired protein, contributing to the production of a highly pure product. Chromatography utilises a stationary phase which remains motionless throughout the process, as the name infers. A mobile phase moves towards, over or through the stationary phase. Based on the affinity and interaction of a species within the stationary phase and mobile phase, separation of the species into its constituents is enabled. Five predominant chromatography techniques include Size Exclusion Chromatography (SEC), Ion Exchange Chromatography (IEC), Hydrophobic Interaction Chromatography (HIC), Affinity Chromatography (AC) and Mixed Mode Chromatography (MMC). The characteristics, purification phase suitability and conditions of the techniques are described in Figure 5 (Jozala et al., 2016).

Method	Typical characteristics: Resolution	Typical characteristics: Capacity	Purification phase: Capture	Purification phase: Intermediate	Purification phase: Polishing	Sample start conditions
AC	+++ or ++	+++ or ++	+++	++	+	Various binding conditions
SEC	++	+	+		+++	Most conditions acceptable, limited sample volume
IEX	+++	+++	+++	+++	+++	Low ionic strength. pH depends on protein and IEX type
HIC	+++	++	++	+++	+++	High ionic strength, addition of salt required
RPC	+++	++		+	++	Ion-pair reagents and organic modifiers might be required

Figure 5: Chromatography Techniques and suitable phases (Sigma-Aldrich, 2018)

The three-phase column chromatography approach can be referred to as the Capture, intermediate Purification and Polishing step (CiPP). CiPP can be used to perform the initial capture of the protein of interest which is represented in figure 6.

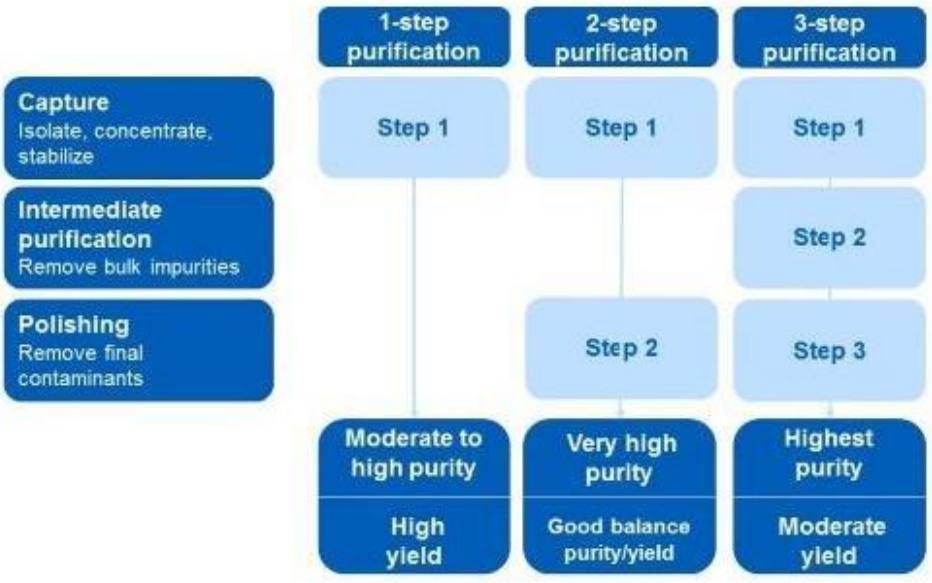

Figure 6: CiPP (GE Life Sciences 2018)

This step will concentrate the pool by reducing the volume and will stabilise the protein. The intermediate purification can be used to remove bulk contaminants, whilst the final polishing step removes the most difficult to remove impurities, such as aggregates. Initial research requirements may only require the application of one chromatography technique (GE 2018).

A rule of thumb exists that involves the higher the purity requirements, the more chromatography steps that will be needed in a purification process (GE 2018). However, although more steps may benefit the overall improved purity of the protein, there will be a negative impact on the yield. Each step will offer a balance between capacity, resolution, speed and recovery (GE 2018).

2.2 Chromatography Columns and Components

This chapter examines the different all the chromatography columns options available and common components.

Chromatography can be described as an indispensable unit operation used in the downstream processing of mAbs. The scaling of each chromatographic step will typically involve adjusting the column diameter and in commercial manufacturing, will mostly involve columns with diameters ranging from 1.4 to 2.0 meters (Johnson et al 2014). At this scale, the flow distribution contained in a packed column will be critically impacted by design. Vendors that supply the industry will have varying design features that impact the flow distribution, column efficiency and cleaning requirements. These variations will have an implication to the process and the corresponding impact needs to be thoroughly understood to prevent issues later in the project (Johnson et al 2014).

The materials used to construct a column must be non-leaching materials that are deemed suitable for good manufacturing practice (GMP) manufacturing and must include properties that make the column resistant to chemicals (Bemberis et al. 2003).

2.2.1 Glass Columns

Glass is a first-choice option for pilot-scale operations, when high pressure is not a process requirement. Glass is inert, offers good chemical compatibility and is clear which allows the user to visually check the bed for deficiencies around the circumference of the column. Glass is manufactured in cylindrical tubes to form the column. As large-scale industrial requirements have increased, the practicality of glass has diminished. Manufacturers of glass columns struggle to meet the tolerance specification required to make seals as scales increase for larger columns. An area of concern is the intrinsic properties of glass which lead to its low material to strength ratio. It was also challenging to form the tube to deal with the high pressures required, particularly as resin particle size decrease and operating pressures increase (O'Neill 2003). Traditionally, users have had a preference to view the packed bed for visual confirmation. However, due to column diameter increasing to meet industrial demand, the portion of the bed that can actually be inspected, relative to the amount of packed material present, has been greatly reduced (Jagschies et al., 2017).

2.2.2 Plastic Columns

Plastics of various compositions have been used but the most common is acrylic formulations. The composition will depend on the tolerances required to seal the column (O'Neill 2003). Using acrylic formulations provides a safe and efficient alternative to glass. The first major advantage is that acrylic formulations are close to half the density of glass at a range of 1150-1190 kg/m³, compared to a density of 2400 to 2800 kg/m³ for glass. Another advantage is the acrylic material has higher impact strength than glass and possesses greater integrity when exposed to high strain. An additional advantages of acrylic over glass is that it transmits more light than glass, providing 92% optical transparency. Transportation and handling considerations are also helped by reduced weight and the fact the acrylic is less fragile (Hydrosight.com, 2019). GE Healthcare (2009) published a user manual for their AxiChrom columns which range from 30cm to 1m diameter columns, supporting bed heights of 30 to 50 cm. The GE range of columns are supported with an acrylic ring or a stainless-steel tube. GE highlights a disadvantage and important observation with acrylic tubes. Care must be taken to ensure the surrounding area is free from sunlight and is not susceptible to temperature changes (GE 2009). If there are significant temperature changes, the acrylic ring is susceptible to shrinkage or expansion. This will result in undesirable tension on the tube itself, the dome nuts attached to the adaptor, on the dome nuts attached to the tie rods and the bolts on the bottom bed supports. In effect, each will become either tightened or loosened. Care must be taken when transporting the column from areas of differing temperatures and specified torque settings must be adhered to. Another negative is that acrylics provide poor resistance to commonly used organic solvents, typically used in the cleaning and storage of resin, such as ethanol. GE recommend that a concentration no higher than 20% ethanol can be used with an acrylic tube, which is low when compared with 70% ethanol for stainless steel (GE 2009).

2.2.3 Stainless Steel Columns

Stainless steel is widely used in the manufacture of biopharmaceuticals for a number of reasons. Stainless steel provides a resistance to corrosion, is biocompatible, non-reactive and is extremely durable over a large temperature range. The type most commonly used is stainless steel 316L. This is an iron-based alloy that consists of between 17%-19% chromium, 13%-15% nickel, 2.25% - 3% molybdenum, less than or equal to 2% manganese and up to a maximum of 0.03% carbon (Thompson et al. 2017). 316L is used once the surface has been passivated which forms a protective oxide layer on the surface, providing additional corrosion resistance (Thompson et al. 2017). Stainless steel columns are designed in accordance with the American Society of Mechanical Engineers (ASME) Boiler and Pressure Vessel Code (BPVC). Certain chlorides and especially low pH buffers can result in corrosion and procedures must be created that ensure the correct volume and flow of water is directed over the column, to remove any residuals. Stainless steel parts are susceptible to scratching so care must be provided to prevent this. Large unpacked and packed columns can weigh a substantial amount and take up large spaces (GE 2012). The stainless-steel column will also have materials that are made from acrylics, such as the distribution cell and the nozzle body. Wiper blades are made from polytetrafluoroethylene (PTFE) (PALL 2019).

Chromatography columns for a large manufacturing process can be manufactured but two meters is the largest diameter available. This is as a result of flow distribution limitations (Shulka et al. 2017).

2.2.4 Distribution Cell

Each vendor will have their own design for a distribution cell in which the liquid inlet is delivered to the centre of the column and distributed uniformly across the top of the bed. Specially designed channels will collect the liquid and distribute it, which minimises any hold up and pressure along the channels. A distribution cell will ideally consist of a conical shape plate, designed with thickness reducing from the liquid inlet to the column side wall, and it extends across the full diameter of the column. Figure 7 shows a column configuration with both upper and lower distribution cells (Jagschies et al., 2017).

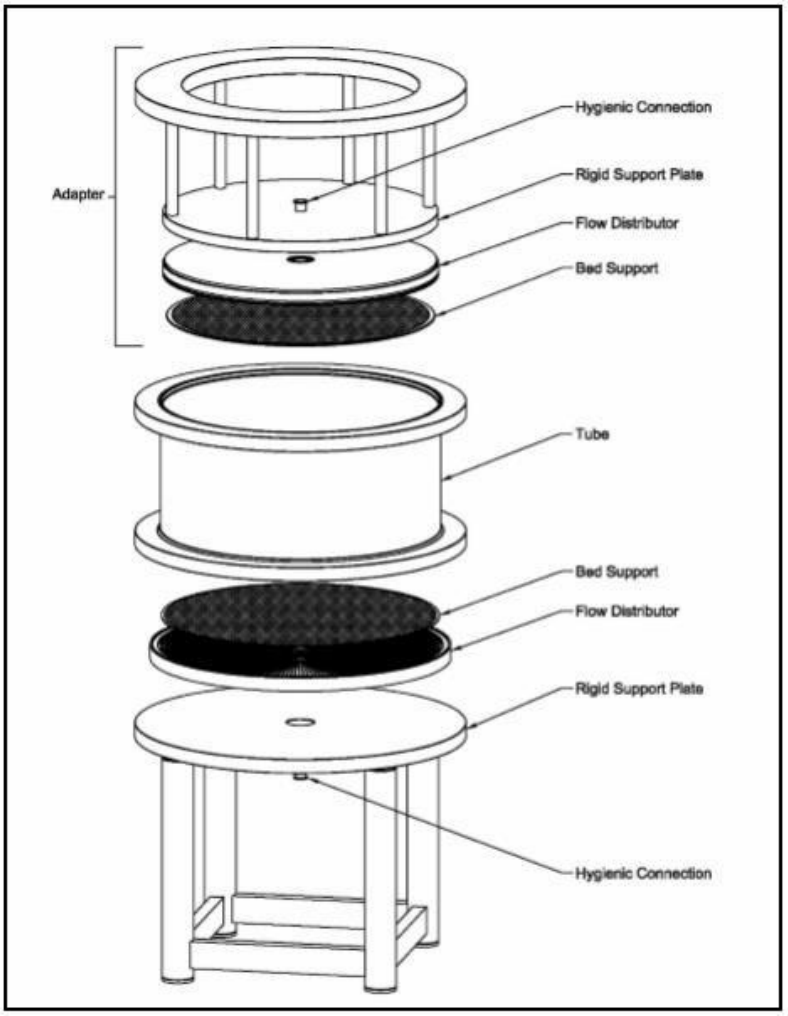

Figure 7: Example of a Chromatography Column (Maher et al., 2015)

2.2.5 Bed Supports

A bed support is positioned between the packed bed resin and distributor cell, and acts similarly to that of a sieve. This bed support acts as a porous particle retaining tool that will retain resin particles of a given size within the packed column (Jagschies et al., 2017). Figure 8 is presented by GE (2016) and shows a bed support for a chromatography column, the positions of the slurry valves and the distribution plates. It is important to remember that these are positioned above and below the packed bed.

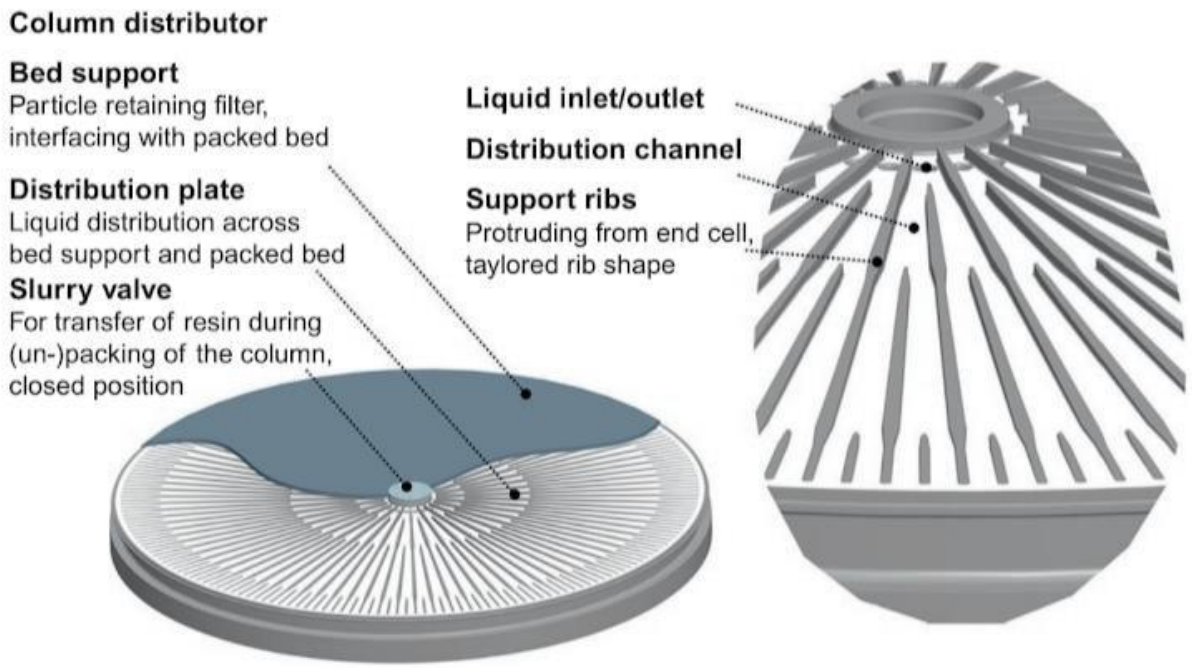

Figure 8: Image of a distributor and bed support (GE 2016)

Depending on the type of column, bed supports can be made from a variety of materials. Plastic bed supports can be manufactured from polyethylene (PE), polypropylene (PP), polyamide (PA) or poly-ether ether ketone (PEEK). Plastic materials however can be prone to damage, mainly due to fatigue and cracking, before and during column packing operations. Procedures must ensure that there is sufficient wetting of the bed conducive to the specific bed support material before using it. Sintered polyethylene can also be used for bed supports but they must be comprehensively degassed and completely dry prior to fitting. Alcohols such as ethanol or Isopropyl Alcohol (IPA) in concentrations above 80% concentration can be used to perform the degassing. Stainless steel bed supports are also an option but they must be inspected for corrosion before and after use. Stainless steel bed supports should be dedicated and procedures must ensure that same bed supports are not used for different modes of operation. Another check must be executed to ensure that the bed supports are not plugged. Specially designed equipment for moving large bed supports and the top adaptor is required for large applications (Bemberis 2003). As a rule of thumb, the pore size should be 1/3 of nominal mean resin bead size (PALL 2018).

2.2.6 Column Nozzles

A nozzle is located at the centre of both the top and bottom of the column. This nozzle controls the liquid entering and exiting the column, depending on the operation being performed. Both top and bottom nozzles are identical to ensure the flow profile is identical in both directions. This also provides that ability to pack chromatography media without exposing the process to the outside environment, aiding microbial control. Figure 9 shoes each nozzle has three positions, depending on the activity; the pack, run and unpack position (PALL 2018).

Figure 9: Nozzle packing Positions (PALL 2018)

2.2.7 Cleaning and Sanitisation

Regulatory inspections will focus on cleaning validation of chromatography resins and multiuse systems. Costs typically dictates that resins will be multiuse so resins must be cleaned post each usage, to ensure subsequent reproducibility on following cycles (Sofer and Yourkin 2007). When a decision is made to reuse a resin, resin life time studies must include information on cleaning performance for continued use. It's widely accepted by regulators that TOC (Total Organic Compound) is an acceptable approach for assessing the effectives of a clean in place (CIP) cycle (Sofer and Yourkin 2007). In situations in which high carbon containing buffers are used, TOC may not be an option so other assays such as HPLC or sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) may be used to detect carry-over (Sofer and Yourkin 2007).

Chromatography columns consist of stationary phase of packed media into a consolidated bed. This consolidated bed consists of fine particles that must be maintained in a hydrated state. The presence of air bubbles or other disturbances within the packed column will result in uniformity issues with the stationary phase. These uniformity issues will have a direct impact on the chromatographic separation performance of the column. The cleaning and sanitisation

of packed chromatography columns creates challenges to the biopharmaceutical industry, due to the packed nature of the resin and the typical systems that require CIP operations. Obtaining turbulent flow either within each particle or around each particle is impossible (Maher et al 2015). As a result, the only way to clean a column is to flow a buffer through the stationary phase, typically this is the same route as the mobile phase. Contact to all of the interior surfaces and stationary phase to the cleaning and sanitisation buffer is delivered by the same distribution hardware that delivers the mobile phase during part of normal operations. Cleaning protocols can be developed with this in mind, as packed columns should be kept stored with a sanitisation agent. Examples of this include storing the column in 20% ethanol or 0.1M Sodium Hydroxide to provide bioburden control when not in use (Maher et al 2015).

Vendors that supply resins will provide information on chemical compatibility of resins and equipment surfaces. This should be filed with data that the company collects to show there is no impact as a consequence of the cleaning regime, such as decreased performance. Cleaning agents used must be compatible with o-rings and the internals of column hardware to protect patients (Sofer and Yourkin 2007). Leachable and extractables must be assessed from resins, and all other product contact parts and accessories. Column qualification will involve writing Standard Operating Procedures (SOPs), training staff, effectively packing and evaluating columns (Rathore et al., 2003).

Honrich (2016) details a meeting of industry experts in which issues concerning column packing was discussed. The exact origin of bioburden issues can be hard to pinpoint; it can be from previous operations or introduced during the handling of slurry. Good knowledge of likely species in the local environment is a good starting point and a good sanitisation method with closed column operations will also help. Resin fouling can occur, highlighting the need for effective strip and CIP steps. An ineffective cleaning regime can also result in a broadening of the elution peak after multiple uses, so a good strategy may include blank runs to ensure minimal carry over (Hroncich 2016).

2.3: Column Packing Methods

This chapter describes the different methods column can be packed and the current trends within the industry.

Methods to pack columns have been described by many commentators as an art, rather than a science, and this has resulted in a more science-based approach to packing columns (Rathore 2003). The goal of packing resin into a column is to produce a packed bed that meets an acceptance specification and is reliable over a long period of time (Johnson 2015). Packing chromatography columns and compression factors are largely determined by the type of resin that are being used and the column diameter (Keener et al. 2004). An important point to understand is that a poor column packing technique may not immediately have an impact on bed integrity but can instead result in gradual, continuous degradation with every cycle over time (Siu 2014). A column that does not meet specification or is packed poorly will result in inefficient separations and will result in another time-consuming re-pack. Repacks, due to their nature will results in increased cost, due to additional buffer requirements, and will negatively impact the schedule. Traditional methods have remained dominant with manual methods being employed to pack columns, requiring experienced operators and detailed standard operating procedures (SOPs). The biggest cause of column packing failure is due to operator error. As a risk mitigation tool, the biopharmaceutical industry is moving to a more automated process for packing columns. There are column packing options available that use semi-automated platforms but it still leaves considerable risk for operator error. Data has been generated for companies that have moved from manual methods, who claim to be benefiting from packing success rates of between 62% and 99%, with a 35% reduction in labour requirements (Johnson 2015). Manual methods involve a detailed SOP and Paper Batch Record (PBR), and requires up to four technicians to perform an intricate task in a reliable and repeatable manner, which is not always possible (Johnson 2015). Other issues experienced by industry include reliance on column packing operators who may not be available to complete activities on a given day or who may leave the company, impacting project timelines. Manual methods of repack also involve removing the media from the column, increasing the cleaning requirement, buffer requirements and timelines. Transferring the process to other geographical areas are also prone to error, as SOPs can be interpreted differently by less experienced operators. Excess media methods require slurry to be left over post pack, Protein A resin can cost as much as $15,000 per litre (Johnson 2015). Therefore, if 50L of resin (within slurry) remains post pack, the cost

associated represents as an area of inefficiency (Johnson 2015).

Name	Vendor	Main Packing Method	Slurry Handling	Scale	Comment
ReadyToProcess	GE Healthcare	Prepacked	NA	Pilot/manufacturing	Single use column
OPUS	Repligen	Prepacked	NA	Pilot/manufacturing	Single use column
AxiChrom	GE Healthcare	Axial compression	Closed	Pilot/manufacturing	Intelligent packing Automation
AxiChrom	GE Healthcare	Axial compression	Open	Lab/pilot	Intelligent packing Automation
Chromaflow	GE Healthcare	Flow/pressure	Closed	Pilot/manufacturing	Traditional pack-in-place
BPG	GE Healthcare	Flow/pressure	Open	Pilot/manufacturing	
QuikScale	Millipore	Flow/pressure	Open	Pilot/manufacturing	
Resolute	Pall Corporation	Flow/pressure	Closed	Pilot/manufacturing	Traditional pack-in-place
Resolute Autopak	Pall Corporation	Axial compression	Closed	Pilot/manufacturing	Automation
IsoPak	Millipore	Flow/pressure	Closed	Pilot/manufacturing	Traditional pack-in-place

Figure 10: Column Packing Vendors and corresponding techniques (Gebauer and Tschop 2018).

Figure 10 above, shows the different column packing vendors and their corresponding column packing techniques. Increased production titres have resulted in larger columns to accommodate the increased batch sizes. In industry, it is typical to see columns of 1 to 2 meter in diameter. The columns present a challenge with regard to managing layouts in terms of space, electrical connections, Heating, Ventilation, and Air Conditioning (HVAC) classifications. Weight is also another critical design feature that must be considered. Concentrating on unit operations rather than the support operations like column packing at early design stages will present issues further down the line. Figure 11 shows the slurry vessel requirement and weight considerations that need to be included into project design. (Bloomingburg and Ganhi 2005). Resins are another important factor which are chosen based on their separation ability and not on how well they will pack in a specific vendor column (Rathore et al 2003).

Column Diameter	Cross-Sectional Area	Approximate Weight of Empty Column[1]	Bed Volume[2]	Slurry Tank Volume[3]
30 cm	707 cm^2	96 kg	21 L	50 L
80 cm	5,027 cm^2	890 kg	151 L	335 L
120 cm	11,310 cm^2	2234 kg	339 L	798 L
160 cm	20,106 cm^2	4294 kg	603 L	1419 L
200 cm	31,416 cm^2	7127 kg	942 L	2218 L

[1] Based on a curve fit of data from 35 cm to 2 m (acrylic columns) with an approximate height of 220 cm

[2] Assumes 30-cm bed height

[3] Assumes 30-cm bed height and 50% slurry (typical for ion-exchange resins; slurry volumes vary with resin type) with vessel sized to be 85% full to top tangent line

Figure 11: Impact of column diameter on facility design (Bloomingburg and Ganghi 2005)

In terms of facility deign an assessment must be performed up front to ascertain;

1. How often will a column packing operation be required in order to meet the manufacturing schedule?
2. Will the column be packed at the position of use (packed in place) or in a separate area with dedicated utilities (packed out to place?)
3. Will the operation be manual, semi-automated or automated?
4. What is the storage requirement of the resin, in terms of delivery to site and normal storage conditions? (Bloomingburg and Ganhi 2005)

2.3.1 Manual Packing

Manual packing of chromatography columns can be referred to as traditional or conventional applications. Dry packing (or commonly referred to as tap packing) was one of the first methods employed for packing columns. As per figure 12, manual packing involves removing the head and placing resin into the column, agitating it to ensure the media settles uniformly and then hydrating the media in the column. When media was eventually supplied in a hydrated form, flow packing became the routine method (O'Neill 2003). Manual column packing has provided many challenges and one of these is the chance of achieving a "good pack". Repacks are often required due to the media delivery and resin often ends up spilled on the floor. Manual packing is also very difficult to scale to larger columns. This involves very active manual participation. As a result of the manual process being an open system, contamination risk is higher (PALL 2018).

Figure 12: Manual pack method (Bio-Rad 2019)

2.3.2 Flow Pack Method

Flow packing (constant pressure or flow) is a widely accepted technique for consolidating chromatographic packed beds, due to its ability to pack particles in suspension more quickly

and uniformly than gravity settling (Gebauer and Tschop 2018). Flow packing can be used for manually packed columns but when pack in place nozzles were created, it solved a lot of issues for “lid off” packing. These nozzles resulted in the top adjuster staying in place for the pack which limited resin waste and allowed packing, unpacking and CIP steps to seamlessly take place. Flow packing utilises the constant flow rate of the mobile phase that is pumped over the sealed column. This results in suspended particles being packed much quicker and reduces the bias of larger particles settling first. A higher packing flow rate, up to 30% higher, ensures that there is minimal settling or bed compacting post packing operations (Bemberis et al.2003). Flow packing is regularly used for resins that would be considered more delicate. These include dextrans, agaroses and cellulose media. For flow packing, the bed can also further compress during the operation so it is advised to have an adjustable cell, to remove any void that could develop (Bemberis et al.2003). When using constant flow to pack a column, the flow rate and pressure differential must be maintained and controlled in order to achieve a bed with the correct compression (Gebauer and Tschop 2018). As per figure 13, the flow packing method can be broken into three main steps;

1. Prepare slurry to correct percentage and transfer into an empty column. Re-suspend to ensure homogeneity – lower top adjuster onto slurry.
2. Allow flow to pack the column.
3. Lower plunger to final bed height and secure into position (Cheng 2009).

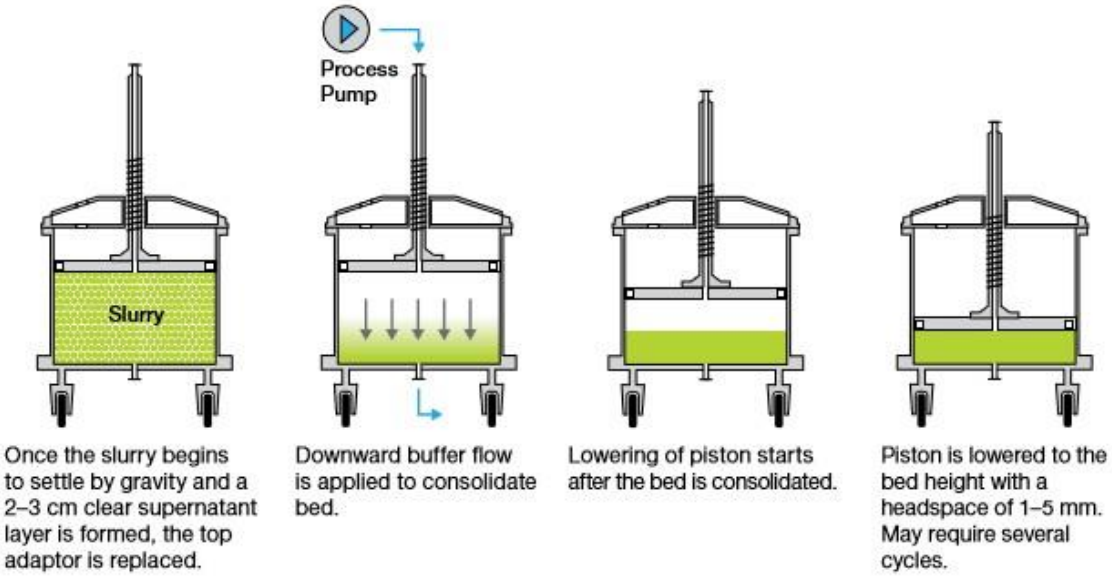

Figure 13: Flow Packing Method (Bio-Rad 2019)

By dropping the top adjuster onto the bed after the flow has compressed the column, it ensures the bed will not decompress (Gebauer and Tschop 2018).

Figure 14 shows the different type of profile that can be obtained when you are either doing a flow pack with contact flow or with constant pressure.

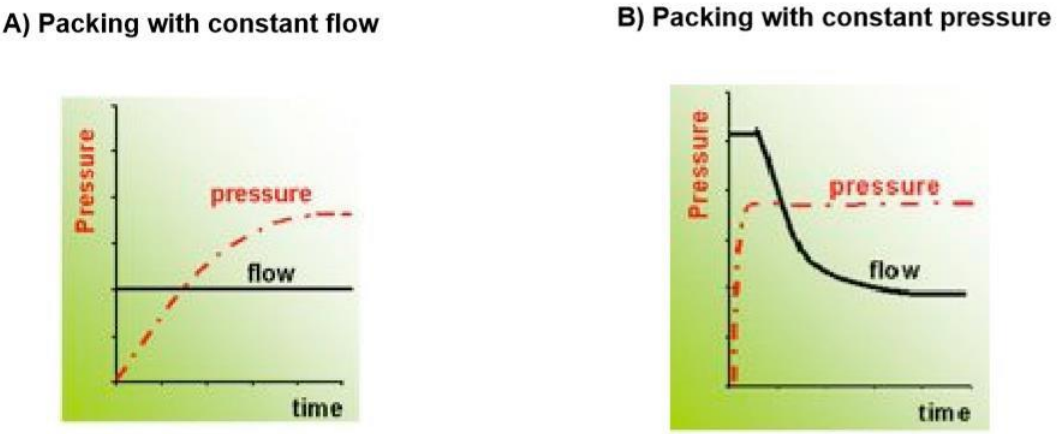

Figure 14: Pressure and Flow profile during Column Packing (Cheng 2009)

2.3.3 Dynamic Axial Compression

Of the various different resins available for large scale chromatography, each group will have different characteristics in terms of column packing. Different column packing approaches can also result in a packed bed that has different structural characteristics. Some resins are soft and compressible but will not function optimally when even moderate pressures are applied. As a result of this, when a column is packed with this soft group of media, up to 30% more media volume is required to achieve the specified column volume at the standard operating pressures (Siu et al., 2014).

Axial compression technology is supplied to industry by various vendors. Axial compression utilises a movable column adaptor or end piece to form the packed bed by compression. When compared to flow/pressure packing, axial compression can give a user significant improvement in the quality of the packed bed which produces higher chromatographic performance. Axial compression can also provide more reproducible results and a higher process robustness. When utilised alongside pack in place technology and leveraging the highest level of automation available, users can expect to gain higher performance and efficiencies compared to other column packing approaches (Gebauer and Tschop 2018). Axial compression speeds up the packing process and minimises size segregation caused by gravity settling by providing a constant pressure over the entire bed. The

piston that exerts the force over the bed eliminates any voids or channels that may form in the bed, resulting in a uniform distribution of the mobile phase. Unlike flow and pressure packing, Dynamic Axial Compression (DAC) can be performed in a single step. The control strategies can be configured to deliver the specified compression factor and because the process is automated, it delivers better control and repeatability. DAC is particularly suited to small rigid media and is the preferred method for industrial operations (Bemberis et al., 2003).

One disadvantage with DAC is the interference from the bed support that can result increased compression in areas around the moving adjuster. This can be reduced by increasing the velocity of the adjuster to execute the pack quicker. Essentially, this produces the effect of flow packing as the fluid is displaced at the bottom nozzle which gives an improved bed compression and compensates for reduced compression at the base of the packed bed (Bemberis et al., 3003).

Different methods to pack columns will impact on bed the heterogeneity and hydrodynamic stability. Experiments completed by Dorn et al (2017) concluded that flow packing tended to result in increased compression at the column outlet. Conversely, DAC resulted in increased pressure at the top of the bed, nearest the moving adaptor. It's understood that column performance issues can be attributed to bed non-uniformity.

2.3.4 Column Packing and Lean Manufacturing

Within the biopharmaceutical manufacturing Industry, there is continued pressure to advance the efficiency and productivity of large-scale manufacturing activities using new technologies. There is significant pressure to improve column packing processes, to make them more efficient. Scale-up requirements have introduced the need for the creation and development of packing and cleaning methods, mainly due to the lack of standardisation offered between the various vendors. This has extended the time required to get products to the market which has caused the industry to focus on bottle necks that exist with current downstream chromatography equipment. An example of improved column packing system is GE Healthcare's AxiChrom™ intelligent packing system uses axial compression which is offered from 50mm to 1.6 meters for industrial operations (Lundkvist, 2016).

Figure 15 below is presented by GE Healthcare (2019) as a time study that was conducted between a traditional hoist dependant pack-in-place column packing method and their hoist free axial compression method. The figure shows that the packing process is faster with the disassembly and cleaning of the AxiChrom™ method proving to be more efficient.

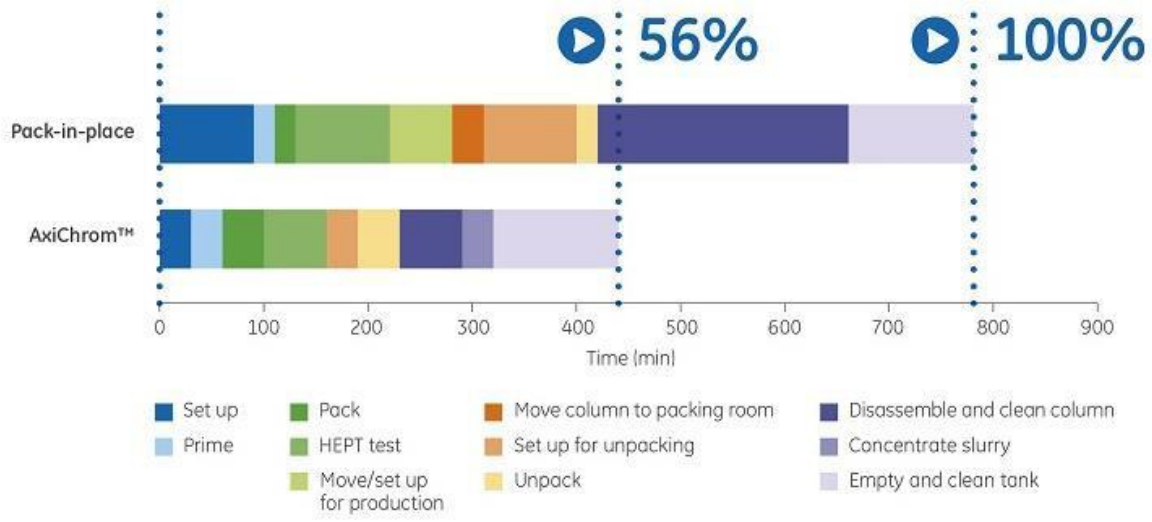

Figure 15: GE AxiChrom pack efficiency versus traditional PIP method (GE 2019)

Column packing operations for pack in place systems that use axial compression systems are not fully closed or isolated from the environment. For example, the receipt and handling of resin alongside the transfer of resin to the slurry vessel is into completed in a closed system. Figure 16 outlines these steps;

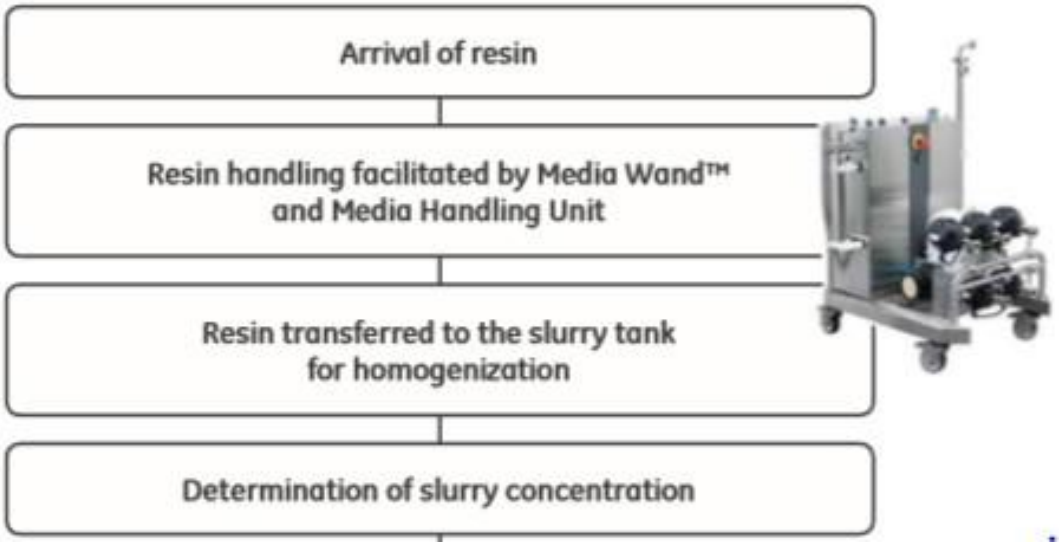

Figure 16: Typical open operations required pre-packing activity (GE 2016)

Typically, the initial column packing operation involves taking a carboy of resin, removing the storage buffer, manually agitating the resin and transferring the content into the slurry vessel. Vendors now offer media unload probes that can perform all these tasks. Media unload probes are made for large scale operations that involve multiple carboys and large amount of resin. These probes can deliver air to break up resin, suction to remove slurry and buffer to

rinse the container (GE 2019). Another issue is that by excessively agitating and stirring resins "fines" can be generated. Fines are produced by the shearing of beads creating small particles that can occlude the bed supports. Although fines are not deemed to be a contamination, they can cause back pressure issues, disrupting flow and impeding uniformity in flow (Bemberis et al. 2003). Decanting supernatant after a settle period can reduce the amount of fines present but procedures should be put in place to avoid creating fines in the first instance (Bemberis et al. 2003). Figure 17 below shows a 60% time saving, with a significant reduction in manpower from using an automated resin handling unit rather than traditional manual techniques.

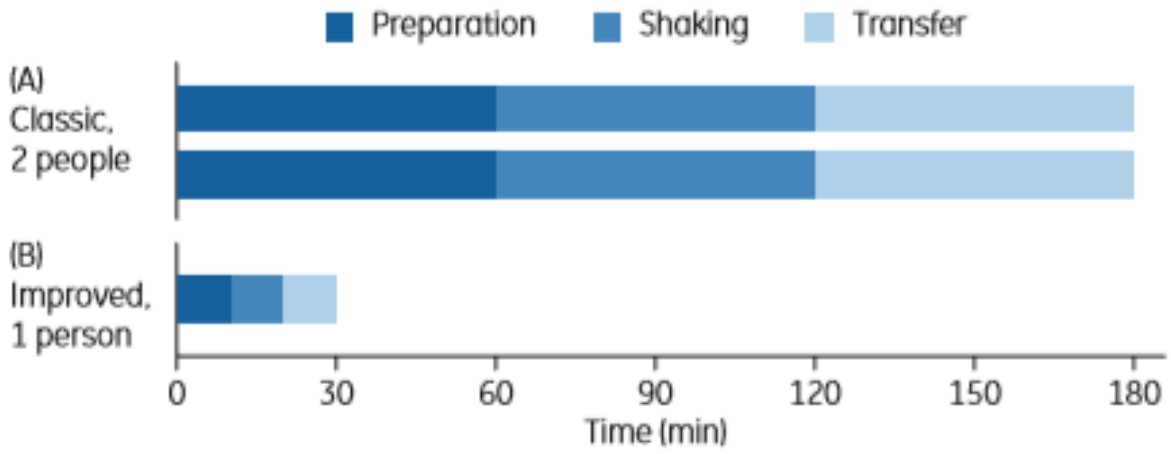

Figure 17: Resin Handling Efficiencies (GE 2016)

2.3.5 Slurry Concentrations

Slurry concentration refers to the ratio of media to buffer in a "settled" slurry. This measurement needs to be ascertained because quantities supplied by vendor containers are not always accurate or reliable (PALL 2018).

Determining precise slurry concentrations can be susceptible to meniscus error, people variation or incomplete mixing and suspension issues (PALL 2018). A precise slurry concentration along with the compression factor is paramount to calculating the correct amount of resin required in order to achieve a certain bed height. Each pack will require a volume of resin to be mixed with a volume of buffer. The resin type will dictate the buffer but typical buffers include salt (e.g. 10-250mM) or a hydrophobic solvent such as 10% ethanol to minimise particle to particle interactions. A common concentration is a ratio of 50% resin to 50% buffer, but this can vary between 30% and 70% resin. If the slurry concentration is not calculated correctly, the correct compression factor will not be achieved and the bed will be packed either

too loosely or too tightly. Another important factor relates to ensuring the resin is homogeneous as this may also impact on sampling (Gebauer and Tschop 2018). An SOP should specify an agitation time, followed by sampling and centrifugation. Slurry Concentration sampling can be determined as follows;

1. Slurry tank concentration (SC) = Settled height / total Height (Figure 18).

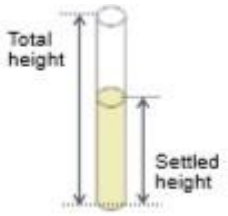

Figure 18: Slurry Concentration (SC) (PALL 2018)

2. Volume of packed bed (Vpb) =Radius^2 X π X bed height (Figure 19).

Figure 19: Volume of packed Bed (PALL 2018)

3. Volume of resin Required (Litres) = Vpb x Compression factor.

4. Amount of slurry required

   = Slurry Litres = Media / SC.

2.3.6 Syringe Pack

PALL (2016) offer a fully automated column packing system and believe that this can turn "art into a science". The system is similar to other fully automated systems offered by other suppliers (GE, Bio-Rad), removing the manual interventions and benefiting from predetermined methods for CIP, pack and unpack operations at the selection of a recipe.

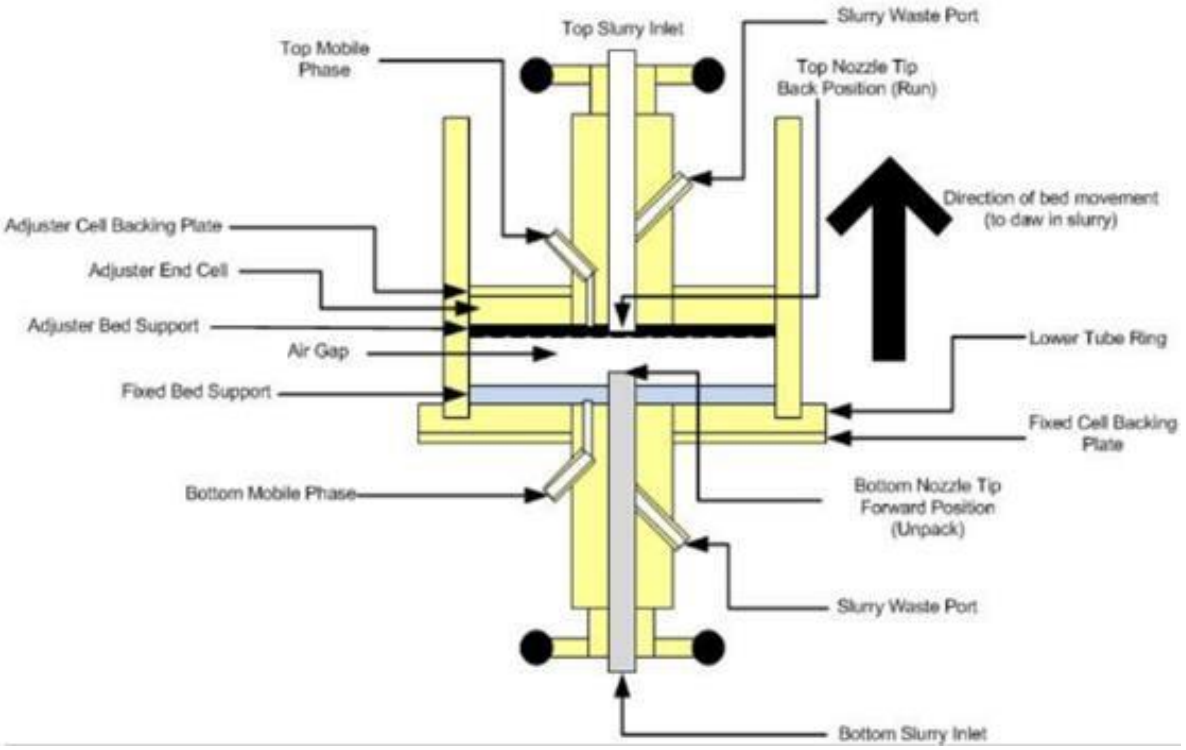

Figure 20: Syringe Pack - Column cross section (PALL 2016)

For the syringe pack method, operators initially calculate the slurry concentration. Using a Programmable Logic Controller (PLC), a user can enter in;

- Bed Height
- Compression factor
- Slurry Concentration
- Velocity

Once input parameters have been entered into the PLC the system, it will calculate the amount of slurry to be drawn into the column. Initially, a priming buffer will be drawn into the column to remove air, as per figure 20. The piston drives the adaptor up sucking in the buffer. With the bottom nozzle closed, the top adaptor will push down and remove the air from the headspace. Once the top nozzle is flooded a liquid sensor will indicate the column is primed. The top adaptor will then drive the remaining buffer out through the bottom nozzle. The PLC will transition to the pack recipe and pull in the required volume of slurry from the slurry vessel. The unit can also be configured to have no excess media in which all slurry is used, or by allowing for excess media post every pack. Once the column has the required amount of slurry, the top adaptor will push down and displace the buffer out from the bottom nozzle. The velocity of the compression can be predefined and will be vary based on the type of media used in the

packing. It is typical to perform the pack in two stages. Initially, the slurry is adjusted to meet the preferred bed height, typically at a faster speed. Once achieved, the actual bed compression will occur with a slower more controlled velocity (PALL 2016).

Figure 21 below, is a visual representation of valves and hoses configuration to perform a syringe pack.

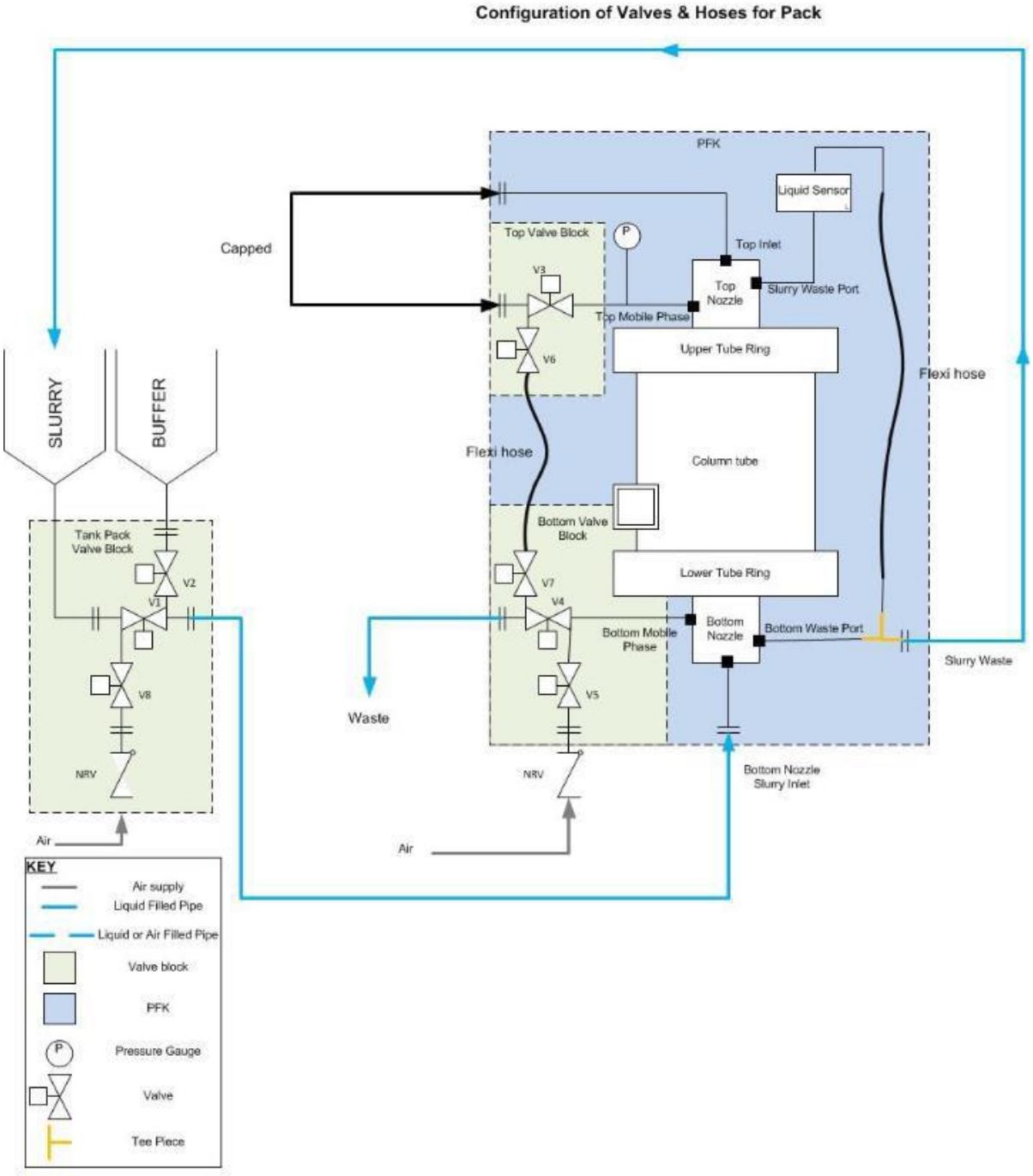

Figure 21: Configuration for an automated syringe pack (PALL 2016)

2.3.7 Pre-packed Chromatography Columns

Large biopharmaceutical manufacturers can gain a competitive advantage by using prepacked columns. The manpower required to manually pack columns, the associated costs, coupled with the flexibility requirements are strategic factors that must be considered. Pre-packed columns are currently available for both clinical scale and commercial scale processing (Grier and Yakubu 2016). Figure 22 shows a cross section of a pre-packed column with parts and materials of construction.

Part	Materials of Construction
1. Inlet port	Polypropylene
2. Outlet port	Polypropylene
3. Unpacking port	Silicone and Polypropylene composite
4. Flow distributor	Polypropylene
5. O-rings	Silicone
6. Return line	Silicone and Polypropylene
9. Side guard	Composite
10. Tube	Polypropylene glass composite
11. Integral casters	Composite

Figure 22: OPUS 80R: Commercial prepacked 80cm in diameter column - cross section (Repligen 2019)

Currently, the largest column on offer is an 80cm column with bed heights that ranges from 10 cm to 30 cm. This area represents a growing trend towards pre-packed columns. Repligen (2018), a supplier of prepacked columns, noted its first shipment of an 80cm to a manufacturing facility in Q2 of 2018.

The advantages of using pre-packed column are similar to advantages associated with single use technologies (SUTs). Quality groups could consider these columns as consumables, removing the need for capital investment, qualification, asset management, routine calibrations and maintenance. Resources can be dramatically reduced as outlined in figure 23. Costs associated with the column in terms of initial capital investment, materials required and storage can all be reduced by 67%. The reduction in the total man hours of Full Time Employees (FTEs) can be as high as 81% (Repligen 2019).

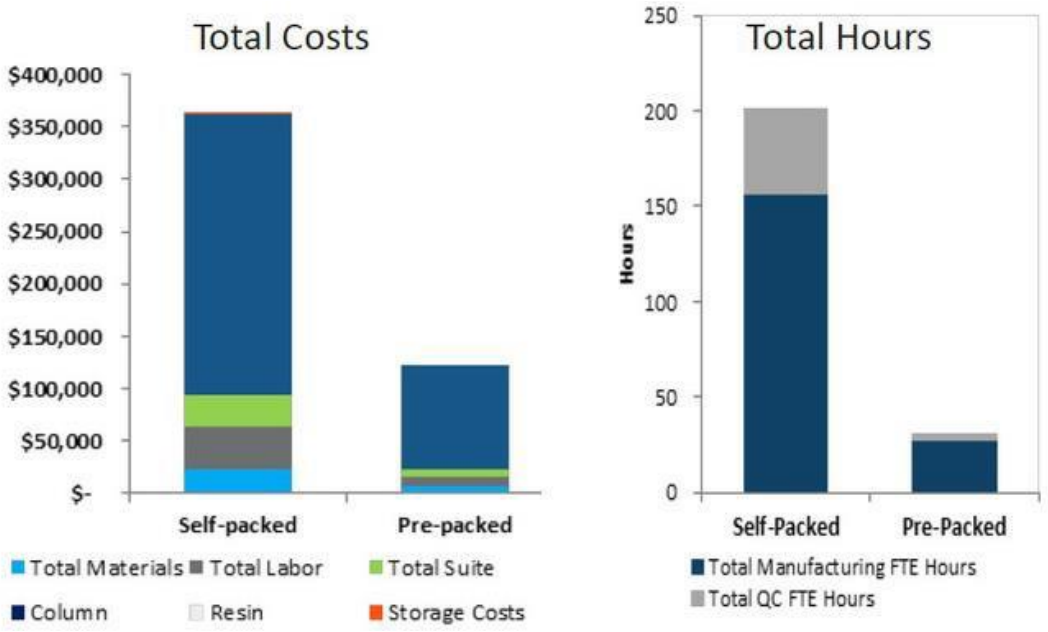

Figure 23: Time and Manpower saving of prepacked columns (Repligen 2019)

Whether packing columns using axial compression or flow packing, it is highly complex with many variables that must be controlled. These variables range from hardware, packing parameters (e.g.; slurry concentrations), testing, determining acceptance specifications, documentation and personnel. Pre-packed columns considerably reduce the variability that can present between packs. Thus presents as an excellent risk reduction strategy for manufacturers of commercial biopharmaceutical products (Repligen 2019).

In a study at 20cm bed diameter and 10cm bed height, both a prepacked column and stainless-steel column were axially compressed with the same Protein A resin and identical compression factor. When the prepacked column was spiked, it was found that the vendor qualification and post shipment qualification for asymmetry, number of plates and retention time differed but still met the acceptance criteria (Grier and Yakubu 2016). For the plate count, the onsite test was higher. In contrast, the asymmetry and retention was comparable. Ten capture cycles were performed on both columns and results were compared for step yield and HCP removal. It was determined that the prepacked column performed marginally better. However, analysis of the chromatographs showed the stainless-steel column was superior in relation to mass transfer and separations. It was concluded that these minor differences would have no impact to product or impurity profiles. The draw back to pre-packed columns includes transport requirements as consideration is needed to ensure the column and packed bed is not negatively impacted in transit. Additionally, there will be additional leachable and extractable information required; and manufacturers are limited to the current column diameters that are available (Grier and Yakubu 2016).

2.3.8 Extractables and Leachables (E&Ls)

Repligen (2019) outline the advantages pre-packed columns present to manufacturers and state these are much aligned with advantages offered by SUTs. Lopes (2015) details benefits associated of SUTs, such as flexibility, initial cost outlay, time efficiencies and prevention of cross contamination. However, Jordi et al. (2018) points to the limitation that SUTs pose in terms of susceptibility to physical damage, process control issues and product quality assurance concerns. A key concern relates to the leaching from polymeric materials into product streams. Leachables are compounds that migrate from material in which the product comes into contact with. Extractables are compounds that migrate under harsh or worst-case conditions. It is important to highlight that not all leachables are a subset of extractable compounds. Leachables can negatively impact the product and in turn, can impact patient safety (Jordi et al. 2018).

SUTs have additional validation requirements such as assessing for the potential impact both E&Ls pose. E&Ls are considered product related impurities that arise when a product comes directly into contact with single use components during the manufacture, storing or delivery of a biopharmaceutical product (Wakankar et al. 2010). Figure 24, presented by Lalor et al. (2019), looks at some of the advantages and current day challenges. The waste disposal issues regarding these non-recyclable materials should also be considered.

Benefits	Challenges
Elimination of CIP and SIP requirements	Potential for leachables and extractables
Reduced facility footprint	Limited scalability
Increased flexibility for changeover	Increased supply-chain risk
	Not fully realised for all unit operations
	Increased operating costs
	Single-use instrument calibration and reliability
	Increased potential for human error
	Robustness of materials of construction
	Potential increased contamination risk during perfusion campaign

Figure 24: Some of the Benefits and challenges to Single Use (Lalor et al. 2019)

The pharmaceutical and biopharmaceutical industry have been provided with a variety of guidelines and draft guidelines from various regulatory agencies and concerned groups. These include the Food and Drug Agency (FDA), the European Medical Agency (EMA), Biophorum Operators Group (BPOG), Bioprocess Systems Alliance (BPSA) and the Product Quality Research Institute (PQRI). These regulatory agencies and groups have provided examples of Risk Assessments (RAs) to industry on how to screen and select components as well as providing advice on extraction studies and conditions (Jordi et al. 2018). In addition, testing standards have been outlined in the United States Pharmacopeia (USP) and the European

Pharmacopeia (Ph. Eur).

Elder (2017) highlights that the International Community for Harmonisation (ICH) provide safety guidance to manufacturers which is outlined in ICH 4,5,6,7,8. However, this does not discuss E&Ls as they are regarded as contaminants rather than impurities.

The Extractables and Leachables Safety Information Exchange (ELSIE) consortium was created so that manufacturers could share their experiences with E&Ls. These companies come from different sectors and include biopharmaceutical, pharmaceutical and medical device industries. This consortium uses a Quality by Design (QbD) approach and helps manufacturers by presenting an opportunity of identifying issues with components at the early design process stage. This reduces the risk and potential financial impact that can result from the discovery of E&Ls later in the project. ELISE also provides a forum for collaboration, aiding companies in removing duplication of work which directly increases the speed of regulatory filing (ELSIE 2018).

2.4 : Evaluating a Packed Column

This chapter describes how a packed column is evaluated post column packing operation and what criteria deems a pack successful.

Once a column is packed, a number of tests should be performed to evaluate the pack. These tests will not predict how effective the column will be in the field but will evaluate the performance of the packing procedure. The results obtained will allow manufacturers to compare the column packing operation in terms of reproducibility. The reproducibility will be of interest to regulatory bodies such as the FDA (Kennedy et al. 2003).

Choosing the correct metric to evaluate a column is important. The ability to pack columns repeatedly with sufficient precision will allow the user to compare the performance of the pack over time. Throughout industry, Height Equivalent to a Theoretical Plate (HETP) and asymmetry (As) can be used to support this and help with controlling the chromatographic process. The process can start at initial qualification and can be applied for in process testing. HETP and As are used to ensure bed integrity throughout the period that a column remains in service. Columns and resins can be product specific and therefore may be stored away for periods of time, awaiting the product specific campaign. HETP and As testing can be used to ensure a column is fit for purpose after long periods of storage (Bemberis et al. 2003).

2.4.1 Pulse Infection for HETP & Peak Asymmetry (As)

Pulse injection is widely used to evaluate the uniformity of the bed or to check its integrity. Many chromatography systems use a UV (ultra violet) absorbing molecule to complete the test. P-aminobenzoic (PABA) or acetone standards can be used for UV detection systems. Alternatively, sodium chloride (NaCI) or sodium hydroxide (NaOH) standards can be spiked and measured using a conductivity probe. Using such buffer standards, that are non-reactive with the resin, recreates the flow of a protein of interest through the column and creates a tracer on a chromatogram that can be used to determine the HETP and As. Typically, 1% to 2.5% of column volume is used to determine the volume of “spike” buffer required. Pulse injection volumes should be large enough to ensure it is not diluted by any hold up (Bemberis et al. 2003). GE (2010) recommend that the tracer be selected so that the molecular weight results in full penetration of the porous bed structure. The data generated from a column evaluation can be performed by manual calculations or by using a computer system (O’Donnell 2003). Figure 25 shows the possible tracers and the background eluent with the corresponding detection unit required.

tracer	eluent	detection
water	150 mM NaCl	conductivity
1M NaCl	150 mM NaCl	conductivity
1 % - 3 % acetone	150 mM NaCl	UV at 280 nm

Figure 25: Possible Test System Configuration (Cheng 2009)

There are a number of calculations that can be used to evaluate a packed bed. The number of theoretical plates (N) is a common concept that is mathematical in origin. However, the actual packed column does not contain any physical distillation plates. Theoretical plates are an indirect measurement of a peak's width at a specific point and at a specific retention time (Cheng 2009). Equation 1 shows the formula to evaluate the number of theoretical plates.

$$N = 5.54\left(\frac{V_e}{W_{1/2}}\right)^2$$

Equation 1: Theoretical Plate Equation (GE 2010)

- N = Number of theoretical Plates
- 5.54 = Constant
- Ve = Retention time
- W1/2 = Measurement of width of peak at half the peak height

A column that results in a higher plate count will be more efficient than a column with a lower plate count. A column that results in a higher plate count will generate a narrower peak and longer retention time than a column with a lower plate count. Column efficiency is dependent on the column and bed measurements (e.g. diameter and bed height). When comparing columns, the number of plates per meter is also commonly used. The height equivalent to a theoretical plate is expressed at HETP = L/N and is a measurement of peak broadening. (Kennedy 2003).

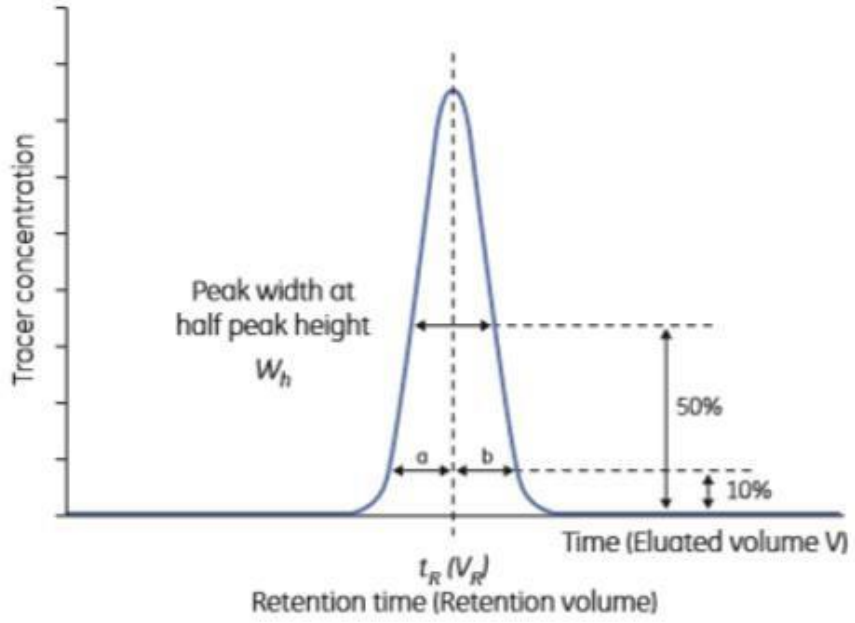

Figure 26: Evaluation of Tracer (GE 2010)

Figure 26 shows a chromatogram with the typical peak dimensions that need to be measured to calculate HETP and asymmetry. Peak asymmetry is an important concept and is referred to as "As". The peak is divided in two sections from the highest point of the peak, a and b. The width of the a and b sections are measured at 10% of the peak height from the baseline. The distance from the leading edge to the centre is measured and represented by "a". The distance from the centre to the trailing edge is measured and represented by "b". The ratio of a to b is then calculation to determine the As. As values will vary depending on the resin but typically a result between 0.8 and 1.4 are acceptable (Kennedy 2003).

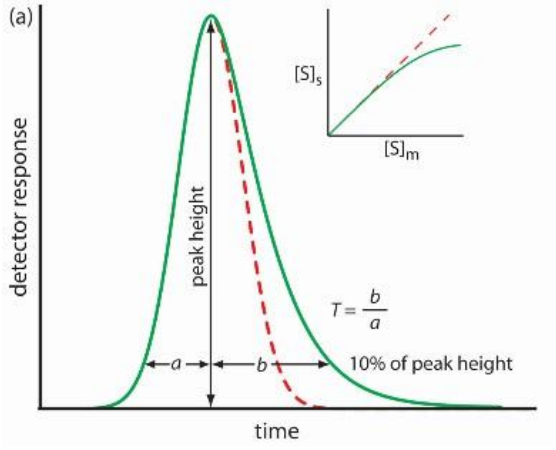

Figure 27: Illustration of Tailing (Harvey 2013)

An As of >1.4 indicates that the column is not packed or compressed enough, as illustrated by the green line on figure 27. A possible root cause may be a clogged bed supports at either the top or bottom of the column or a poor pulse injection technique (O'Donnell 2003).

An As of <0.8 indicates that the column may be over packed, packed at higher than required pressure or the column bed is compromised. It may be indicative of a cracked column bed. Reference the green line on figure 28 (O'Donnell 2003).

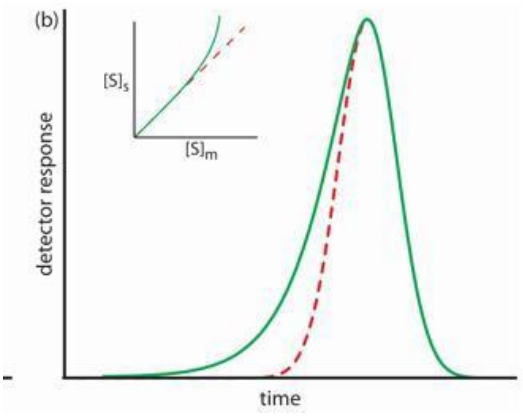

Figure 28: Illustration of Fronting (Harvey 2013)

PALL (2018) graphically illustrate how a non-uniform bed may present in a tracer in figure 29;

Figure 29: Non-uniform bed and resulting Peak (PALL 2018)

In another illustration, represented in figure 30, dye band tests were conducted at PALLs laboratory during column development. For the bad pack, you can see the die bands as non-uniform, indicating the solute will move at different speeds, resulting in an atypical chromatogram. The next image, illustrating a good pack, shows uniform dye bands and the typical tracer attained in column evaluation which represents one dye band; and would result in a chromatogram with a passing As criteria (PALL 2018).

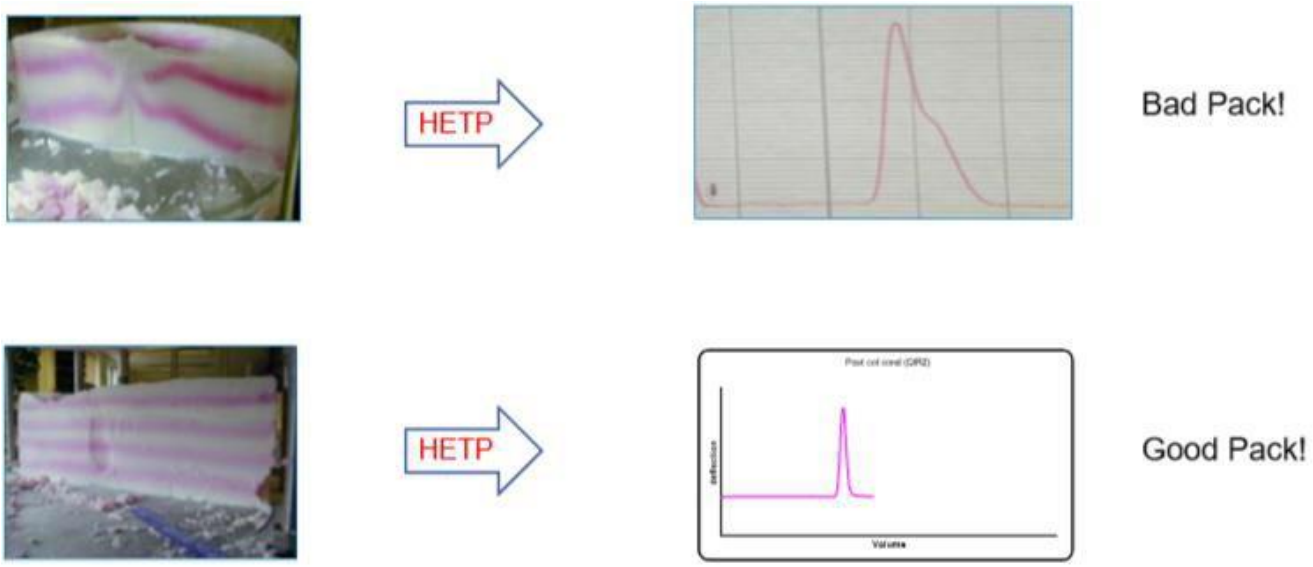

Figure 30: Dye bands applied to packed beds (PALL 2018)

2.4.2 Van Deemter Equation

The Van Deemter equation considers all the factors that impact HETP and band broadening. The factors or variables in the van Deemter equation need to be investigated and manipulated to ensure the theoretical plate count for the column is high. The first factor or variable is eddy diffusion (Meyer 2015) and is illustrated in figure 31.

Figure 31 illustrates how some particles will leave the column early by chance, having travelled through the column via an unobstructed path. Other particles will have their path obstructed, essentially increasing the distance they must travel to exit the column (Meyer 2015). This is defined as eddy diffusion.

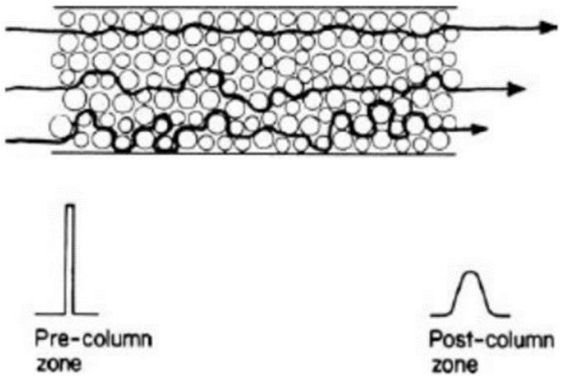

Figure 31: Eddy Diffusion (Meyer2015)

Flow rate and distribution is also a contributing factor whereby the flow will be faster between the channels i.e. between the resin particles. Eddy and flow distribution can be reduced by packing a column with evenly sized particles and with an overall similar size distribution. The third factor relates to the diffusion of the sample molecules within the mobile phase. It's

understood that there's higher friction located around the perimeter of the column, resulting in higher concentration of the sample molecule passing through the centre of the column. To reduce the impact on band broadening, higher velocities are required so the sample molecule spends less time on the column. The fourth factor relates to resistance to mass transfer. The stationary phase has absorptive properties and attracts the sample molecules that can either absorb or desorb. Sample molecules can be retained by the structure of the bead and the time taken by some molecules to diffuse back into the mobile phase can vary, resulting in band broadening (Meyer 2015).

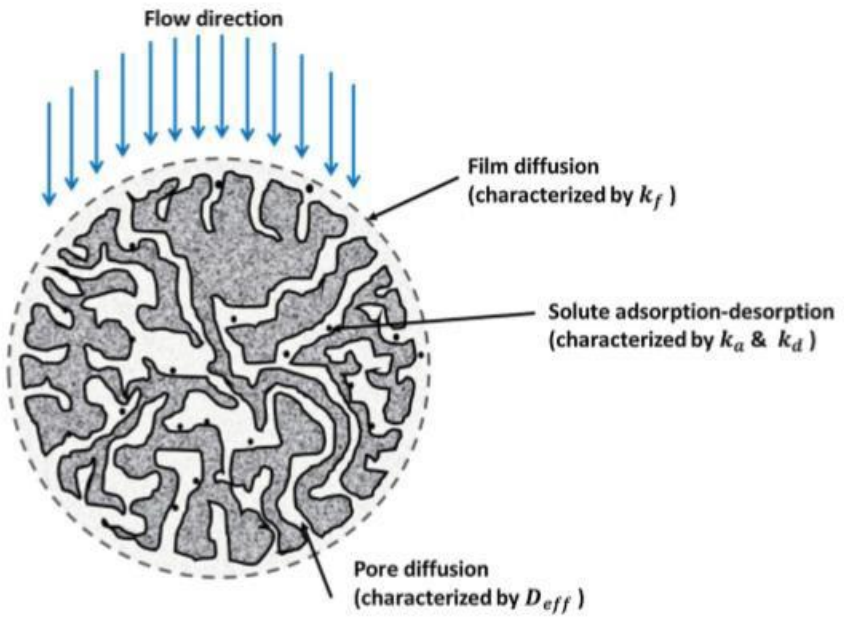

Figure 32: Mass Transfer Mechanisms (Shekhawat et al 2018)

In a chromatography process, there are certain physical phenomena's that occur. Figure 32 shows the solute mass transfer in three scenarios;

1. Absorption & desorption
2. Film diffusion
3. Pore diffusion (Shekhawat et al. 2018)

Johnson et al (2014) describe the classical Van Deemter equation as seen in equation 2;

A = Eddy Diffusion or unevenness of the flow

B = Contributions from Longitudinal Diffusion

u = Speed or Velocity of the mobile phase

C = Mass transfer resistance

$$\mathrm{HETP} = \frac{B}{u} + A + Cu$$

Equation 2: Van Deemter Equation (Johnson et al. 2014)

2.5: Chromatography Media

This chapter describes the resin used for liquid chromatography.

Increased titres from the upstream process have placed further burden on the downstream platform to remove the many impurities present, such as HCP, DNA, process related contaminants and product related impurities. A combination of different chromatography steps are used with different resin chemistries to purify the molecule of interest to acceptable levels. The number of chromatography steps and choice of chromatography method are dependent on the type of molecule and the nature of the contaminants present in the process stream. There are primarily four chromatography techniques used to purify biopharmaceutical, namely affinity, ion exchange, multi-mode and hydrophobic interaction chromatography (Challener 2017).

2.5.1 Resin advancement

Globally, the resin market was valued at $1.63 billion in 2016. Expected sales are forecasted to expand by 6.5% every year until 2025. This is also fuelled by the growing number of contract manufacturing organisations entering the market. Table 1 below forecast the US resin market by type (Grandviewresearch.com, 2016).

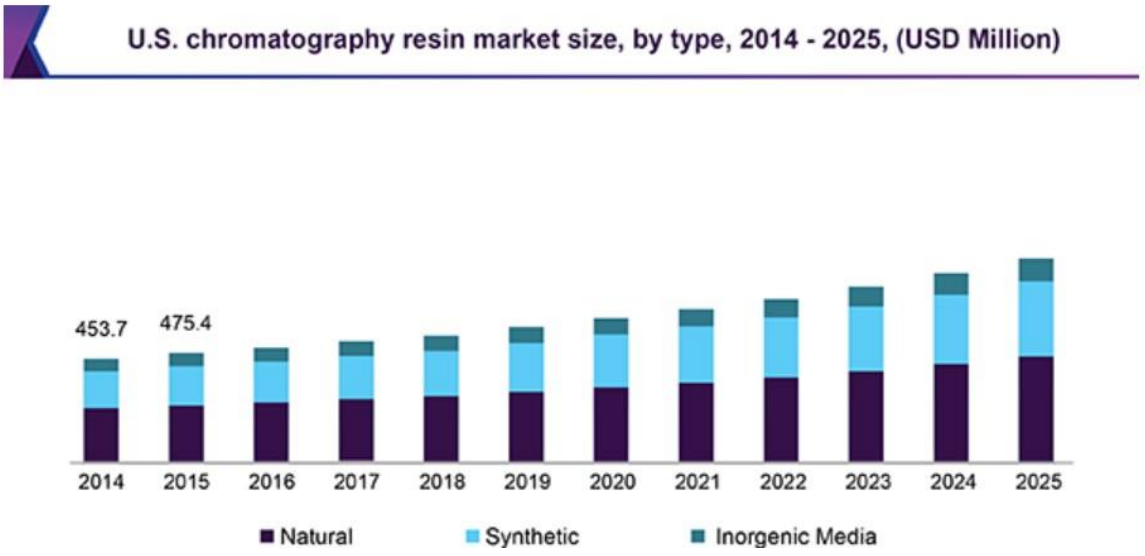

Table 1: US Chromatography resin market by type (Grandviewresearch.com 2016)

The requirement of modern-day biopharmaceutical manufacturers has resulted in improvements regarding the stationary phase, in base bead and with ligand chemistry (Rathore,

Kumar and Kateja, 2018). Other requirements include safety aspects, such as E&Ls, and the cost implication resin has on the overall downstream process (Nweke 2017). Current resins for large manufacturing processes are limited by a 2m column diameter and 30cm bed height due to pressure drop limitations. This impacts the amount of product that can be processed. As a consequence of this, it is typical for large manufacturers to run multiple cycles which increases hold times (Shukla et al. 2017).

Significant effort has been directed at the base bead, in terms of chemical and physical stability. As a result, a number of base materials are currently commercially available that are applicable to processes that require high pressure. Modern day polymeric base beads have been designed so that they are rigid enough to allow the required pressure flow characteristic and can be compressed so that a uniform packed uniform bed can be achieved within the column. This is a critical enabler for the manufacture of large-scale biopharmaceuticals and allows for large columns to be sufficiently packed, to allow highly concentrated batches to be purified. Another important element refers to chemical robustness of the resin beads to withstand high and low pH buffers, for instance with CIP buffers. Chromatography resins have also seen improvement, as pore size, structure and volume have been optimised which has increased the mass transfer ability and subsequently the DBC (Rathore, Kumar and Kateja, 2018). The DBC is related to the amount of target molecule that can bind to a column under normal flow conditions. This value is always below the static binding capacity (SBC) which relates to the saturation capacity of the matrix you are utilising (EMD Millipore 2005).

Auroa et al (2017) define the classes for an affinity chromatography in terms of class, see table 2 below.

Matrix Class	Examples	Commercial Names
Natural	Agarose	Sepharose
	Dextrans	Sephadex and Superdex
	Cellulose beads	Cellulose beads
Synthetic	Acrylamide	Bio-Gel
	Polystyrene	Polystyrene
	Polymethacralate	Separon HEMA
Inorganic	Porous silica	Porous silica
	Glass fiber	Glass fiber
	TiO_2	TiO_2

Table 2: Example matrices for each class (Arora et al. 2017)

2.5.2 Ligand advancement

A ligand is a molecule that has the ability to recognise a target protein with high selectivity (Roy, Mondal and Gupta, 2007). Further improvements have been achieved through improved ligand arrangement and the density of these on a bead. Manufacturers of biopharmaceuticals have asked for increased DBC of the resins they use. The driver for this is that as the DBC goes up, the column gets smaller. As a result, less resin is required to pack the column and buffer volumes are decreased (Rathore, Kumar and Kateja, 2018).

Limitation in protein A and increased bioreactor titre pose a significant threat to large scale manufacturers. Bioreactor titres in 1987 were 500mg/L and this has increased to 5g/L today for fed batch processes. Now ligands immobilised on beads, with single or multiple linkage sites, offer increase accessibility for proteins to bind and subsequently increase DBC. Adjustments to the protein A domain and the amino acids present have also increased protein A capacity, lifetime, stability with NaOH, elution pH and specificity. Productivity and capacity are still improving and this led to requiring new higher performing resins, such as the Mabselect Sure LX and Toyopreal AF-rProtein A HC0650F resins that have been released recently (Bolton et al. 2016). Table 3 shows the new high performing resins at specified residence times.

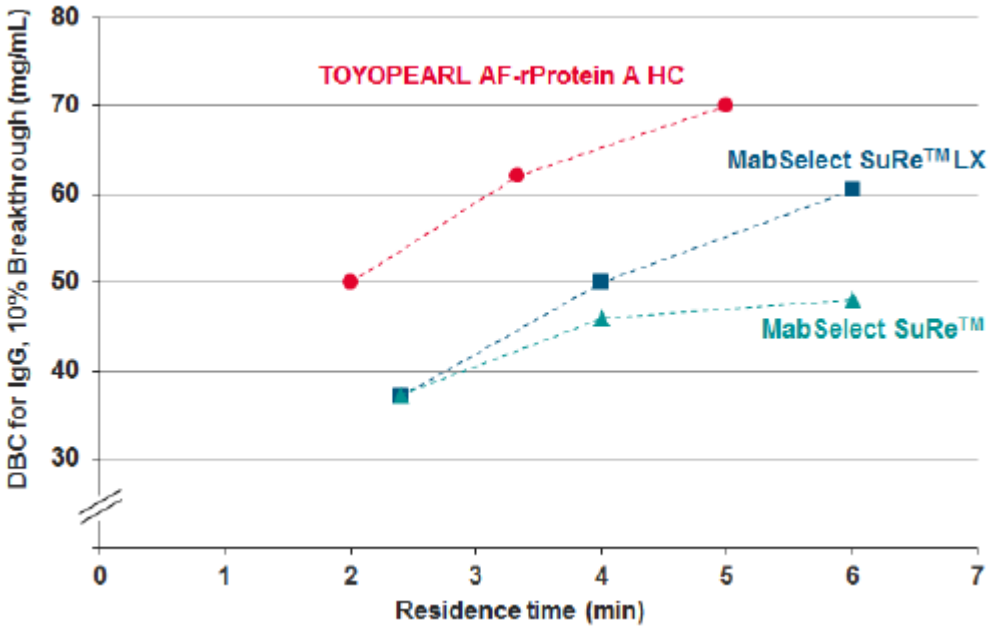

Table 3: DBC of High Capacity Protein A resin (Toshiba 2017)

There have been several attempts to replace protein A, such as with thermal responsive protein A (TRPA), His-mutants, amidation and hydrophobic charge induction chromatography (HCIC). Another option designed to address cost, low elution pH and leachables, is mabsorbent and multimodal. None of these have been able to offer industry the specificity and DBC of

protein A. However, mabsorbent and multimodal chromatography is now adopted as a polishing step. Its multiple interaction modes has found increased interest as the current platform utilises two polishing steps and manufacturers can now complete the same task in one step. Removing process steps is a major advantage for manufacturers and reduces cost which increasing process efficiency (Rathore, Kumar and Kateja, 2018).

In another development, a salt tolerant IEC resin is available that allows loading at different pH conditions. This is another area of interest for manufacturers as it removes dilution and buffer exchange steps from the critical path (Champaign et al. 2013). Table 4 is presented by Rathore, Kumar and Kateja (2018) and highlights some of the advancements in chromatographic process.

Type of chromatography	Advances	Remarks
Protein A chromatography	Dual flow loading strategy to improve the binding capacity	High flow rate is used when all binding sites are available and a lower flow rate when all the readily available sites are used up
	Chromatin extraction of protein A load	Histone components of chromatins bind strongly to protein A resins
Cation exchange chromatography (CEX)	Use as platform capture step for high titre antibody processes	Loading > 65 mg/mL of resin HCP reduction factor: 9–44
	Used for charge variants separation	pH gradient based platform developed for charge variant separation of mAbs
	Use of PEG to enhance aggregate clearance	Solubility enhancers to prevent PEG induced precipitation of proteins
Anion exchange chromatography (AEX)	Simultaneous removal of both acidic and basic protein impurities	Strong binding of both acidic and basic impurities on AEX resins while weak retention of mAb monomer
	Virus clearance using weak partitioning mode	DoE based staged approach to establish optimum operating ranges for robust viral clearance
Mixed mode chromatography (MMC)	Use as capture step	Uses hydrophobic charge induction chromatography
	As a polishing step	Unique selectivity facilitated in elimination of one chromatography step in GCSF purification
Hydrophobic interaction chromatography (HIC)	Under no salt conditions	A combination of strong HIC resins + optimized pH binding conditions to promote protein binding

Table 4: Advancements by Resin Type (Rathore, Kumar and Kateja, 2018)

2.5.3 Resin life times

Chromatography resins, once packed and passing relevant criteria, will be reused multiple times for multiple batches in the downstream process. The key reason these are reused is due to the cost of the resin. This is certainly true when considering the high cost of protein A media. Manufacturers must ensure that the resin they are using can perform and deliver product that meets the predefined critical quality attributes (CQAs). Regulatory authorities expect maximum number of cycles to be defined. Lab scale tests will be the starting point and concurrent validation of resin lifetime should be performed in parallel with initial routine manufacturing. The concern is that when the column is used, stored and multiple cycles have been passed through, the column performance will degrade and its ability to clear viruses will diminish. Other areas of concern relate to a reduction in step yield, HCP and DNA removal (Jiang et al. 2009). A study on step yield demonstrated that a column cycled continually experienced a large decrease in yield on run 44, with the column lifetime concluding on cycle 66 at which the step yield was 70%. This is presented in table 5 below;

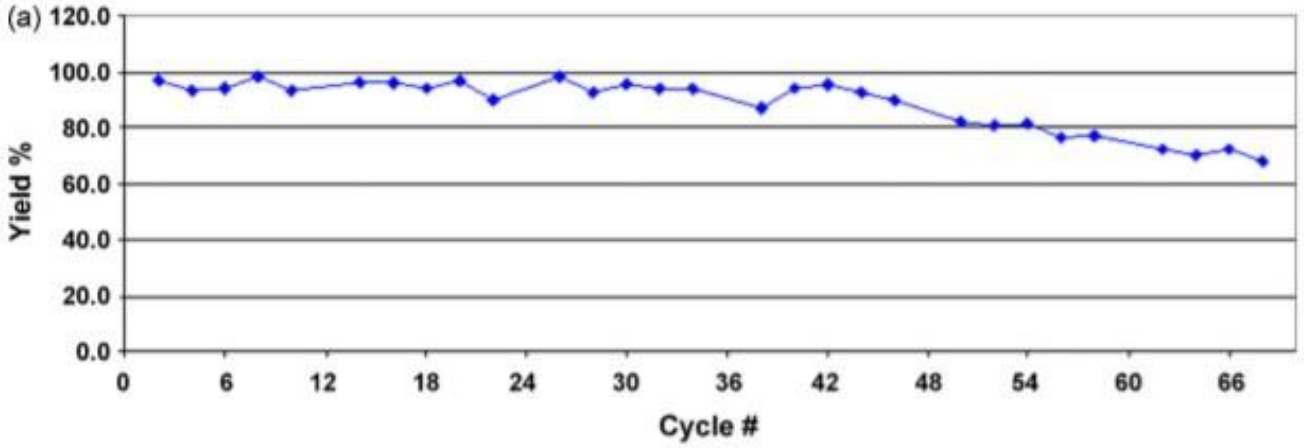

Table 5: Step yield and Column lifetime (Jiang et al.2009)

Lintern et al. (2016) completed a study examining if residual proteins remained on a column from previous cycles. A scanning electron microscope (SEM) was used to examine the resin at distinct time intervals. It was found that morphological changes arise on the surface of the agarose matrix. Virgin resin was examined under SEM, see figure 33A, and 100 cycles later the same resin was examined, refer to figure 33D. This shows a visual difference in surface fouling from time point A to D. This was found to be residual HCP but although the HCP on the resin increases 10-fold after cycle 50, there was no significant issues with HCP on the eluate.

The increase in HCP on the resin was associated with loss of DBC but not associated with decrease in product quality.

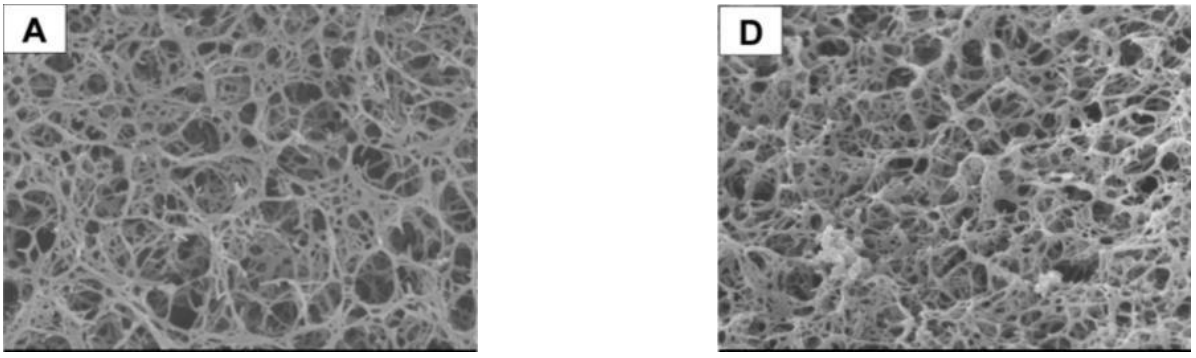

Figure 33: Agarose Resin and Time Intervals (Lintern et al 2016)

2.6 : Chromatography Techniques

This chapter discusses the chronological sequence of steps applied to a column, the different types of chromatography and alternatives to packed columns.

Chromatographic separations are critical to both the manufacture and analysis of biopharmaceuticals. When correctly applied, chromatography can deliver excellent specificity when separating different molecules contained within the one stream. The separation of the protein of interest from the mobile phase occurs based on understood physiochemical properties of the molecule of interest; this can be charge, hydrophobicity, or affinity. Large scale industrial columns can remove endotoxins, HCP, nucleic acids, provide viral reduction, and remove process intermediaries from a concentrated harvested material (Scott 2012).

2.6.1 Operational steps of a chromatography column

All chromatography columns are operated in a series of defined steps, as per figure 34; each one with its own objective. Once a column is packed successfully, it is stored. This involves flowing a storage buffer over the packed bed and then the column is isolated and left until the column is required for a production run. The storage buffer is typically bacteriostatic that will not allow bioburden to grow and proliferate (low concentration of NaOH / 20% Ethanol). All chromatography columns are operated in a series of defined steps, each one with its own objective (Fetterolf 2009).

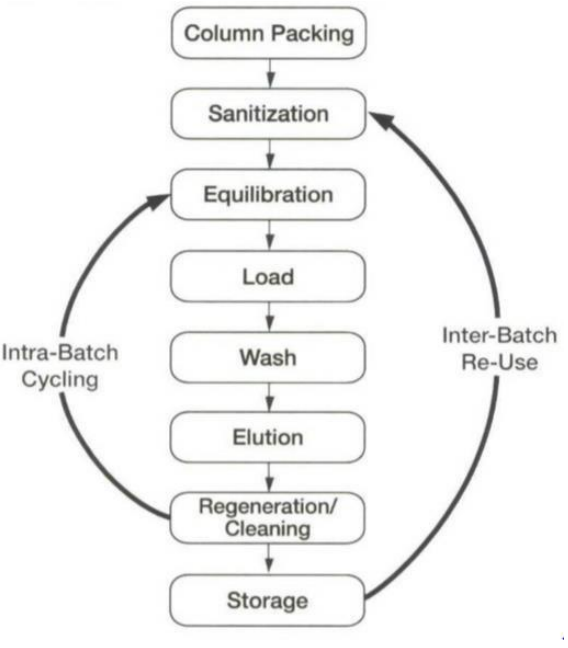

Figure 34: Operating steps of a chromatography Bind and Elute Column (Fetterolf 2009)

To ensure the equipment and flexibles (e.g. hose) being used are bioburden and endotoxin free, a pre-use sanitisation buffer is flushed over the column and the system (Fetterolf 2009). This removes any storage buffer on the column and it is typical to see concentrations ranging between 100mM and 1M NaOH. Vendor recommendations on each type of resin must be

adhered, to ensure the resin is not negatively impacted. It is also typical to have a static hold time if a specified contact time is required. Once complete, a Water For Injection (WFI) flush will remove the sanitisation buffer and samples can be taken at this point, to confirm the absence of bioburden and endotoxin (Fetterolf 2009).

Equilibration steps bring the internal of the column and associated pipework to the required conditions to support product load. pH, conductivity and temperature specifications are achieved after a number of column volumes of buffer have flowed over the column. Online probes will monitor these parameters and offline samples can be taken to ensure they are achieved. DBC studies should be conducted to ensure the flow rate is sufficient to allow the specified residence time, as high flow rates will result in a decrease of DBC. The product will then be loaded on the column to a max load concentration, based on a calculation using the volume in the hold vessel and the absorbance at A280 result. Certain scenarios will require the batch being split into a number of cycles, to prevent the batch being overloaded. A wash step will remove any loosely bound material and UV absorbance will be monitored. The elution step will see the pH being reduced to change physiochemical properties within the column. For ion exchange columns, increasing the salt concentration of a buffer, using 50mM Tris & 200mM NaCI, will remove the interaction between the molecule of interest and the resin particle. A UV detector will trigger the opening of the route to the pool vessel, once it starts to see protein levels increase with increased absorbance; and decreased absorbance to a baseline will trigger the closure of the route. As discussed, resins are reused for various applications so it is cleaned again, possibly with a high salt containing buffer to remove any bound material. A decision can be made to perform another cycle in which the operator can go back to repeat sanitisation or store the column for the next production run (Fetterolf 2009).

2.6.2 Affinity Chromatography

Protein A is the industry's choice of chromatography for direct capture of mAbs from a products collection vessel, post centrifugation and depth filtration. The high selectivity that protein A resins give allow for non-target proteins to flow through. Another advantage of protein A is its strong affinity to the Fab region of the mAb which affords the user the choice of using harsh wash buffers, to remove lightly bound variants to further increase purity. The protein A step provides greater clearance of HCP than any other unit operation and can remove >90% of the HCP in the product stream. The wash step for protein A is a critical step and will remove HCP reducing the responsibility and strain on further downstream steps. Typically, the

wash step is carried out between 5.0 – 5.5pH and will remove weakly bound HCP from the ligand. When a column is loaded near its DBC, low pH wash buffer can remove some mAbs so this may be represented in a UV spike and yield issue (Li, 2017). Figure 35 below shows the arrangement of resin with a large scale packed column, a schematic of the resin with ligands and a SEM image of the porous structure (Nweke et al. 2018).

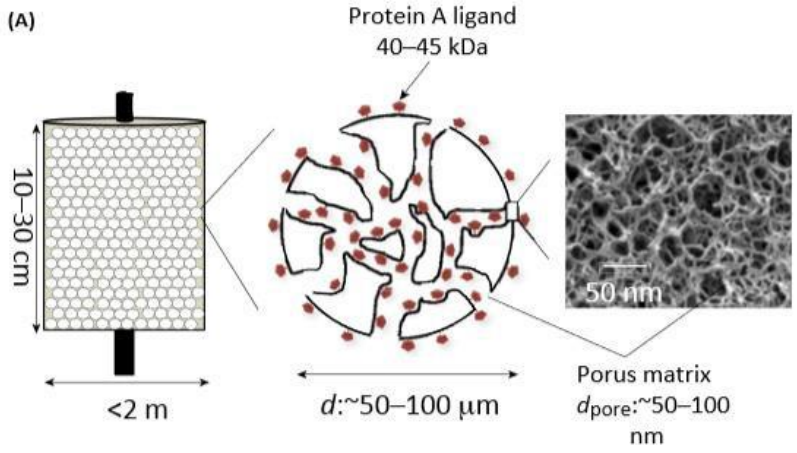

Figure 35: Typical large scale column, Pro A resin Structure and SEM image (Nweke et al. 2018)

Protein A has a high affinity for the Fc area of IgG type monoclonal antibodies. The elution step is typically carried out between 2.5pH and 4.0pH. For a large manufacturing process, the bed height for the capture step will be between 10cm to 30 cm; and consideration should be given to the type of resin you are using (Liu et al.2010).

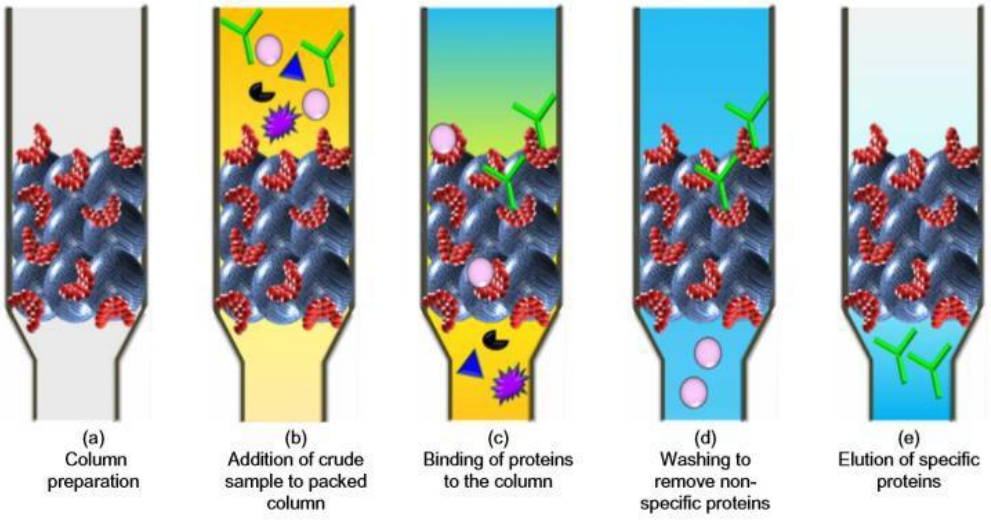

Figure 36: Steps involved for Protein A Capture (Ayyar et al. 2012)

Figure 36 illustrates the steps required in a protein A, bind and elute sequence. The column is prepared and equilibrated before a crude sample is passed over the column. The crude sample

will have a mixture of proteins and biomolecules. The specificity of the ligands will dictate what is retained on the column and the rest will pass through. A wash step will remove lightly bound material, before altering the pH to remove the protein of interest (Ayyar et al. 2012).

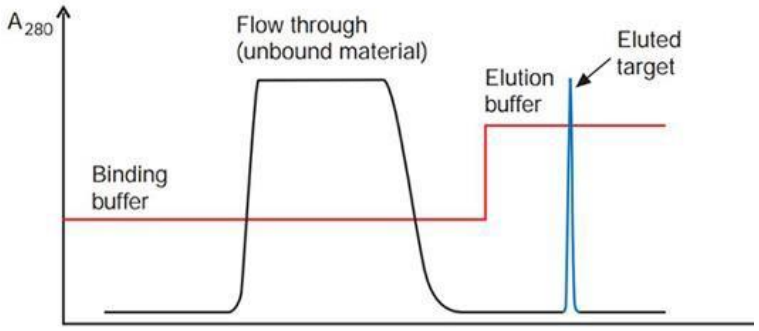

Figure 37: Chromatogram for Bind and Elute operation (Sigma Aldrich 2007)

Figure 37 shows a typical chromatogram for a bind and elute step. A tracer can be constructed using absorbance readings at A280. The absorbance will increase as the load and wash steps are running. The blue line shows the elution of the protein of interest.

In 2002, the FDA published the "Pharmaceutical Current Good Manufacturing for the 21st Century – A Risk based approach" with the hope that manufacturers would modernise their process. The FDA saw that there were advantages to using Process Analytical Technologies (PAT). PAT is defined as a "system for designing, analysing and controlling manufacturing" by making decisions based on online equipment. PAT technologies can be applied to the way chromatography columns pool their eluate in the biopharmaceutical manufacturing process. Online reading of A280 can dictate when to start collecting and when to stop collecting the protein of interest (Rathore, Bhambure, and Ghare, 2010).

2.6.3 Ion Exchange Chromatography (IEC)

Most large-scale manufacturing facilities will utilise IEC chromatography for at least one step. The resins used at this step are not as expensive as protein A, so it can be positioned early or late in the process. For antibodies that have a basic isoelectric point (pI), cation exchange chromatography can be used as a capture step; but it would be more likely to see it as a polishing step. IEC chromatography is suited for removing HMW, LWM, charges variants, residual protein A (rPA), HCP and DNA (Liu et al 2010).

When using IEC as a chromatography technique, it is critical that you understand the condition of the stationary phase. The pI of the protein in the mobile phase will be dictated by

the pH of the buffer it resides in. The given charge of a protein will depend on the quantity and type of ionisable amino acids groups within the protein. Arginine, lysine and histidine when ionised will have a positive charge. On the other hand, glutamic acid and aspartic acid will be negatively charged when ionised. The different side chains will have their own pKa. As a result, the overall charge of a protein at a specific pH will be dependent on the number of ionisable amino acid groups. The pI of a given protein will have no net charge when the pH is equal to the pI, and will results in no interaction occurring. If the pH is greater than the pI, the surface is negatively charged and it will bind to an anion exchanger. If the pH is less than the pI, the surface of the protein is positively charged and will bind to a cation exchanger (refer to figure 38). The pI of the protein will determine the pH of the buffer and salt gradient. Cation exchange mode should use a buffer with a pH below the pI of the protein (refer to table 6). Alternatively, for anion mode, pH should be above the pI (Fekete et al., 2015).

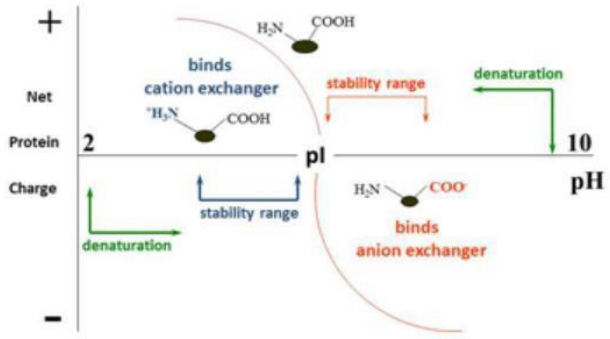

Figure 38: Protein Charge Versus pH (Bio-Rad 2019)

Resin Type	Cation Exchanger	Anion Exchanger
Net charge of molecule of interest	+	−
Charge of resin	−	+
Running conditions	0.5–1.5 pH units below the pI of the molecule of interest	0.5–1.5 pH units above the pI of the molecule of interest

Table 6: Resin Selection and Charge (Bio-Rad 2019)

In a study of 23 therapeutic mAbs currently approved by regulatory agencies, it was found that the pI of the mAbs was wide ranging when plotted against each other, from a pI if 6.1 to 9.4 (Goyon et al 2017).

Anion Exchange Chromatography (AEC) is referred to as weak partitioning chromatography. Due to the point highlighted above, the pI for mAbs tends to be relatively high. Impurities such as HCP, DNA and viruses are generally acidic and can bind to resin more tightly than product; and this makes this mode a good choice for flow though mode. If the pH is below the pI of the protein of interest, and conductivity is low, the product will pass through the column and impurities will bind, this will give a superior impurity clearance step. Under certain pH and conductivity condition, this step can give a 4 log reduction value (LRV)

reduction in terms of viral clearance that makes a case for a two-column process (Li 2017).

AEC uses a positively charged group that is immobilised to a resin bead, commonly diethylamino ethyl (DEAE) or dimethyl amino ethyl (DMAE) which are weak basic, or quaternary amino ethyl (Q) which is strongly basic. Antibodies with a pI above 7.5 will generally use flow through mode. This will operate with a mobile phase at pH 8.0 to 8.2; and conductivity will be no greater than 10mS/cm for buffers used for equilibration, load and wash. As this is in flow through mode, it can process a much more concentrated product (up to 100g/L) stream as it is only binding impurities. Alternatively, AEC can be processed in a bind and elute mode which uses a buffer up to pH 9 to give a net negative charge on the surface of the protein. The product pool is then loaded onto the column which results in the protein of interest and impurities being bound to the column. A high salt buffer can then remove the protein of interest using a step or linear gradient. The impurities can then be removed using CIP or regeneration steps. A lower ionic strength is required for this operation and manufacturers may need to introduce a buffer exchange system, hold vessels or use inline dilution (Gagnon 2012). Figure 39 below shows chromatogram of a flow through operation for and AEC.

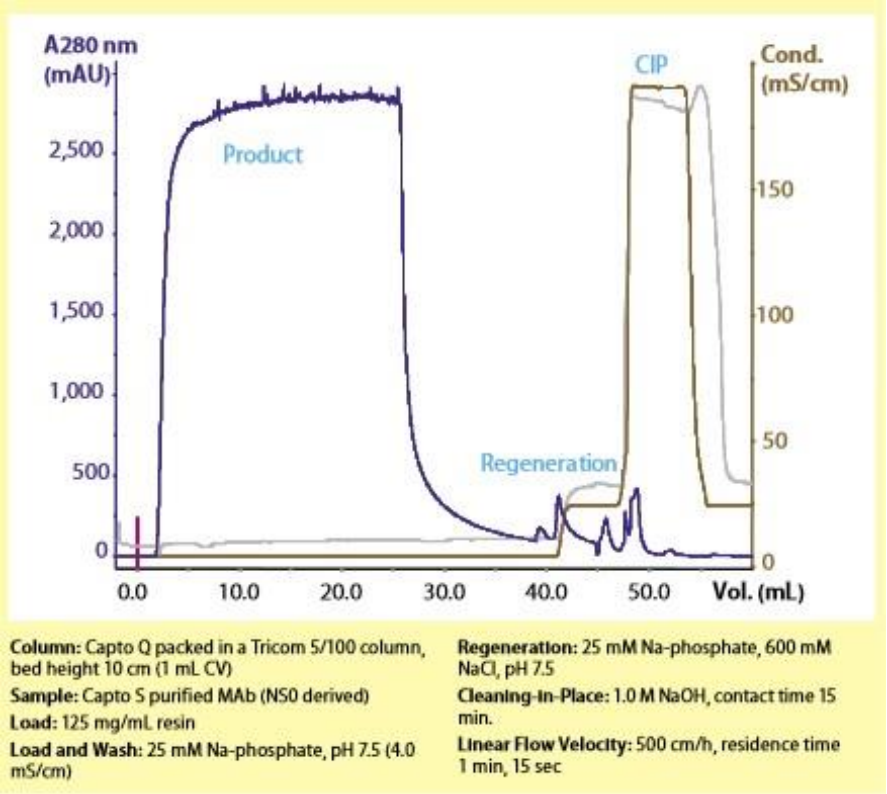

Figure 39: Flow through Profile AEC (Gronberg et al.2007)

Cation Exchange Chromatography (CEC) uses a negatively charged column and can be applied to products that have a pI that range from neutral to basic. The operating pH is typically around pH 6; and the column is equilibrated and then loaded. The protein of interest binds to

the column and impurities reduce during the load and wash steps. CEC can also provide superior separation properties and can remove variants, deaminated products, oxidised species, N-terminal truncated forms and HWM forms. The binding capacity is dependent on the pH and conductivity. At a high net charge (low pH and conductivity), it was found the DBC was reduced (Liu 2010).

2.6.4 Hydrophobic Interaction Chromatography (HIC)

HIC is typically used in the manufacturing of proteins as a polishing step. HIC offers an orthogonal approach to IEC chromatography and is effective for HCP, impurity clearance and aggregate removal (Ghose et al. 2013). Separation is based on the hydrophobic interactions between the protein of interest and the stationary phase; and the order of elution will be based on the protein of interest's relative hydrophilicity. HIC uses pH and does not use any denaturing conditions like the use of organic solvents or high temperature. HIC is completed using a reversed salt gradient, starting with a high salt and then lowering this for elution. At high salt concentration, proteins are retained and bound to the column due the hydrophobic interaction of the salt in the buffer. The more hydrophobic a protein of interest is the less salt is required to promote binding. The mobile phase is normally a salt containing 1 – 2M ammonium sulphate or 3M NaCI; and a buffer to control pH to between 6pH – 7pH. High concentrations of salt have been known to precipitate proteins so studies must be performed to understand the solubility of the molecule being manufactured. The strength of the interaction will decrease as the pH increases and the protein will be unbound from the column (Watson 2017).

The process throughput of HIC can be greatly improved by using this step in a flow through mode and this uses a lower salt content so there's a reduced risk of precipitation. Flow through mode can be referred to as negative mode. In this mode, the protein of interest flows through the column and impurities are retained. Operating in flow through provides several advantages;

- High pool concentrations can be used as manufacturers no longer are concerned with the DBC of the column and protein of interest. As HIC is typically a polishing step, the amount of impurities that are still present are relatively small as a percentage of the pool.
- Flow through mode does not use high concentration of salt; and impurities will bind to the resin due to their higher hydrophobicity.
- Not requiring high salt, results in less raw material usage and waste.

- Reduced salt concentrations in the subsequent pool is favourable if the next phase is an ultra-filtration/ Diafiltration (UF/DF) step, as high salt and high concentration may result in precipitation and possibility membrane fouling.
- Higher process throughput is attained as the step will be quicker as more material can be processed (Lu et al. 2009).

2.6.5 Mixed Mode Chromatography (MMC)

MMC is another chromatography technique that is growing in terms of interest within the industry. This has been supported by the introduction of new media and greater understanding of multimode interactions. MMC exploits two or more interaction mode on one stationary phase (Zhang and Liu, 2016).

MMC is generally used as a polishing step and involves the use of mixed mode resins in which ligands can operate using two different interactions. Proteins can be described as multi-modal due to their distribution of hydrophobic and charges regions (Li 2017).

Mixed mode resins contain ligands that use different binding modes. Figure 40 below shows a representation of a ligand for capto adhere resin and highlights hydrogen, ionic and hydrophobic functionalities for interacting with a target molecule. Mixed mode resins as sophisticated and require the user to thoroughly study its application prior to use (Liu 2019).

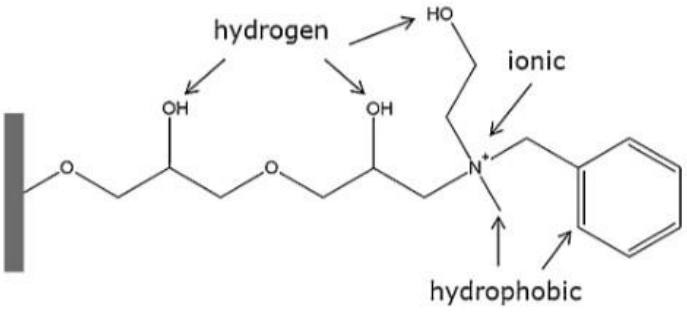

Figure 40: Capto Adhere MMC ligands (Liu et al. 2019)

This mode differentiates the protein of interest from HCP as the charge and hydrophobic areas on your molecule of interest will be different to that of any other molecule. Due to this, mixed mode chromatography can give manufacturers selectivity and specificity that traditional ligands don't offer. The nature of the interaction means that manufacturers need to study and optimise this into their process. GE supplies Capto-Adhere and Capto-MMC, two recently new resins to the market. Capto-Adhere is similar to AEC and is used in the flow through mode.

Capto-MMC used ligands that utilise cation exchange and hydrophobic interactions (Li 2017). Liu et al. (2019) has used MMC as a capture step using a two-column platform.

2.6.6 Alternatives to Packed Columns

Whilst there have been a number of significant improvements in traditionally packed beds used for chromatography steps in the large scale manufacture of mAbs, research has also continued in alternative chromatographic formats (Rathore, Kumar and Kateja, 2018).

2.6.6.1 Membrane Chromatography

Protein A is the most common step employed in the initial capture and purification mAbs. Due to the fact that protein A is so expensive, manufacturers are investigating in alternatives. Membrane chromatography is an alternative to traditional resin packed columns which has gained increased focus as a potential downstream large scale chromatography platform. Unfortunately, to date this technology is limited to ion exchange chromatography, completed in flow through mode (Hou et al 2015).

The main benefit to membrane chromatography is that diffusive pores are eliminated which results in the mass transfer of the protein of interest to the binding site being convective (Kumar et al. 2013). There have been recent advancements in relation to membrane chromatography that include higher binding capacities, increased mass transfer, higher processing efficiencies and higher flow rates. Due to the disposable nature of this product, low buffer requirement, lower equipment cost, and small footprint, membrane chromatography will continue to be investigated as an option. Membrane absorbers have a low pressure drop and don't have compression issues or suffer from channelling. The fact they are disposable means reduced cleaning and validation requirements. Issues still pertain to poor binding capacity, poor device design, irregular physical issues such as pore size distribution and membrane thickness. Accumulated bubbles can be difficult to remove without compromising sterility (Orr et al. 2015).

Liu et al (2019) highlights that binding capacities have recently improved with the introduction of improved base matrices but this technology is still only suited to lab and pilot scale. Muthukumar rt al. (2016) also points out that using membrane absorbers removes any column packing and unpacking studies or validation requirement. There's also a cost saving with this technology for short campaigns (>10 batches) as the resin maybe discarded. The future for membrane technology may be aligned with greater use of SUTs in downstream.

2.6.6.2 Monoliths

Monoliths are similar to disk stacked membrane modules and have similar limitation in terms of decreased binding capacity based on reduced surface area (Orr et al .2013). Monoliths have been described as fourth generation chromatography, following on from membrane chromatography. A monolith is a single structure of homogenous stationary phase that has multiple interconnecting channels. Monoliths are versatile in terms of scale, pore size, material type, and cover the most common forms of chromatography such as AC, IEC and HIC which makes them suitable for manufacturing of large biomolecules. The advantages monoliths have over packed columns is that it uses convective flow based separation, allowing for high flow rates, high bed porosity, high surface area which lends to high DBC and good scalability (Rathore, Kumar and Kateja, 2018). Monoliths are also user friendly and can easily be installed, cleaned, stored and housings can offer different separation mechanisms within the same step (Rathore, Kumar and Kateja, 2018). The main drawback to monoliths include clogging, irreversible fouling and large footprint required (Orr et al. 2013).

Figure 41 shows the comparison of different stationary phases. Mixed membrane chromatography, can also be referred to as particle-loading membrane chromatography and incorporates resin particles within a membrane support. This approach pulls together the benefits of using resin, due to the high resolution and increased binding capacity they possess, along with the benefits of increased flow rates, reduced fowling and pressure drops associated with microporous membranes. Whist flow rates are improved with this approach, it was found that DBC decreases when flow rates are increased which indicates there are still mass transfer limitations (Orr et al 2013).

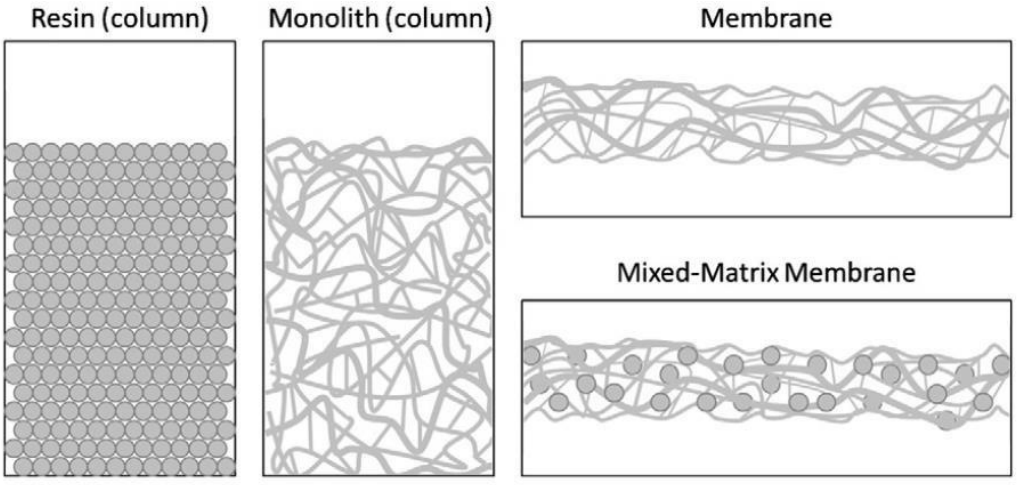

Figure 41: Comparison of stationary phases (Orr et al. 2013)

Despite the disadvantages of monoliths and membrane absorbers, they do have potential to replace the packed column format, due to its current low productivity. Cycle times for monoliths and absorbers are much fasters and do open the possibility for use in future multicolumn chromatography operations (Bolton et al 2016).

2.6.6.2 Expanded Bed Absorption (EBA)

EBA is seen as a potential to enable the replacement of multiple unit operation on the traditional batch platform. Figure 42 below shows the potential impact this has to the traditional batch process and the removal of centrifugation, filtration and the protein A capture step. The advantage of this is that it will reduce process time, increase yield and reduce the initial investment and operating cost for manufacturers (Jin 2015).

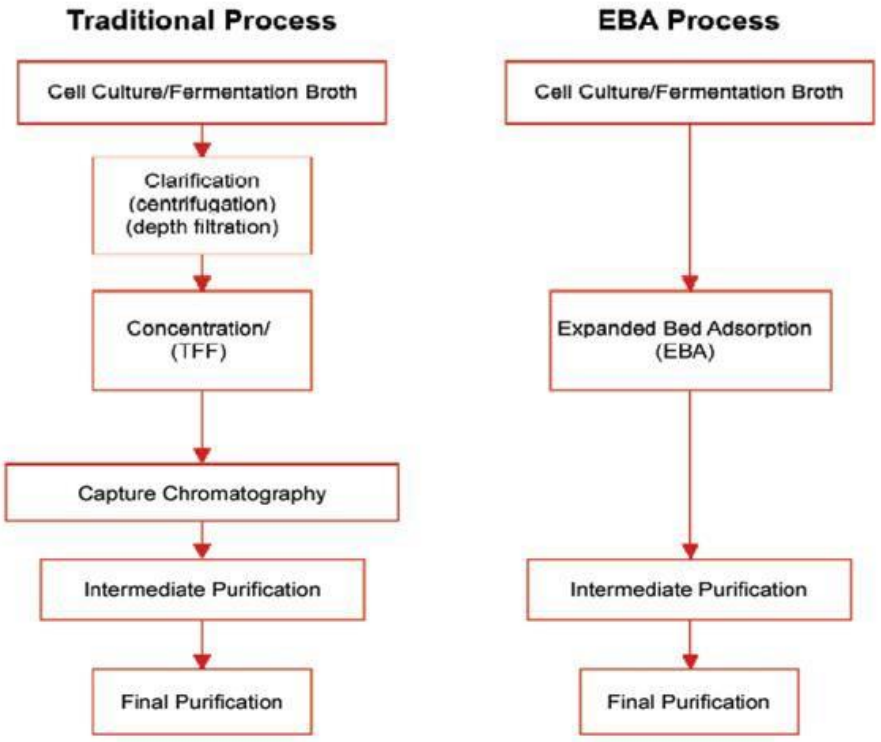

Figure 42: EBAs impact to Downstream Platform (Jin 2015)

As per figure 43, the sequence of activities are similar to column processing but with this approach the settled bed is expanded by directing upward flow that is sufficient to fluidise the bed. The column is then equilibrated and loaded with feedstock in which the target molecule binds to the EBA absorbent and other contaminants are allows to flow through. A new wash buffer is applied to remove any loosely bound material. The top adaptor is them lowered to

form a packed bed and elution buffer recovers the bound material. The column can then be CIP'd, regenerated and used again (Jin 2015).

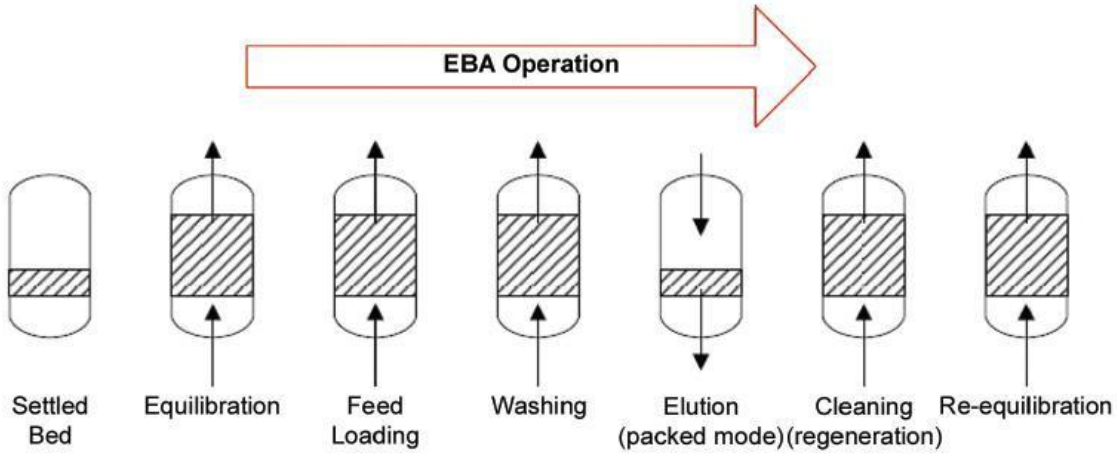

Figure 43: Expanded Bed Operation (Jin 2015)

By increasing the interstitial volume between absorbent particles, cell debris and contaminants can pass through the column unobstructed. Advancements in EBA have seen the second generation Rhobust Technology deliver an improved, correctly sized alternative to the capture step. Using this approach, manufacturers can process in one third of the time. Relative to the traditional approach, it uses 50% less buffers and gives a 12% yield improvement. In addition to this EBA, it gives superior DNA removal and comparable purity (Jacquement et al. 2016). The second generation is described by Jin (2015) as a failure due to issues with design. The new distributor design creates back mixing in the lower part of the column and there are issues in relation to media grinding, reliability and maintenance cost. In addition to these drawbacks, elution can only be performed when the bed is expanded, resulting in a less concentrated process stream.

2.7 : Chromatography Platforms

This chapter examines the background behind chromatography platforms and details some industry examples.

MAbs, as a specific class of molecule, exhibit certain shared properties which make them ideal candidates for a platform approach, in terms of the sequence of downstream unit operations. This platform approach enables efficient processing to cover research and initial development, followed by subsequent clinical phases and finally manufacturing (GE 2019).

The platform approach to biopharmaceutical development emerged in the 1990s and was primarily developed with mAbs in mind. It was observed that, as companies began to develop and add to their pipeline to include different molecules, there were advantages to consistently using the same tools across the development program. Advantages included increased quality and consistency, cost savings due to efficient resource utilisation, and increased speed in relation to process and product development. Another key improvement brought about by adopting a platform approach is the benefit to patients as products with a proven performance and safety profile can be delivered quicker to the market (Moran et al 2013). Figure 44 below shows how platform approaches have developed from having none to current day widespread use;

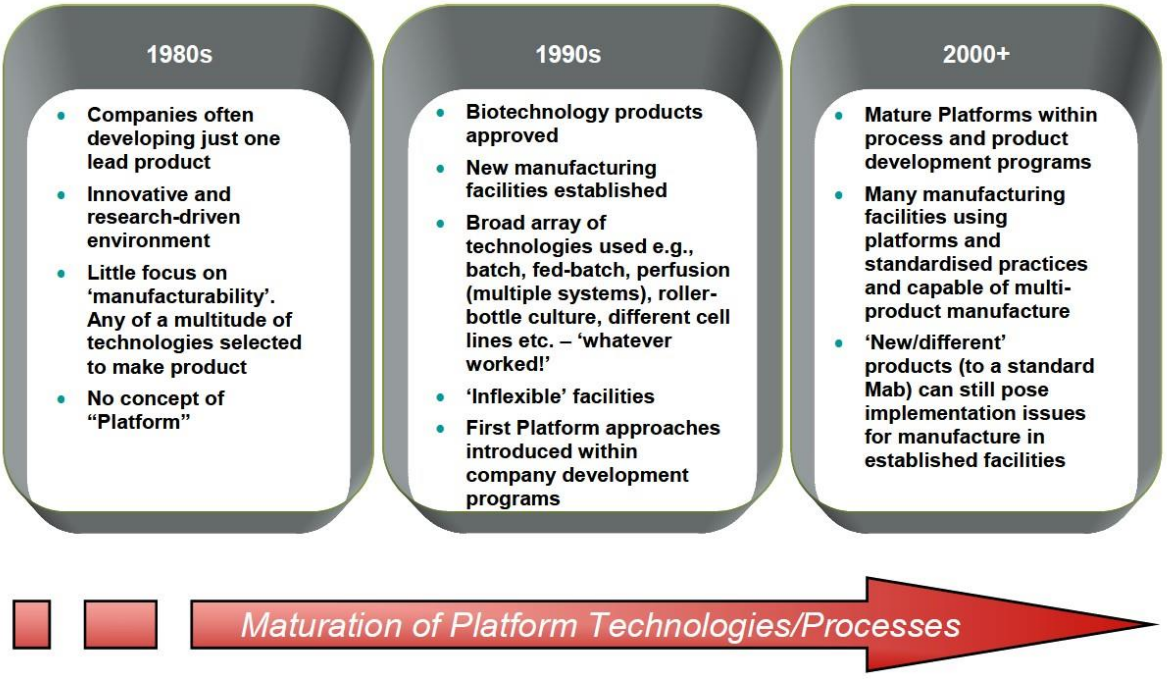

Figure 44: Evolution of Biopharmaceutical Platforms (Moran et al 2013)

Shukla et al. (2017) also highlights that platform approaches offer manufacturers a competitive advantage from a business standpoint. Speed to clinical trials can be a key

determinant to a company's success. MAb platforms have proven to enable companies to go from gene to Investigational New Drug (IND) within one calendar year, by removing the development efforts that can result in years of a delay. A reduction in testing and experimentation also reduces costs; and a template document approach can offer time benefits.

2.7.1 Platforms used in Industry

The increase in the number of therapeutic biopharmaceutical products being evaluated at early stage clinical trials has resulted in companies developing their own manufacturing platform to accommodate most, if not all, of their future pipeline (Kelley et al. 2008). Amgen was one of the first companies to communicate its thinking in relation to their downstream platform. Their template approach for all molecules was not possible due to the differing requirements of each molecule, including; dissimilar elution pH requirements and the subsequent polishing steps, depending on the impurities present. Amgen used a platform that included protein A as the initial capture step followed by a polishing step. CEC was used in a bind and elute mode or, if HMW impurities were a concern, they used a HIC flow through step. Another step that was Amgen utilised was AEC for products in which HCP was a concern. Amgen also employed hydroxyapatite as a final chromatography step if there were concerns over process related impurities (Shukla et al. 2017).

2.7.2 Industry examples

Early platform approaches applied by Pfizer to enable the supply of clinical material were adapted to cover antibodies derived from NSO and CHO cell lines. Figure 45 outlines the traditional method as the original process, consisting of a three phase chromatographic approach. Clinical manufacturing involved the use of a 1,200 L bioreactor, Process 2, which consisted of centrifugation, due to its efficiency. This was followed by depth filtration at a reduced surface area to reduce cost. The polishing and intermediate chromatography steps were flipped to remove the requirement for diafiltration pre anion exchange column. This was also a cost reduction tool as it removed one unit operation, along with its associated buffer requirements and increased yield. Process 3, developed from Process 2, removed the cation exchange step and replaced the anion exchange resin with a membrane absorber. By employing a membrane absorber, the loading capacity was increased significantly. Process 3 was acceptable for most but not all of Pfizer's products. Certain products from high titre cell lines resulted in a higher

levels of impurity and viral clearance issues. As a result, process 3 was further developed to add in a cation exchange chromatography step (Hagerty et al. 2009).

Original process	Process 2	Process 3	Current process
Depth filtration	Centrifugation	Centrifugation	Centrifugation
Concentration	Depth filtration	Depth filtration	Depth filtration
Protein A chromatography	Protein A chromatography	Protein A chromatography	Protein A chromatography
Low pH inactivation	Low pH inactivation	Low pH inactivation	Low pH inactivation
Cation exchange chromatography	Anion exchange chromatography	Anion exchange membrane	Anion exchange membrane
Diafiltration	Cation exchange chromatography	Nanofiltration for virus removal	Cation exchange chromatography
Anion exchange chromatography	Nanofiltration for virus removal	Final diafiltration	Nanofiltration for virus removal
Nanofiltration for virus removal	Final diafiltration		Final diafiltration
Final diafiltration			

Figure 45: Platform approach at Pfizer (Hagerty et al. 2009)

Recently, Pfizer have used their current process, as defined in figure 45, and applied it to a single use platform. Pfizer were looking to increase speed to market for two biosimilars they wanted to study and gain access to the Chinese market. GE transferred Pfizer's original process using stainless steel to a single use 200L scale up process. The project reduced Pfizer's typical timelines by 6 to 12 months and the study was completed within 5 months (GE 2019).

Amgen's new facility, built in Singapore, is another example of a company utilising a combined single use and continuous manufacturing approach. Amgen's new next generation facility uses disposable systems, modular designs, aseptic connectors and reports to have real time analysis. It is expected that a 2,000L bioreactor will be able to achieve the same output as a 20,000L bioreactor; and 45 miles of pipework that would have required CIP and SIP was removed (Amgen 2016).

Figure 46 outlines the processing steps that Amgen, Genentech, Biogen and KBI Biopharma use.

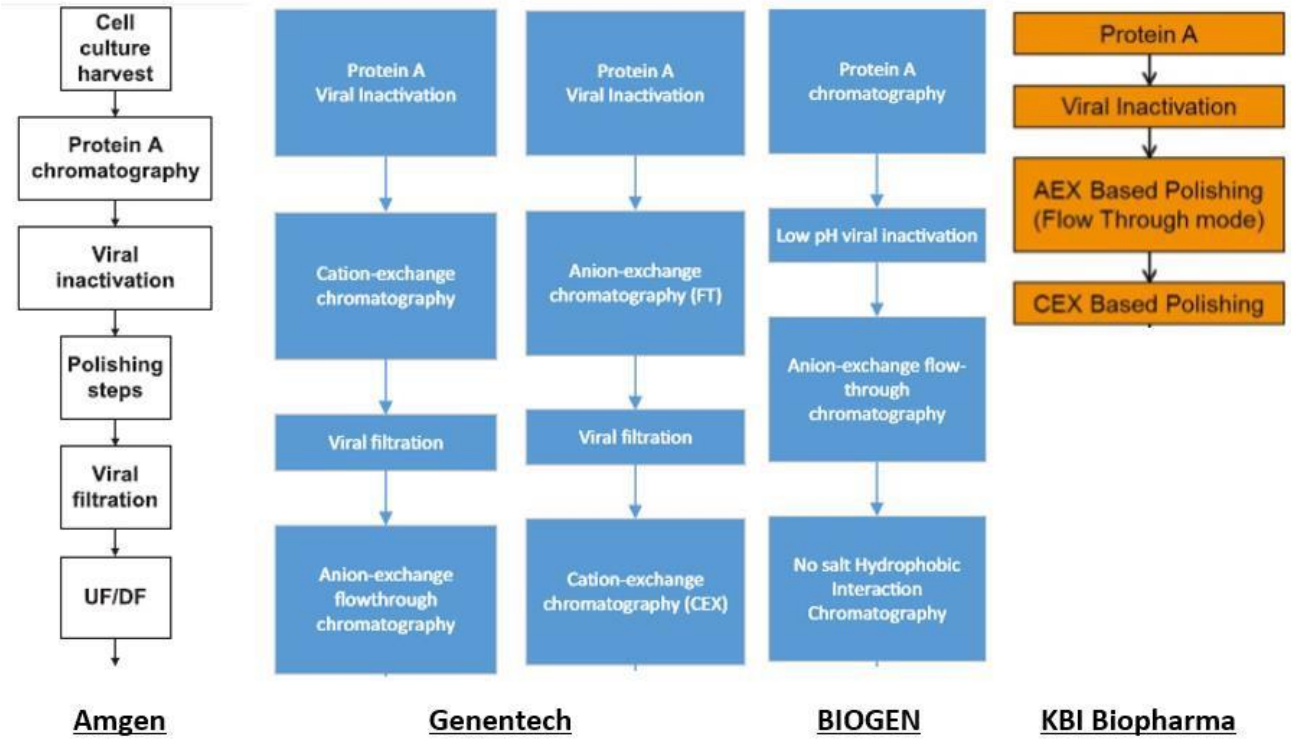

Figure 46: Chromatographic platforms used in Industry (Shukla et al. 2017)

Genentech operate with a 3-column platform, using protein A, CEC (bind and elute) and AEC flow through mode. As mAbs are typically basic, they bind tightly to a CEC column and relatively straight forward processing can be achieved through an AEC column. The issue with this process is that whilst CEC can remove HCP and HMW species, the subsequent AEC column requires low conductivity for the load step, resulting in a bottleneck. Mainly, due to the requirement for large buffer tanks. Another drawback of this approach is that CEC and AEC, depending on the protein, are not sufficient to remove HMW species. This added difficulty may be overcome through weak partitioning AEC (Shukla et al. 2017). The use of new mixed mode resins, such as Capto Adhere, could reduce the requirement for large dilutions.

An alternative approach to the typical loading in HIC is to perform the step at high concentration, without salt conditions. Using no kosmotropic salt and a highly hydrophobic HIC resin (Hexyl Toyopearl), manufacturers can yield positive outcomes. Optimised pH conditions enable HCP reduction; and the reduced volume will also help the viral filtration step that requires expensive filters as the surface are is decreased (Ghose et al. 2013).

The HIC has also been shown to be operable during overloading condition (>200g/L). Biogen currently use this step as part of its large manufacturing platform, in combination with a flow through AEC step. Employing flow through mode at high concentrations has the added

benefit of reducing column cycles and increasing process efficiency. KBI Biopharma use multimodal chromatography as part of their platform for either their AEC or CEC steps. Although there is a lot of experimental work and tailoring to be performed for each mAb, the approach by KBI may support a wide range of possible products (Shukla et al. 2017).

Manufacturers of today can create flexible manufacturing platforms. Stainless steel, which was once the only option, can now be integrated with single use equipment. Single use technologies compliment continuous manufacturing as the advantages of reduced footprint, flexibility and improved process performance are combined (Pollard et al. 2016).

Figure 47 depicts the possible end processing scenario. Today we are using stainless steel and a mixture of single use technologies. Continuous processing combined with single use technologies is currently being evaluated.

Through process intensification, the last process illustrated in Figure 47 has fewer unit operations and is fully end to end (Pollard et al 2016).

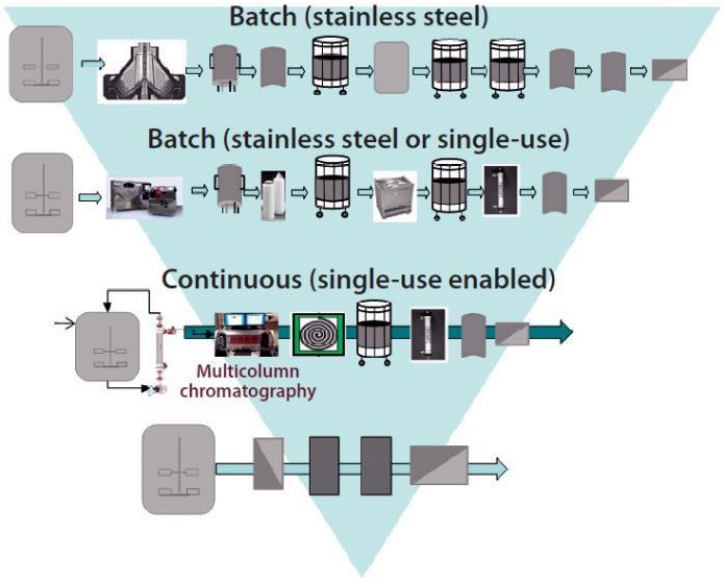

Figure 47: Flexible Production Platform (Pollard et al. 2016)

Fully single use facilities are also becoming more common. Amgen have completed building a state-of-the-art single use facility that differs from more conventional designs. It boasts a manufacturing suite, from cell culture to viral filtration, with a closed system that utilises aseptic connectors. Due to the background environment being an ISO 9 standard, it reduces the burden, environmental monitoring and garbing requirements (Mire Sluis 2018). Figure 48 highlights the reduced footprint and cost of the Amgen single-use facility in Singapore.

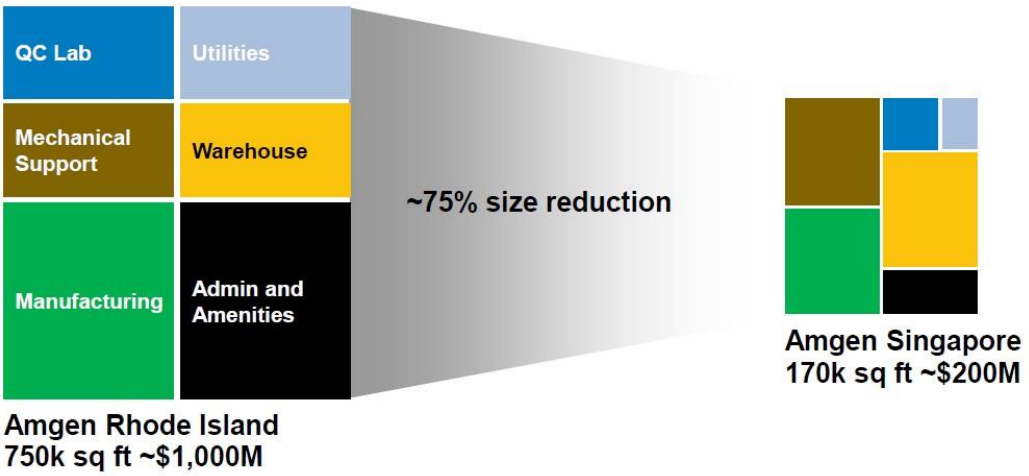

Figure 48: New Manufacturing Platform, Same Productivity, 75% Smaller (Mire-Sluis 2018)

It is worth noting that it is not only Amgen that are building fully single use facilities, but Patheon (Brisbane) is another example. Amgen completed their build and process qualification runs within 26 months; and 95% of their fluid lines were disposable. The Amgen facility in Singapore was originally built on the premise that downstream chromatography would be performed using glass columns and packing facilities were incorporated into the site. However, this decision was adapted. Amgen now use pre packed columns (Tingley 2017). Figure 49 depicts a two column, semi-continuous process which uses a surge tank, to pool and control the flow before processing over the second column (Mire-Sluis 2018).

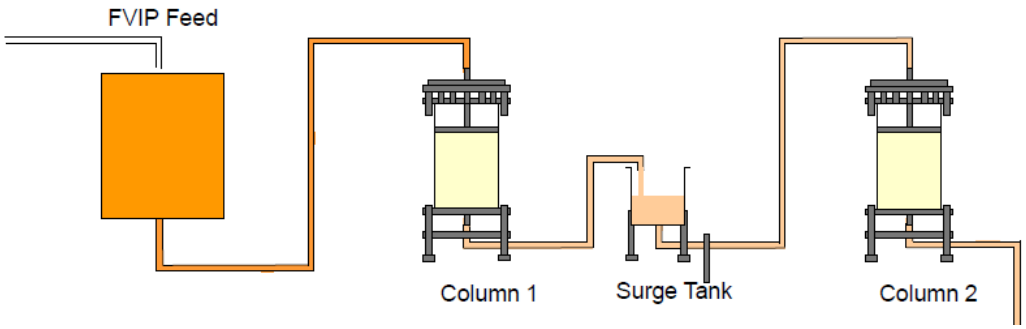

Figure 49: Amgen 2 column platform (Mire-Sluis 2018*)*

WuxiUP (ultra-high productivity) continuous process platform, presented in figure 50, couples a perfusion system directly with a continuous capture step. Wuxi describe this process as suitable for proteins that are difficult to express, mAbs and Fc-fusion proteins. Wuxi claim this system provided rapid development, scale up and can be implemented at large scale. At lab scale, they utilise between 1L to 15L bioreactors. For pilot scale and clinical cover, they

use 15L to 250L bioreactors and this increases to 1000L to 2000L bioreactors for commercial, all utilising Repligen's Alternating Tangential Flow (ATF) cell retention systems (Wuxi Biologics 2019). Wuxi have also claimed to have reached a breakthrough of 51g/L by using a 1000L bioreactor in a continuous process. One batch can yield 30kg of active product which is 10 times greater than traditional productivities using stainless steel at 3-5g/L (Wuxi Biologics 2018).

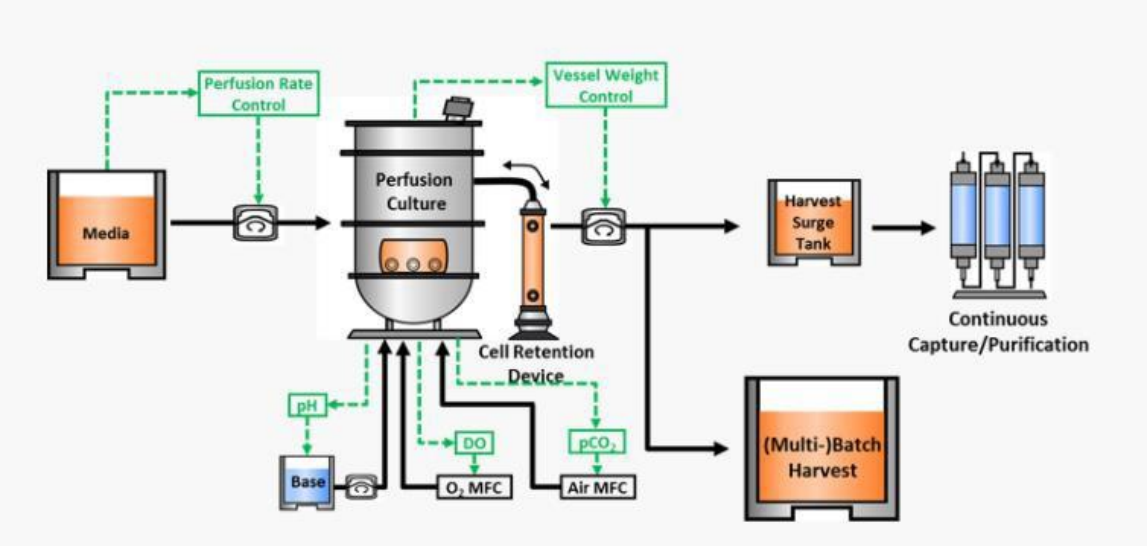

Figure 50: Wuxi UP Continuous process (Wuxi 2019)

2.8 : Batch Processing versus Continuous Processing

This chapter describes the traditional batch approach and gives details on new, more efficient chromatographic methods that the industry is currently evaluating.

Different operating modes have different definitions. Yang et al. (2019) describe these modes in table 7. The differences between batch and continuous will be discussed in more depth, later in this report. Semi batch refers to fed batch; and semi continuous is similar to continuous but occurs over a more discrete time period.

Operating mode	Definition
Batch	Materials are charged before the start and discharged at the end of process
Continuous	Materials are simultaneously charged and discharged from the process
Semi-batch	Materials are added during processing and discharged at the end
Semi-continuous	Materials are simultaneously charged and discharged from the process within a discrete time period

Table 7: Definition for Operating Modes (Yang et al. 2019)

The current downstream manufacturing landscape for processing commercial-scale high value products is based on batch processing. Batch processing splits the downstream process into unit operations that are completed in sequence. This involves moving the product from one unit operation to another; and using holding tanks to stage the product for the next unit operation. This type of approach allows for optimisation of each singular unit operation and allows for offline sampling to determine CQAs before processing to the next step (Zydney 2015). Figure 51 shows a possible hold tank configuration for a typical batch processing manufacturing operation.

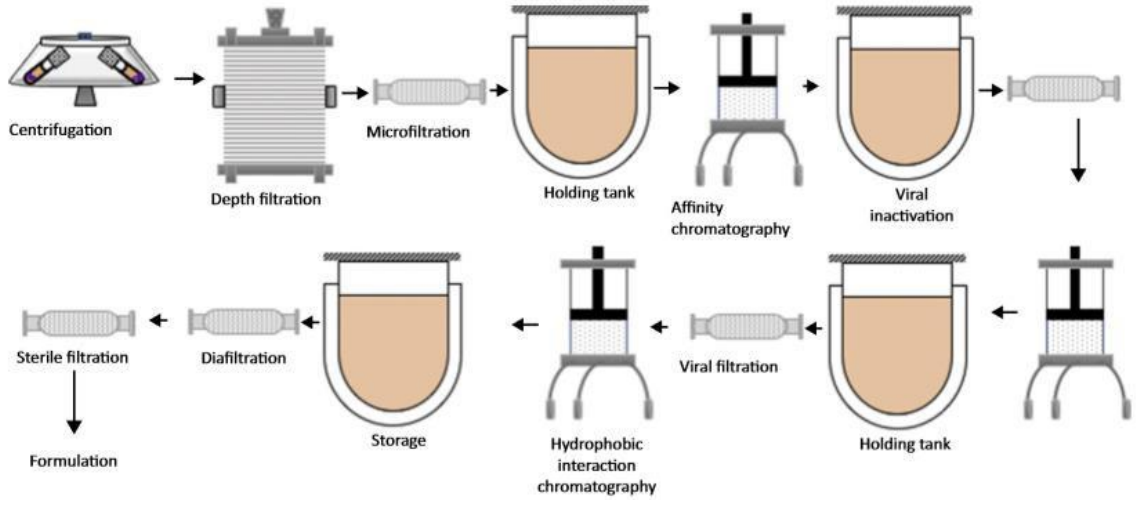

Figure 51: Stainless Steel Holding Tanks required for batch processing (Fisher 2019)

The large scale manufacturing of biopharmaceutical therapeutics has barely changed over the last 20 years. During this time the industry has expanded considerably, delivering innovative products to market which have benefited patients throughout the globe. These products have generated extremely high revenues and profits for the industry. It has been alluded to that the wealth generated over this period has led to legacy, resulting in a manufacturing platform that is accepted. The acceptance of this platform has meant manufacturers have not fully considered the cost and effectiveness of their assets. The manufacturing industry now faces new realities that are forcing them to consider their approach in the face of increased competition, biosimilars, patent expiries and time to market (Walther et al. 2015).

As discussed, the biopharmaceutical manufacturing industry is maturing and is extremely profitable. However, the manufacturing paradigm has changed little over the previous decades. In a survey conducted by 222 industry experts, the respondents indicated that the current downstream manufacturing paradigm required immediate attention in terms of continuous processing technologies (Estes & Langer 2017).

Continuous processing is currently being adopted into the upstream process and this change has not just occurred recently. Remicade was approved by the FDA in 1998 to treat Crohns disease and is manufactured via a perfusion system (Poggioli et al. 2007). Currently, approximately 20 FDA approved biologics are manufactured using a perfusion system and these companies include, Pfizer, Genzyme, Biogen, Bayer, Baxter and Shire to name a few (Fisher et al. 2019).

Companies such as Merck have made great strides to develop continuous processing. Merck is using its perceived disadvantage of having very little stainless-steel capacity in comparison to most other biopharmaceutical companies – refer to figure 52. Merck's goal is to harness the advantages offered by continuous processing and single use to deliver a multiproduct capable facility (Brower et al. 2015). Merck is also increasing its stainless steel capacity by retrofitting an existing site for the batch manufacture of biologics (MSD Ireland, 2019).

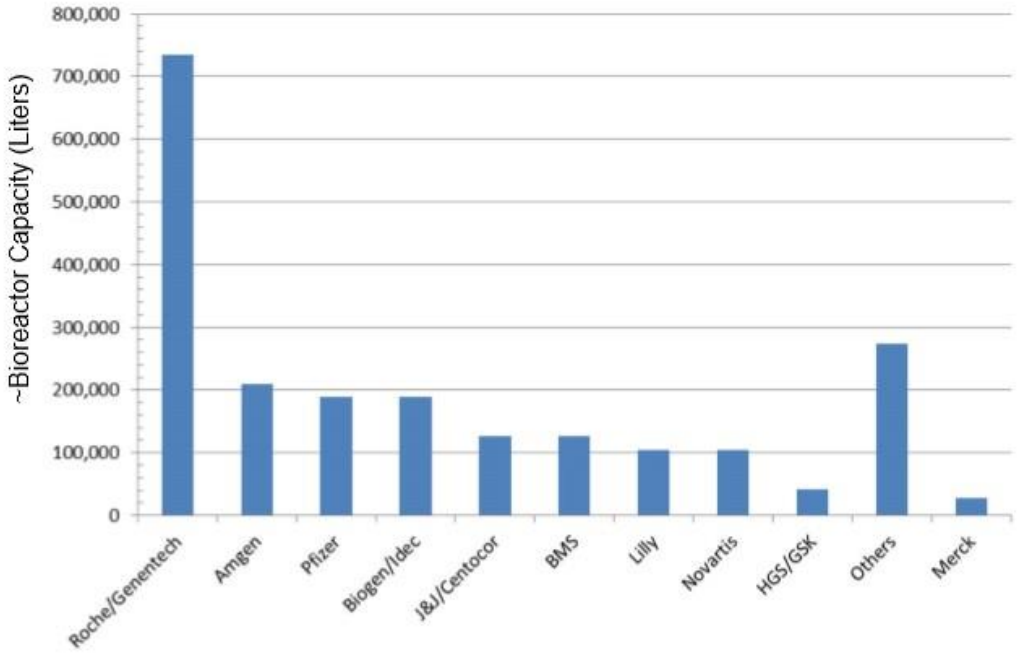

Figure 52: Stainless Steel Bioreactor Capacity (Brower et al. 2015)

Another company, Bayer has created and developed their own continuous process for downstream manufacturing, using SUTs and a 200L perfusion bioreactor in Chempark, Leverkusen (Klutz et al. 2015).. The downstream operation uses a multicolumn approach which will be discussed later in the report Figure 53 shows how the different operating modes impact the production schedule.

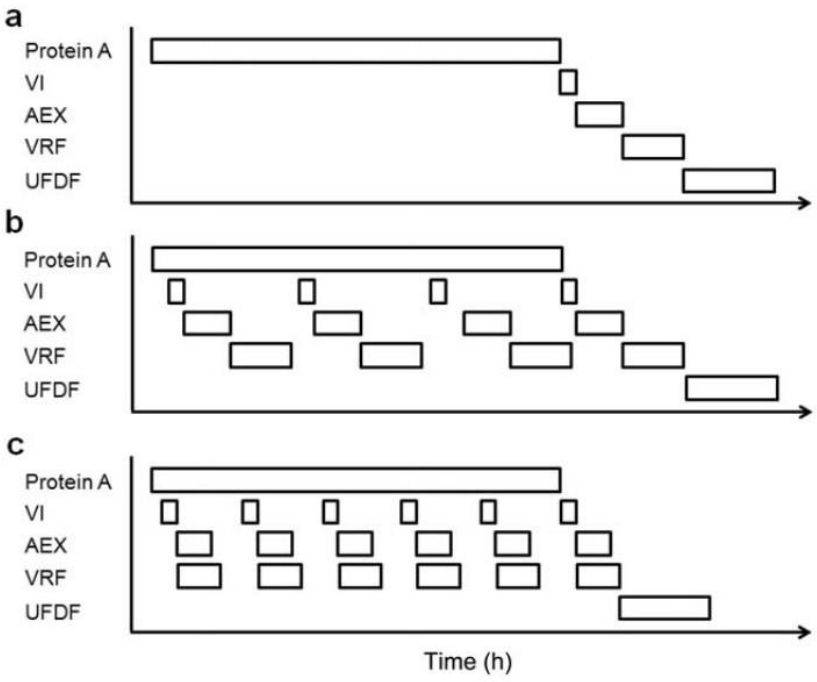

Figure 53: Visualisation of Batch versus Semi-continuous (Pollock et al 2017)

Figure 53a; represents the traditional batch sequence with each step completed before the next step commences. Figure 53b represents a semi-continuous process in which smaller elution volumes are pooled together and uses a semi-continuous capture and UF/DF step. Figure 53c highlights the continuous nature of AEC and a viral retention filtration step. The product flow stream will directly flow from the AEC system directly over an appropriately sized filter area and flow rate, which should maintain transmembrane pressure (Pollock et al 2017).

2.8.1 Benefits of Continuous Processing

The benefits of continuous processing deliver operational flexibility. Different products will have different characteristics. The ability to adapt the manufacturing process will give manufacturers a competitive advantage and facilitate more rapid delivery of the product to the market than their counterparts (Croughan, Konstantinov and Cooney, 2015). The industry has recently displayed great interest in immunotherapies that target PD1/PD-L1 pathways and 11 mAbs were identified to be targeting this same pathway (Alsaab et al. 2017). If a manufacturer is not first to the market, they must compete for other indications, and do that at significant cost, which underlines the advantage of being first to market (Schofiled 2018).

Levine et al. (2013) estimate that facilities required to produce therapeutic products, using both continuous processing and SUTs, will take 2 years less to complete than a traditional stainless steel facility. This is due to the savings gained from eliminating the requirement for stainless steel design and manufacturing. Additionally, there is no requirement for utilities such as CIP & SIP systems, ultimately reducing validation requirements.

A continuous process is more simplified, requiring a smaller footprint; and can be scaled by increasing volume or by running operations in parallel. Smaller equipment of uniform size aid process development, clinical stages and eventually the production of commercial products; and reduces the risk that technology transfers pose. SUTs can be used to compliment continuous manufacturing processes through modular standardisation. Also, a reduced residence time will allow manufacturers to produce stable and unstable proteins (Croughan, Konstantinov and Cooney, 2015). Schmidt (2017) states that in general most of the cost saving is realised with the reduction in overall footprint. This approach could potentially translate into smaller facilities that are easily expanded or duplicated at multiple sites for redundancy measures. Stanton (2018) highlights a recent newcomer to the Irish market. Wuxi Biologics announced their plan to build a state-of-the-art biologics manufacturing facility in Co Louth in

2018. Wuxi have increased their bioreactor volume from zero to 222,000L within the last 10 years. Wuxi intend on utilising continuous processing and single use technologies at their site in Co Louth; and deliver 54,000 L of single use bioreactor capacity and utilise their next generation bioprocessing platform. This will take advantage of new disposable technologies coupled with modular, continuous platforms that are easier to replicate globally at reduced cost.

Croughan, Konstantinov and Cooney (2015) highlight that product quality can be increased by reducing the time a product spends in bioreactors while being exposed to harsh conditions. In downstream, the removal of hold steps increases processing time and removes the need for hold time and mixing studies.

To produce a large-scale process using batch mode, manufacturers need to acquire and store large equipment at low utilisation rates. Resin capacity is increased by using the multicolumn approach and buffer volumes are decreased. The result is a simpler closed process, with a smaller and simpler facility. Increased automation can also reduce the number of trained personal required to run the facility which will reduce costs (Konstantinov 2015).

For multicolumn operations, the first column can be overloaded beyond past breakthroughs so that the resin can meet its static binding capacity. Before the column reaches the static binding capacity, the flow is routed to the subsequent column to collect unbound material. This approach enables high capacities at low residence times and significantly improves productivity (Bisshops et al. 2009).

Despite the advantages continuous processing can give, manufacturers of blockbuster mAbs are still building traditional facilities and using stainless steel in the manufacturing process with no single use aspect. Biogen are completing their purpose-built plant for “Aducanumab”, a new treatment for Alzheimer’s in Switzerland. Their process utilises four stainless steel 18,500L bioreactors, and operates in fed-batch mode. Building a plant of this size before FDA approval is a major risk. If this treatment is not approved by regulatory agencies, finding a replacement drug to manufacture at this site will be difficult. The reasons for not implementing continuous processing may be attributed to the time the decision was made to build, as there have been advancements made since that time (Schofield 2018). Unfortunately this risk did not pay off and Biogen announced that it had stopped clinical phase 3 trails due to efficacy issues in March 2019. This knocked 18% off the value of the company and its stock price fell by 28.65% (Hargreaves 2019).

2.8.2 Disadvantages of Continuous processing

As with any novel technology, manufacturers may see continuous processing as not yet mature enough, needing more time to warrant further investigation. For continuous processing to garnish more support, manufacturers will want support from regulatory agencies so that the challenge of regulation does not overshadow the technological benefits continuous processing can provide. Another area of concern relates to old legacy facilities that been on the receiving end of large capital investment projects. These have not yet paid back on the initial investment and a corporate strategy rethink will be required for this approach to change. Reliance on single use technologies means that manufacturers are more reliant on suppliers to maintain manufacturing schedules (Konstantinov 2015).

For certain multicolumn operations, the introduction of new equipment may incite the belief that more can go wrong. Issues over contamination in operations which require the stream of one column to be directed on to another, may be of concern from a cross contamination viewpoint (Fisher et al. 2019).

For certain therapeutic products, market demand is not enough to warrant a continuous process. Another issue relates to the requirement for a new registration if the process is changed. Therefore, manufacturers may see this as a potential hurdle (Jungbauer 2013).

2.8.3 Validation of Continuous Process

There is currently an increasing interest, not only as stated by manufacturers of biologics but also from regulatory agencies, in respect to continuous processing. Regardless of the benefits and advantages that this new approach yields, its fate will largely be determined by the regulatory requirements for this new approach. Central to these regulatory requirements lies quality risk management (QRM), control strategies, real time testing, viral safety, process monitoring, process validation and verification. There's growing consensus that to attain the modernisation of current biopharmaceutical manufacturing, a collaborative approach must be adopted that needs to include experts in the industry, regulators and academia. Regulatory agencies have actively supported the continuous processing paradigm. In 2017, the FDA created the "Emerging Technology Program" that contained a draft guide, the setup of a new task force and several articles on advancing innovation. The European Medicines Agency (EMA) have set up several teams to investigate QbD and PAT. These teams include experienced inspectors and assessors. The PDMA (Japanese Pharmaceutical and Medical

Device Agency) have also established working groups to set out their position (Nasr et al. 2017).

Even though the FDA and other regulatory agencies have publicly outlined their approval of continuous processing and have stated there are no barriers for this in relation to the manufacture of biologics, all is not resolved. Regulatory expectations for a process that is continuous is identical for one that is not. Concerns with a continuous process include issues such as quality assurance, quality control, the definition of a batch and traceability (Hernandez 2015).

Allison et al (2014) highlights the regulatory considerations of continuous processing and the requirement for a different approach for several reasons which include;

1. The definition of a batch – is it applicable?
2. In process control (IPC) – Due to the mode of manufacture, sampling requirements may change. The stage at which the sample is taken, the sample size and the frequency at which it's taken, due to not pooling, will all need to be assessed.
3. Process deviation procedures need to be assessed and the scope of investigations need to be understood.
4. Manufacturers will have to justify testing and consider the time and quantity of material impacted.
5. Controlling variability of incoming raw materials
6. The evaluation of change for a continuous process may need to be assessed differently than that of traditional batch processes.

Johnson et al. (2016) also highlights viral safety concerns will also need to be evaluated.

Batch definitions for a continuous process are critical in relation to material traceability; especially in times of product recalls and other possible regulatory actions. Figure 54 defines "a batch" with no specific mode of manufacturing and it includes reference to a continuous process (Nasr et al. 2017).

- Batch means a specific quantity of a drug or other material that is intended to have uniform character and quality within specified limits and is produced according to a single manufacturing order due the same cycle of manufacturer.[12]
- Lot means a batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by continuous process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits.[12]

Figure 54: Batch and Lot definition (Nasr et al. 2017)

Vogg et al. (2018) states that due to this definition of a batch, it can be defined based on time, volume, mass or completed unit operations, which allows manufacturers the flexibility to define a continuous batch for a given process.

Process validation and continued process validation are utilised to ensure the process is robust and results are reproducible. The following must be assessed;

1. Are process conditions defined allowing the user to determine if the system is in a state of control, including verification of the CPPs and CPAs being within the target range?
2. Are the process control systems sufficient to detect the manufacture of acceptable product? Start-up and shut-down activities should be included in this and completed based on a risk analysis unique to a particular process step, taking CPAs and CPPs into consideration?
3. Can the system achieve the process conditions specified and maintain these conditions over the duration of the manufacturing activities? Worst case durations should be included as part of validation activities?
4. Can the system identify excursions and how can it deal with non-conforming product. Figure 55 shows the system identifying an excursion and placing material to waste?

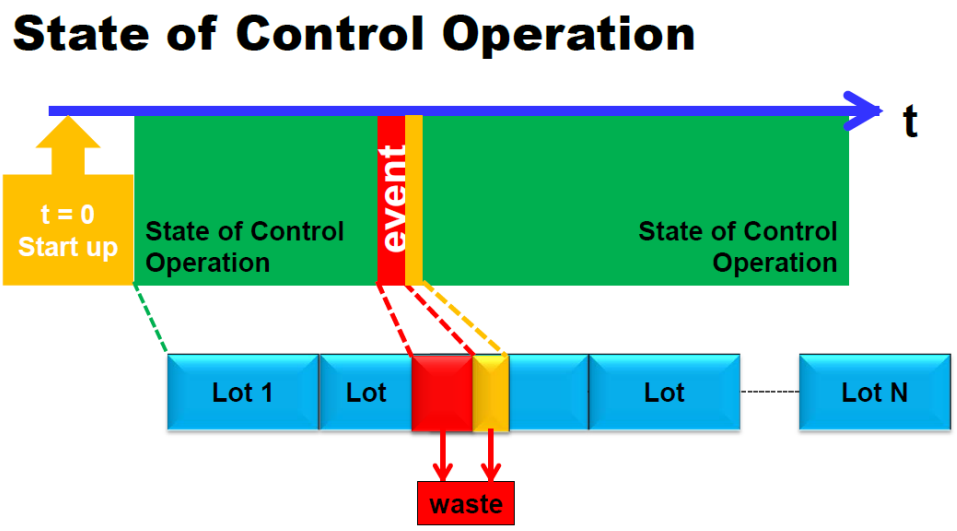

Figure 55: PQRI's State of Control and Non-conforming material (PQRI 2017)

5. How does a change in run rate or equipment scale up impact the system?
6. Can the process be maintained in a state of control for the duration of commercial manufacturing campaign? (Allison et al 2017).

2.8.4 Continuous Chromatography Platforms

Recently, continuous chromatography has entered the manufacturing industry of biologics as an area of interest. The principle driver for this is the overall downstream process which accounts for between 70% and 80% of the overall manufacturing costs (Vogg et al. 2018).

As with traditional batch processing, protein A bind and elute is the method of primary capture. Processing through the Protein A column in batch mode is restricted by the DBC, which makes it a time-consuming unit operation. Due to this, researchers have investigated continuous chromatography as a replacement for this unit operation. This is not a new idea as it was first developed in the 1940s and was referred to as simulated moving bed technology (SMB). This concept was first applied to oil products (Ötes et al., 2018). By reducing the volume of protein A resin required for the process, increased productivities can be availed of, along with the other advantages detailed above (Hummell 2018).

As discussed previously, packed columns are typically batch operated, with column equilibration, load, wash and elution performed as sequential operations and the resin is used for each operation. There have been a number of chromatographic approaches designed for primary capture (Zydney 2015). Some of these approaches are discussed below;

2.8.4.1 Periodic Counter-current (PCC) Chromatography

PCC was first introduced as a bind an elution step for a continuous process by GE in 2011 (GE2011). PCC offers users an alternative to large single use columns and reduces processing time, increases resin utilisation, reduces resin cost by using less and uses resin more at the DBC. As a result of increased loading, less buffers are required, but all of these come at the cost of increased equipment complexity and hardware cost. PPC operates with 2 to 16 columns. As more columns are added, the complexity rises. Figure 56 shows two images – the first is a graphical representation of a batch cycle using a chromatography column. For this instance, the column resin is not efficiently used as the column is not loaded near its DBC and loading is always stopped prior to product break through. The later image shows the sequence of a two column PCC (Angelo et al. 2018).

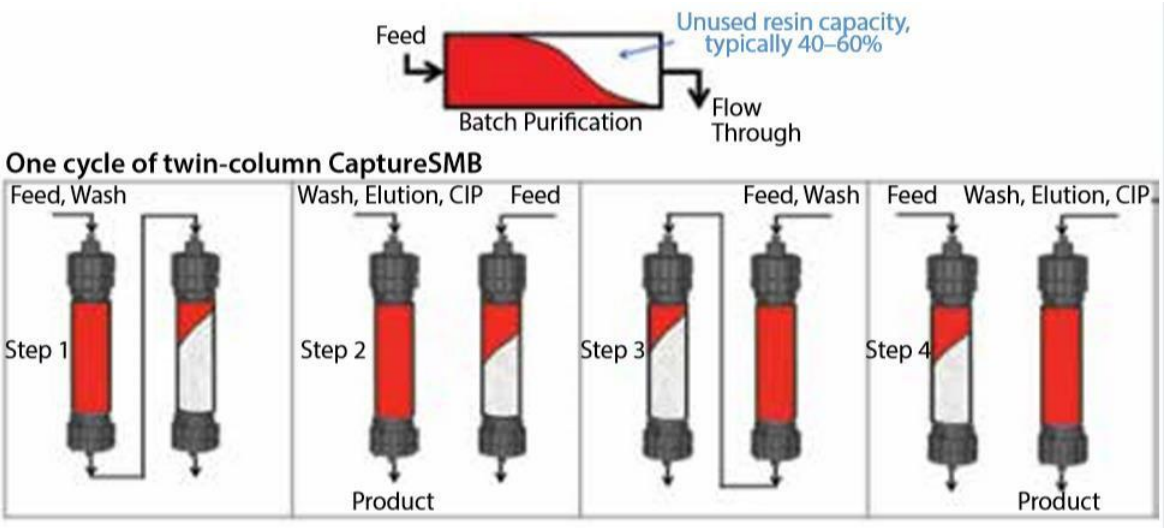

Figure 56: Batch resin usage and One Cycle SMB (Angelo et al 2018)

The concept of PCC is that the first affinity column is loaded beyond the DBC and the eluate is directed onto the next column whist the first column is washed and equilibrated. The steps of PCC can be separated into 4 steps (Angelo et al. 2018).

The 4 steps are described by Mahajan et al. (2012) for a three column PCC;

- Column 1, 2 and 3 have been equilibrated and ready for product load. Column 1 is loaded with harvest material until 1% breakthrough is achieved.
- The eluate from column 1 is then directed over column 2 so that any product that is unable to bind on the first column can find a binding site on the second column. Column load is completed on column 1 when 70% breakthrough is achieved.

- The product stream is then directed at column 2 and column 1 initiates a wash cycle. Due to product loss during the wash cycle the stream from the wash cycle on column 1 is directed towards column 3.
- Column 1 is eluted, regenerated and re-equilibrated whist column 2 and 3 are loaded.

Figure 57 is presented by Majahan et al. (2012) as a graphical representation of a 3 column PCC in operation.

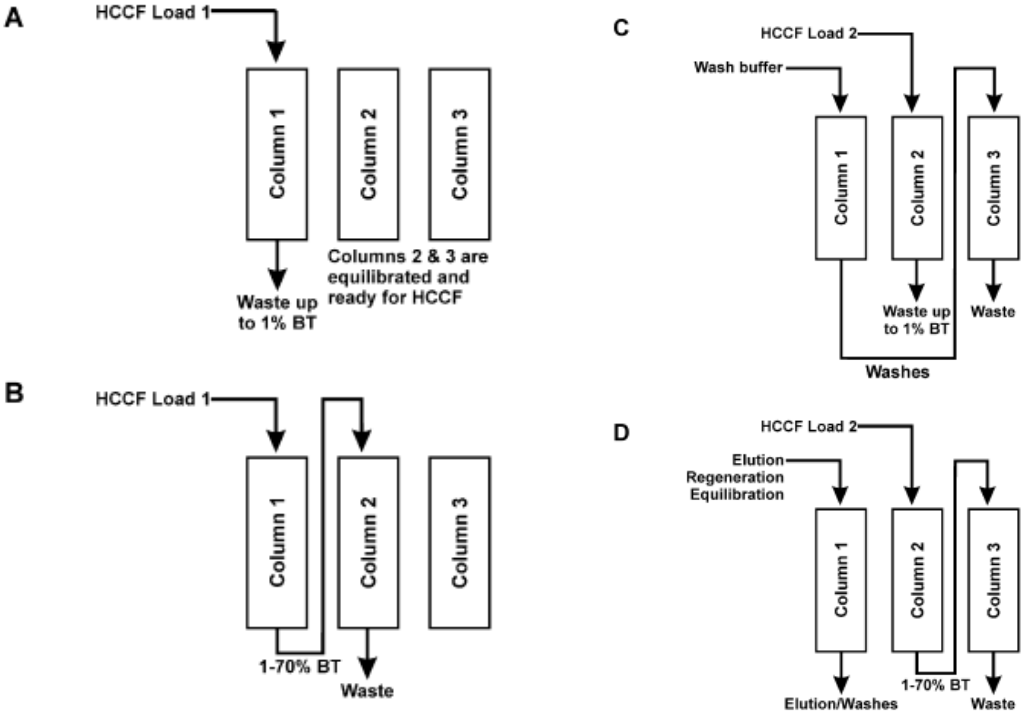

Figure 57: 3 Column PCC (Mahajan 2012)

A single column approach can be used if the material is directed back to the harvest tank until a 70% breakthrough is achieved (Mahajan et al. 2012). A perfusion system can be successfully used in conjunction with PCC; and an online UV meter is used to determine column switching (Warokoo 2012).

Large biopharmaceutical companies have evaluated GEs PCC system to assess the ability of a semi-continuous system to deliver clinical and commercial product. Pfizer's study concluded that to enable a change from batch to semi-continuous would require a process change and a retro-fit to the facility; but the cost of the changes would pay back the initial capital investment with the delivery of 8 proof of concept batches (Pollock et al. 2013). Other companies such as Jansen, Genzyme and Amgen have been reported to be evaluating the advantages of a PCC system (Pollock et al. 2017).

2.8.4.2 Multicolumn Counter-current Solvent Gradient Purification (MCSGP)

MCSGP is suited to difficult separations of weakly absorbing (early eluting) impurities or variants, protein of interest (product) and strong absorbing (late eluting). MCSGP separates the product stream into three fractions in order to extract the product from the stream. MCSGP is used for CEC and AEC systems; and reduces buffer consumption whilst providing high productivity. MCSGP uses a counter current motion of the stationary and mobile phase and gives superior productivity for peptide purification (Muller-Spath et al. 2008).

Figure 58 is a graphical representation of a chromatogram for a batch that is processed using MSCGP. The stream containing the protein of interest (P) consists of weakly absorbed impurities (W) and strongly absorbing impurities (S). The MSCGP batch is separated into five steps;

1. Elution of W (weak absorbing).
2. The recycle of W and P (Columns are interconnected for this step).
3. The removal by elution of P.
4. The recycle of P and S (Columns are interconnected for this step).
5 The last of S is removed during the column regeneration step.

The columns for step $I1_2$ and $I2_2$ need to be connected together for the recycle steps and the elution phase is completed in batch mode (Steinebach et al. 2017).

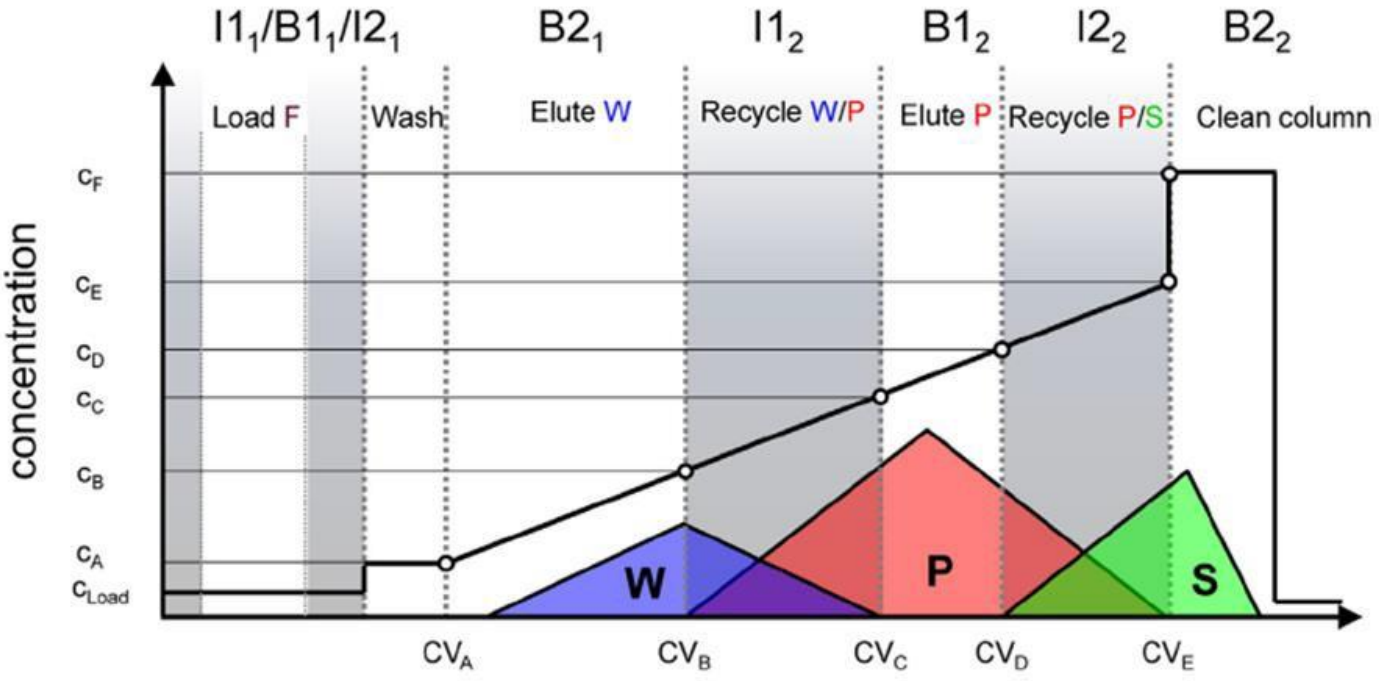

Figure 58: Batch Chromatogram for MCSGP (Steinebach et al. 2017)

2.8.4.3 Simulated Moving Bed (SMB) Chromatography

SMB can be applied to the separation of binary streams and is another continuous chromatography process which can be used to remove product variants and oligomers. SMB uses multiple packed columns on a smaller scale, and utilises periodic switching of the product stream, mobile phase and recovery points that gives a countercurrent flow, without moving the stationary phase. A four-column configuration is shown in figure 59 (Zydney 2015).

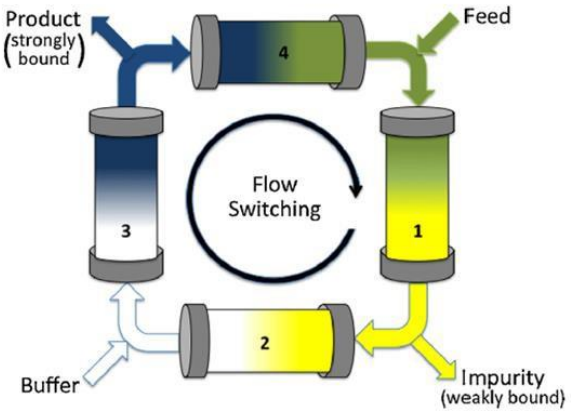

Figure 59: 4 Column SMB (Zydney 2015)

The process starts with the products stream is loaded onto column 1. The stream will be separated with more weekly bound material, eluting first and leaving the column via an exit port. Another mobile phase is added between column 2 and 3 and more strongly bound material is recovered from the exit of column 3. When the product has moved through column 1, the location of the feed, mobile phase and recovery ports are rotated clockwise to mimic counter-current flow. This type of arrangement is only possible if the regeneration phase is longer than the loading phase (Zydney 2015).

2.8.4.4 Continuous Counter-current Tangential Chromatography (CCTC)

CCTC is another continuous processing platform that can be applied for continuous processing. Dutta et al. (2015) proved that by using commercial protein A resin in a CCTC format, the system was able to process both low and high titre process streams. CCTC proved comparable for HCP removal, yield and purity. CCTC was far superior in terms of productivity when compared to traditionally packed columns. CCTC not only offers advantages over traditional batch processing but other multi-column continuous processes also. CCTC eliminates the requirement for column packing activities and slurry tanks, cleaning, validation and resin storage. CCTC uses resin in the form of slurry and it takes approximately 30 minutes to charge the system and does not involve complex switching. The system directs the slurry

into a number of static mixers and hollow fibre membranes. As per figure 60, the separation is completed with six chromatography steps (binding, wash1, wash2, elution, strip and equilibration). The flow rates are maintained by using peristaltic pumps. The hollow fibres retain the large resin particles and allow the protein of interest to flow through. Due to the low pressures (<70kPa), this step is compatible with a fully single use system (Dutta et al. 2015).

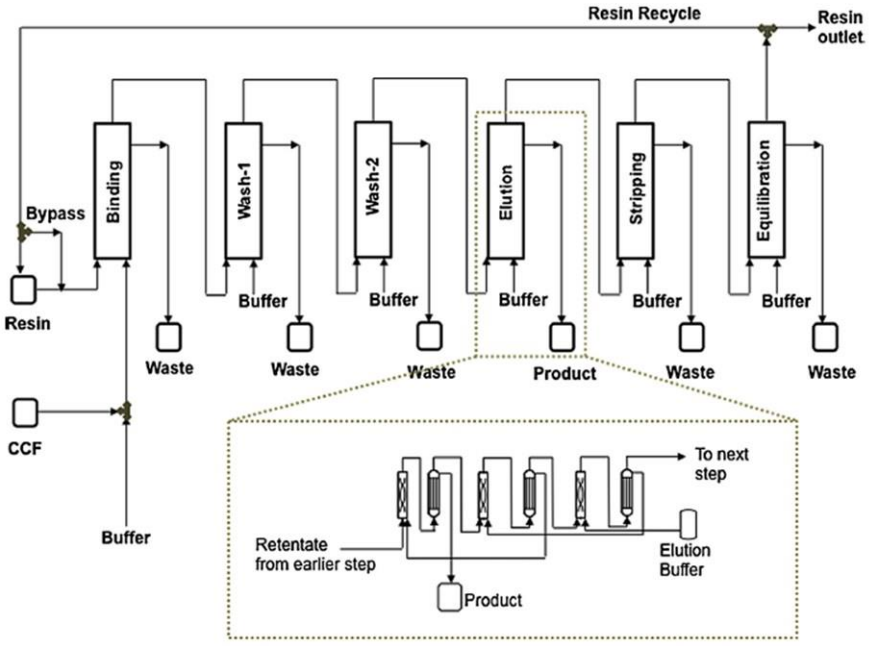

Figure 60: Schematic of CCTC (Dutta et al. 2015)

2.8.4.5 Integrated Perfusion and Protein A Capture Continuous Process

The first successful attempt to integrate a perfusion system and a protein A capture step occurred in Uppsala Sweden using a GE Healthcare AKTA 4 Column PCC system. This was the first time the continuous processing paradigm made its way past the capture step; and it highlighted the operational efficiencies associated with having no hold steps (Warikoo et al. 2012). Then in 2014, Genzyme corporation created a continuous protein A, viral inactivation and continuous polishing operation using a two multicolumn system (Somasundarum et al. 2018).

In 2015, another study was completed examining a fully automated, end to end continuous process for an antibody process and aimed at demonstrating the feasibility of an uninterrupted, fully continuous process over an extended duration. This study also showed that this proposed process train using new continuous technology was considerably simplified. Using a 12L perfusion system upstream, they were able to incorporate two PCC systems, refer to figure 61.

The first one operation was a typical capture step and the next chromatography step was a CEC step. The system ran for 31 days and the product was batched every day (31 batches) with an output of ~8g mAb per day (80% yield). Each CQA was monitored over the duration and all were comparable with batch mode (Godowat et al. 2015).

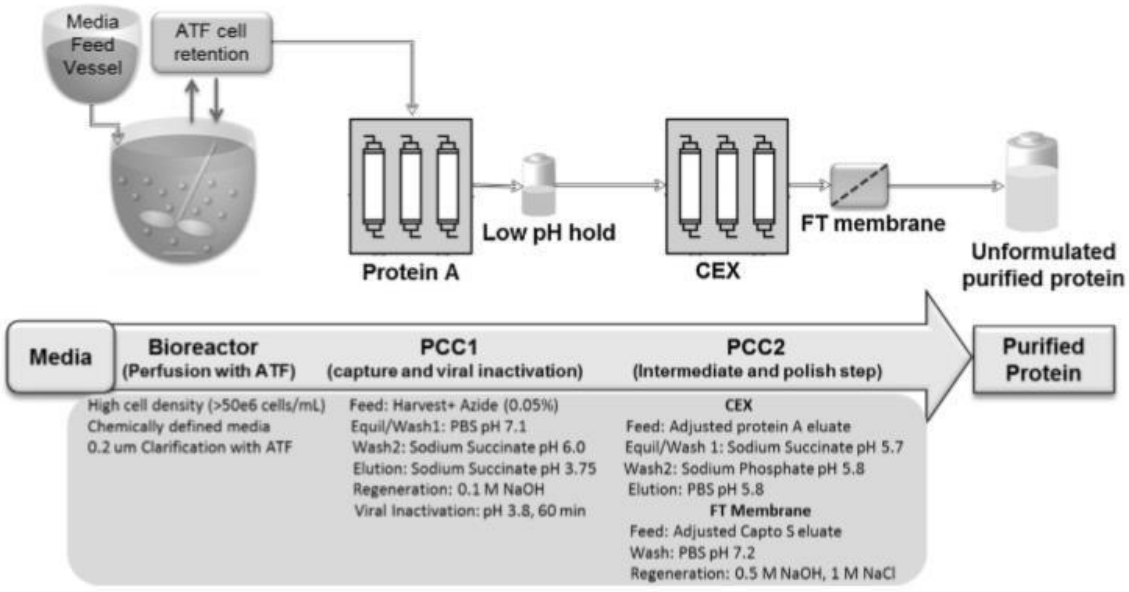

Figure 61: End to end Continuous bioprocess configuration (Godowat et al 2015)

The data also supported this end to end process for increasing throughput, decreasing equipment footprint, removing several non-value added steps, such as hold vessel steps (Godowat et al. 2015).

In 2017, a similar study was conducted which examined what operational efficiency that could be gained by processing a 20L of clarified CHO Supernatant in a) batch mode and b) continuous. For the continuous process, an eight column (5cm bed height) protein A capture step was employed. The protein A eluate was virally inactivated prior to an AEC membrane and mixed mode CEC resin. This study highlighted the economic advantages of continuous process. It concluded that the continuous operation used 97% less protein A resin, 74% less anion exchange membrane surface area, 97% less mixed mode resin and 44% less processing buffers (Gjoka et al. 2017).

As a possible alternative to fully integrated, fully automated, end to end continuous process, Sanofi have proposed the "Accelerated Seamless Antibody Purification (ASAP). ASAP is a simplified version, in which all chromatography steps are carried out in continuous mode (Mothes at al. 2016).

In a study to compare traditional batch processing and continuous process, a simulator was developed. The process simulator was used to compare fed batch and a continuous process, including examination of the cost for both. The process parameters deemed most important are presented in table 8. In this study, the output for the plan of record (POR) was exceeded by using the continuous process. The actual number of batches was considerably less but over an extended duration (Yang et al. 2019). Table 8 below compares fed batch versus continuous processing operating conditions;

	fed-batch processing	continuous processing
inoculation cell density (seed)	1.5×10^6 cells/mL	1.5×10^6 cells/mL
bioreactor cell density (production)	5.5×10^6 cells/mL	6×10^7 cells/mL
max bioreactor volume set	15 000 L	3000 L
perfusion rate		1 vvd
downstream yield	75%	75%
cell cultural time	14 days	24 days
annual number of batches	48	9
annual production rate	618 kg/yr	624 kg/yr

Table 8: Comparison of fed-batch and continuous batch (Yang et al. 2019)

Continuous processing could also be progressed by manufacturers of biosimilars due to their price sensitive strategies. Rather than look at a whole or nothing approach, it may be better to just look at each unit operation and decide which is the easiest to implement that will provide the biggest to gain. Some manufacturers have implemented a continuous capture step only due to the cost of protein A resins. The manufacturing of biopharmaceuticals seems to be coming to a cross roads. Samsung which are new entrants to the biologics industry have built a large fed-batch traditional facility at a time when other more experienced manufacturers, for instance Genzyme, are experimenting with fully end to end continuous processes (Schmidt 2017).

As stated, continuous upstream processing is much more advanced than in downstream. For the semi-continuous capture and polishing steps, the two main options are PCC and SMB. Many commentators cite regulatory concerns, but the FDA have given guidance as far back as 1993. Quality by Design (QbD) concept requires deep understanding of the process to enable the identification of critical quality attributes (CQAs). By understanding all the variables, the process can be controlled to ensure the CQAs remain in control. Process analytical technologies (PAT) is identified as a key enabler for QbD as it is known that product quality must be tested

throughout process; and it is preferable that this testing can be built into systems. QbD and PAT can reduce the risk to product quality and also give process efficiency. This is key for integrated continuous processes, as decisions will need to be made based on in-line, at-line or online results (Fisher et al. 2019).

There have been examples identifying the use of a continuous perfusion system and capture system together. A challenge exists to ensure the flow rates are synchronised, to avoid the requirement to pool resulting in a non-continuous process. Issues with columns may need to be factored in and redundancy will need to be considered. To date there is no true end to end process developed and in use by a manufacturer (Fisher et al. 2019). Some commentators question whether an end to end process is even required, stating that maybe the aim should be to focus on the initial capture step as continuous and the remaining intermediate and polishing steps can be completed by pooling and batch loading (Shukla et al. 2017). Another areas of concern relates to lack of control and monitoring and that this is an area that needs to be developed (Fisher et al. 2019).

CHAPTER 3: Conclusion

As detailed in Chapter 1, the primary objective of this report was to review the current large scale chromatography techniques applied by manufactures in order to purify mAbs. Column chromatography is a well-defined purification separation technique that has become the backbone of the downstream processing of biomolecules, such as mAbs. Its popularity can be attributed to its optimal selectivity, scalability and robustness. Despite this, the cost of resins and inherent inefficiencies has stimulated research and development for industrial large scale process to improve established chromatography methods. Protein A chromatography is widely employed as the initial capture step in the purification of mAbs. Generally, it is followed by subsequent intermediate and polishing chromatography steps, influenced by the intrinsic characteristics of the mAb protein of interest. The more chromatography steps utilised within the purification process, the higher the purity of the protein of interest; but the process yield is also negatively impacted (Section 2.1.3)

Chromatography inefficiencies and limitations can be a consequence of the materials of construction for columns. Column materials such as glass, acrylic and stainless steel must be assessed for compatibility and durability under the operating pressures of a given process (Section 2.2.1). Compatibly of column and component materials with the process and cleaning solutions may pose issues, such as corrosion. Prolonged exposure of column materials to bactericidal and sanitisation buffers is inevitable; and is paramount to preventing bioburden accumulation and biofilm formation. This can potentially lead to the leaching of contaminant compounds from the column's constituent materials into the process stream. Therefore, the leachable and extractable profile of materials must be fully understood (section 2.3.8)

Chromatography productivity is predominantly dominated by the quality of the pack. Reproducibility, uniformity and contamination issues related to the packing columns at an industrial scale (up to 2m) has spurred the development of more technologies with a scientific approach. Packing technologies that reduce operator interaction, such as syringe packing, can reduce the risk of contamination. Removing operators from the packing process will decrease the risk of human error and has been proven to improve reproducibility. However, slurry concentration evaluations are still performed manually. Calculation errors in slurry concentration will result in non-uniform, loosely packed or overly compressed column beds, ultimately leading to ineffective separations. Hence, further development is necessary to eliminate the potential for human error in the packing process. (Section 2.3.5)

Improvements in cell lines and increased productivity have resulted in the downstream process becoming a bottle neck for batch manufacturing. Large scale column dimensions are currently limited to a maximum diameter of 2 m and bed height of 30 cm, due to flow distribution and pressure drop limitations respectively. Industry have responded to column size limitations by improving the resin matrix and ligand chemistry to deal with higher titres. Modern Protein A resins now benefit from improved binding capacities to manage increased titres. Despite this, these optimised resins come at an additional cost, increasing pressure on manufacturers to find alternatives to the operation. The infrastructure required to pack these columns, such as hardware, software, and utilities can be expensive. To alleviate this cost constraint, packed columns can be amortised over a defined number of cycles. However, multiple uses can result in product quality and regulatory concerns. (Section 2.5.3).

Over time, packed columns can suffer from edge effects and compression, inevitably impacting manufacturing ability, requiring a repack. Having a column out of service will impact the schedule, unless manufacturers endure the cost of purchasing a backup column to mitigate delays. Prepacked columns are an option that become more viable for larger scale operations. The largest pre-packed columns on the market are 80cm in diameter with a bed depth of up to 30cm. A column of this size can accommodate many commercial requirements. Prepacked columns remove column packing activities from the critical path and eliminate the need for expensive, complex equipment which increases their attractiveness. (Section 2.3.7). Amgen employ prepacked columns in their single use facility and utilise back-up packed glass columns for redundancy.

Column evaluation methods to examine pack quality remain relatively unchanged, with the pulse injection method being the preferred method. The non-reactive “spike” standard utilised in evaluation is dictated by whether conductivity or UV absorbance detection is applied. HETP and symmetry analysis provide a quantitative measure of column pack quality; and rely upon the analysis of the “spike” standard peak on the evaluation chromatogram. Optimally, a narrow symmetrical peak indicates good column resolution and validates the uniformity of the pack (Section 2.4.1)

There are multiple reasons why the traditional platform approach of a three column purification steps is changing. Due to the mode of operation, multi-mode resins are reducing the three column approach in certain cases to a two column approach (Section 2.6.5). For example, Capto adhere resins can target molecules based on their hydrophobicity and charge, essentially providing HIC and IEX in one step. Single-use and continuous processing

technologies are other influences that are impacting this platform.

It can be concluded that the biopharmaceutical industry is at a cross roads in terms of chromatography technologies with developments in continuous systems. This report highlighted the stainless steel bioreactor capacity available to most large scale manufacturers of mAbs. Genzyme were found to have highest bioreactor capacity and they are investing in continuous chromatography platforms. Merck, with the lowest bioreactor capacity, have also been actively investigating continuous systems, whilst dealing with Contract Manufacturing Organisations (CMOs). Conversely, Merck have also announced plans to build a state of art batch facility to manufacture its new blockbuster immunotherapy drug "Keytruda", which shows that they are not fully invested in continuous processing; or in certain circumstances, will opt for a more conservative approach. (Section 2.8)

This report has highlighted that most manufacturers implement a flexible platform approach rather than a product-specific approach. The platform approach, with standardised methods and practices, enables flexibility and quick adaption to support multiple products. A product specific approach can result in future limitations. Case and point was Biogen's decision to take a product specific approach for its anticipated Alzheimer's drug to be produced at a large scale. Biogen may find it difficult to find an appropriate replacement drug to utilise the plant at that scale in order to recoup the cost of the project (Section 2.8.1). Following the adaptable platform approach, as shown by Pfizer, Amgen and Wuxi, is built on a foundation of utilising lessons learned through previous process experience. As companies transition from one product to multi-product, more information is gathered and the knowledge gained allows manufacturers to tailor their platform to suit their needs. Platform approaches help deliver fast and cost effective processes, enabling scale up of potential drug candidates so they can become evaluated quickly. For successful candidates, this is the template for commercial manufacturing and reduces the validation effort required. Using such an approach, it is possible to take less than one year from finding a gene to IND. Each organisation must evaluate their cell line, product, process and the lessons learned when moving from a one product to multiproduct. (Section 2.7)

Continuous chromatography has emerged as a topic of great interest within the large scale manufacturing of biologics, driven by the exorbitant cost of protein A resins. The current batch paradigm also utilises very inefficient resin usage, with packed columns being loaded far short (60%) of the dynamic binding capacity (DBC) of the resin. This is area should be targeted for further investigation. Manufacturers using traditional batch processing with large columns

would be very interested in increasing the efficiency of their current platform. A one column PCC approach, in which the effluent is directed back to the holding vessel, would increase the DBC of the resin. Increased DBC of the resin can increase efficiency, potentially offset the cost of the expensive protein A resin (2.8.4.1)

The fully continuous process, where product flows through all chromatography steps in one, is not realistic. Mainly due to the reliance on binding and elution as orthogonal purification steps. Amgen have taken steps to provide a closed end to end system for the downstream process. Continuous processing for the downstream platform offers opportunities and limitations. It is currently not widely used; and during research for this report, not one commercial platform offering this as an alternative was identified. Of note, Wuxi Biologics are creating a platform that can deliver continuous capture. This is important for Ireland as Wuxi Biologics have announced plans to build two state of the art manufacturing buildings (Section 2.7.1). One of these sites will utilise the knowledge that Wuxi have gained through experience with continuous capture, which has increased productivity relative to traditional processes by tenfold. The industry must take note of this advancement. It gives the manufacturers the ability to deliver large quantities of product to the market quickly, from a relatively small foot space. Continuous processing also affords the opportunity to change volumes or even product. Equipment that is smaller in size and less complicated, further underlines this as a cost incentive that will further drive the uptake of continuous systems. It cannot be ignored that continuous downstream processing is considerably more complicated than traditional batch processing. However, one of the advantages is that the core building blocks are currently available to deliver a continuous and fully disposable process. Manufacturers have been slow to adapt a continuous process but the number of companies using a continuous capture step, followed by completing subsequent steps in batch mode, has increased. The knowledge being gained by companies from trialling this method will place them at a competitive advantage over rivals.

Manufacturers have been successful in developing new treatments but their method of manufacturing, especially in relation to downstream chromatography, has changed little. A number of more efficient methods in relation to column chromatography were discussed in this report. All of these new methods can significantly reduce resin usage, buffer requirements and provide similar productivity. It's worth noting that PCC can reach high production rates under a semi-continuous operation and is the most utilised approach for the continuous protein A capture step. CCTC is another exciting approach as it is compatible with single use technologies and provides the same clearance as column chromatography in terms of impurities. CCTC is

an attractive method as it does not require packed columns, but there was no evidence that this is currently applied to large scale chromatography steps. Therefore, more development on this approach is required, especially with regard to resin lifetimes (Section 2.8.4.1).

Some of the information sourced for this project highlights that the benefits of continuous processing outweigh its limitations. Limitations include increased complexity and long processing lead times, coupled with increased process development. The ability to test representative samples from pool hold vessels is not applicable to continuous processing. Process analytical technologies are required to give inline process information and results to an automated process control system. This new approach requires a control strategy that is not addressed by the literature. Performing the same sampling for batch processing is not feasible or possible. Therefore, sampling frequency must be explored, along with scenarios in which samples cannot be taken. Regulatory agencies will need to be consulted as their expectation for continuous process should be standardised to support adaption of the technology (Section 2.8.3).

Most new processing advancements involve using already developed PAT technologies but in the case of new continuous processing innovations, new technologies are required. Taking into account the number of new approaches and the requirement for PAT technologies, it would be high risk to accept the introduction of all of these technologies at once. A more practical solution would be to implement these changes systematically, which I believe the industry is doing. There is no doubt the risk and reward must be understood. Those who make the move now will bear the brunt of cost and regulatory issues could scupper project timelines, but to the victor go the spoils.

3.1 Future Trends

All indications point to the biopharmaceutical industry currently being at a cross roads. Manufacturers of complex and expensive biopharmaceuticals operate in a highly regulated environment. Therefore, it is unsurprising to discover that they conduct their business with a conservative approach. There are regulatory concerns which need to be addressed and new technologies pose a challenge. The tried and tested method is well understood and, for molecules that have gone through an expensive R&D process, the tendency may be to rely on what is known. There is a push by companies such as Amgen, Genzyme and Pfizer who are developing continuous chromatography as they believe it will deliver a competitive advantage

over manufacturers that use predominantly fed-batch process. Manufacturers would benefit by coming together, similar to ELSIE, to share experiences, build knowledge and reduce risk.

However, it is important to understand that no one-size-fits-all answer to manufacturing biopharmaceutical products exists. Continuous processing may suit sensitive, complex molecules required in small quantities. For large scale manufacturers of blockbuster drugs, the cost saving of continuous processing is only attainable whist the cost of resins remains high. Regulatory concerns must also be addressed and an approach for changing registration from batch to continuous must be facilitated by applicable agencies.

Convective stationary phases have been examined in this report and can handle diffusive limitations that liquid chromatography presents. Membrane absorbers have been discussed and are currently available for large scale manufacturers. Their ability to handle low capacity, limit their applicability to only flow through processes.

Downstream purification is dominated by chromatography processes. These chromatographic unit operations are the most time consuming operation, due to the chronological and sometimes repetitive order that is applied. Coupled with this is the fact that no matter how many chromatography steps a platform has, protein A is typically the first and most expensive in terms of overall cost.

Although the benefits of a continuous chromatography approach are understood, in the medium to short term, a fully continuous platform, implemented in one fell swoop, is doubtful. Replacing individual unit operations by a continuous operation, along with a continuous protein A capture step, is attainable. This will open the door for monoliths and membranes. Despite the current disadvantages of both monoliths and membrane absorbers, they do have potential to replace packed column protein A format, due to low productivity. Cycle times for monoliths and absorbers are much faster and open the possibility for use in future multicolumn chromatography operations, as they are applicable to certain flow through operations.

Although this report looked at chromatography, non-chromatographic approaches are currently being developed and may represent an option to manufacturers in the future. Selective precipitation, utilising specific polymers, can be used to capture the entire contents of a bioreactor. If these polymers can be developed to be highly selective and inexpensive, they may see acceptance within the large scale platform. Current design of these polymers look to achieve different mechanisms which could result in selective precipitation HCP, impurities or the given target molecule. Another approach is referred to as flocculation, which uses a low pH polymeric solution to precipitate cell material, HCP, DNA. Due to this approach, impurities

can be reduced by decreasing the number chromatography steps required, which further diminishes contamination load. Aqueous two phase separation (ATPS) is another non-chromatographic approach that operates by creating two distinct phases within one solution by the addition of a polymer and salt or just two polymers together. Recent work has shown this to be highly selective but the partitioning approach has proven difficult to develop. Until recently, ATPS has not been generic enough to be applied to a mAb platform, but this research is ongoing due to the possibility of increased productivity.

Current factories built to facilitate a single blockbuster drug, on the premise of using stainless steel in a batch processing approach are no longer feasible. There are options for manufacturers to include continuous and semi-continuous chromatography in their platform. For every unit operation in the downstream process, there are alternatives. I believe that modern plants currently in design and construction, along with traditional legacy factories, must include these new continuous and single use technologies into their design. This is based on evidence which, from a business sense, is difficult to ignore. Improvement and standardised PAT technologies will be a key enabler but also require further development. However, due to the conservative nature of the biopharmaceutical industry, it may take at least one manufacturer to implement a continuous chromatography platform for a commercial product before established attitudes change.

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