

Downstream processing of monoclonal antibody biology essay



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Abstract: Present particular task, its relevance for antibody purification, key findings.

The main considerations important to the process are purity, cost, speed of process development, process throughput and yield.

1. 0. Introduction: motivation, brief summary linking the sections together.

The current surge in monoclonal antibodies in the biopharmaceutical industry has sparked the need for highly efficient purification process development. In the last two decades numerous advances in biotechnology has led to remarkable improvement in upstream production of recombinant therapeutics, in monoclonal antibodies. However these milestone achievements have been closely marred by overlooking downstream processing, which is now beginning to receive the much deserved attention. The importance for research in development in downstream processing of bio-therapeutics cannot be overemphasized. First, it is counterproductive optimising and increasing yield of mAb titre when available downstream units cannot accommodate it. Secondly, relatively high therapeutic dosage of up to grams per year per patient has increased the chances of contaminants accumulation with bizarre consequences (Gagnon et al. 2006). Therefore unlike other products of bioprocessing such as bio-ethanol, single cell proteins, industrial enzymes, a purity of up to 99. 999% [4] may be required to guarantee its approval by drug regulation agencies. This demand is made even more difficult since fermenter fluids are complex and contain compounds with close resemblance to the monoclonal antibody. The cost of

bio-therapeutics is now[1] shifting heavily towards purification (up to 80% of total production cost [6]in some cases).

Purification of mAb usually begins with separation of the most easiest-to-separate particle; the most difficult separation steps are carried towards the end of the process. This speeds up the process and hence the speed to market of the drug under development. The variation in properties of different monoclonal antibodies has inadvertently diversified the protocols for separation; hence a single robust protocol that fits different monoclonal antibodies is almost impossible. The use of platform technologies (semi-generic multistep procedures that are applicable to most antibodies and give good purification performance with minimal development-Gagnon et al. 2006) has been widely reported in literature.(Shukla et al., 2007, Gagnon et al. 2006, Baumgartner, 2009,). Although mAbs share basic similarities that permit platform operations, the extent to which they do is limited by some unique features in various mAbs. Resin loading capacity, type and volume of second washing buffer, elution pH and salt concentration has been identified as hot spots for future development of platform technology (Shukla et al., 2007,).

Due to their biochemical similarities, monoclonal antibody purification platform share very similar structures: they all have three to four key stages which include recovery, isolation, purification and polishing. For purpose of convenience, the platform can be split into three key phases. The first involves extraction and clarification of the crude drug from the mixture of cell and debris. The steps involved can be achieved with the following units: cell disruption unit, centrifugation, filtration, and microfiltration. The second <https://assignbuster.com/downstream-processing-of-monoclonal-antibody-biology-essay/>

phase involves the extraction and concentration of product streams. Units such as ultrafiltration, precipitation, crystallisation, liquid-liquid extraction and adsorption can be employed. The third step involves removal of soluble protein similar to product, and selective capture of the mAb using various chromatographic steps, preferentially affinity chromatography. The alternatives provided are far stretching with research. In the final step, also known as the polishing step, the drug is completely purified from all possible impurities.

The key to a chosen platform is the speed of operation of batches, yield and purification factors, and most importantly cost. As a result industries seek the best platform that incorporates bits of these, although it must be emphasized that the purity of monoclonal antibodies especially for subcutaneous administration cannot be compromised. The reluctance of industries to switch from long established methods could be adding to the woes of increasing cost. With Chromatography for example being the dominant technique in downstream processing, it is evident that high cost of its media and relatively long cycle time are responsible for its overly high contribution to cost (more than 70% of cost of purification) [6].

With industries striving to have their drugs being first-to-market, adequate yield and product quality within the shortest development time [2] is main focus. The industry that gets a particular drug first to the market banks 10 times more than the runner-up. Stiff competition in the monoclonal antibody market and high cost encountered in meeting regulatory demands is now causing industries to resort to shorter process development time and more cost efficient manufacturing and purification of mAb, a situation predicted by <https://assignbuster.com/downstream-processing-of-monoclonal-antibody-biology-essay/>

Schmidt in 1989. Therefore the chosen platform for purifying a mAb has to be robust, reliable and scalable.

2. 0. Background/literature survey: Enough info to make conclusion and decisions on alternatives.

Monoclonal antibody is purified from varieties of impurities with different origin, before it can gain approval for marketing. Impurities can be process-related (residual protein A, residual DNA, endotoxin), product-related (aggregates and degraded products), or product-related substances (Glycosylation variants, Disulphide isoforms, Charge (acidic) variants: deamidation, pyroglutamate, sialylated species, Oxidation, C-terminal lysine variants) (Baumgartner, 2009). In this review, the objectives of various purification stages will be assessed for their suitability to fit in an economically feasible platform technology, with high yield and purity. The objectives of the capture steps include stabilisation of the mAb, removal of protease, solids, water, and bulk quantities of proteins, carbohydrates and nucleic acids for further purification steps. The main purpose of intermediary steps is removal of most of HCP, nucleic acids, endotoxins, and viruses, while traces of these are polished off alongside structural variants and isoforms of mAb, leachable protein-A, acids, salts, and additives in accompanying polishing steps.

2. 1. Monoclonal Antibody Structure and Impact on Downstream Processing

The structure of mAb can be used to facilitate DSP. Based on their structure the mAb can have different charge in solution which can be manipulated to enable it bind more firmly unto an ion-exchange column. The degree of <https://assignbuster.com/downstream-processing-of-monoclonal-antibody-biology-essay/>

exposed hydrophobic groups on mAb defines their capacity onto HIC column. N-linked oligosaccharide on the conserved asparagines on CH2 domain of mAb mediates structural changes in the vicinity of CH2-CH3 domain interface at low pH used in protein A chromatography. Gaza-Bulseco et al. (2009) evaluated the effect of the glycosylation states of recombinant mAb on protein A and protein G chromatography and found that unglycosylated mAb were eluted earlier in and later in chromatography than glycosylated mAb using decreasing pH gradient. Glycosylation, a post-translational modification effected by high cell lines, is also important for the bioactivity (efficacy) of the mAb. Most often when the mAb is wrongly glycosylated or not at all, it folds incorrectly and enhanced aggregation which then hampers DSP.

Another threat from upstream and protein structure is the presence of proteases which have the ability to digest mAb such as pepsin. Usually this is overcome by expressing the mAb as a fusion protein. It however present another challenge as a further step will be required to remove enzymes used to cleave off fusion protein.

2. 2. Influence of Upstream Operations on Purification

With antibody titre of 10g/L(in fed-batch culture) and 27g/L(in the XD process) reported (Kuczewski et al., 2010) , it is obvious that downstream processing will be the major blockade if research and optimisation is stagnated. The complexity of fermentation broth delineates the degree of selectivity of media and the number of separation steps required to attain a

high resolution of mAb. Therefore the cell can be engineered to generate less complex supernatants.

High-throughput of product from upstream processing needs to be matched by proportionate increase in the capacity of existing downstream technologies. This is an emerging concern and is attracting response from researchers. The following sections dissect some of the key research engaged in to settle this issue.

2. 3. Major Unit Operations in Downstream Processing

The table below shows the various stages and methods used in downstream processing of mAb (Lee, S. 1989). As previously mentioned it basically have the high resolution stages and low resolution steps. The former characterises purification and polishing (ultra-purification) while the latter correspond with product recovery and clarification as explained further below. Different protein purification process synthesis methods exist such as heuristics, algorithms, high throughput experimentation, etc. The strength and weakness of the various methods are outlined in Nfor et al (2008).

Table1. Various stages and method of downstream processing.(Lee, S. 1989)

Stages	Steps	Typical methods
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Primary recovery	Extracellular proteins:
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Cell removal (solid	Centrifugation: tubular bowl
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liquid separation)	and disc centrifugation
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Filtration: dead-end and tangential

flow filtrations

Intracellular proteins:

(a) Cell recovery (solid Centrifugation

liquid separation) Filtration

(b) Cell disintegration Mechanical: high-pressure homogenization

and bead milling

Non-mechanical: osmotic shock,

organic solvent and enzyme lysis

(c) Debris removal Centrifugation

(solid-liquid separation) Filtration

Liquid-liquid extraction

High resolution Ion-exchange chromatography

Hydrophobic interaction chromatography

Affinity chromatography

Gel filtration

Liquid-liquid extraction

2. 3. 1. Product Recovery and Clarification

The first step in downstream processing is the recovery of the mAb from the cells producing it, and this depends on whether the mAb is produced and secreted externally or into the periplasmic space, or not at all (stored in cytosol). In the latter, the cells are disrupted and the mAb separated from a mixture of proteins and cell debris. This makes purification more complex. Usually, the mAb is engineered to be secreted into the culture medium. In this case extraction is much easier, involving the filtration or centrifugation. Routinely centrifugation is preferentially used for cell and cell debris removal due to its scalability and economical operations for large volumes (typically 2- 15000L/batch) (Shukla et al., 2007). Depth filtration is then used to eliminate residual cellular debris.

A recurrent concern with filtration is the formation of filter cake on the membrane or filter surface. This reduces the performance of depth filters. The use of filtration is not limited to mAb. Its application in industry is far stretching and has prompted enormous research on reducing fouling and improving performance. Dekker and Boom explored ways of improving cross-flow micro filtration. It was found that frequent-backpulsing technique improves both permeate flux and protein transmission.

2. 3. 2. Concentration and Extraction of Product

2. 3. 2. 1. Precipitation and Crystallisation

In the presence of salt or PEG, mAbs will precipitate. Based on research, selective precipitation using PEG coupled with ion-exchange polishing has

been recommended for large-scale purification of mAb [5, McDonald, P. et al. 2009]. With no special facility required for precipitation, it is therefore easier to scale up, even with the use of existing facilities. The use of selective precipitation of HCP was incorporated with CEX to achieve two-step non-affinity purification. McDonald et al identified the conditions of pH and ionic strength under which mAb precipitates using polyelectrolyte such as polyvinylsulfonic acid (PVS), polyacrylic acid (PAA), and polystyrenesulfonic acid (PSS). PVS was found to have the best precipitation effect. Antibody-polyelectrolyte interactions were disrupted by ionic strength thus preventing precipitation with increasing pH. However increasing pH led to decreased HCP and increased mAb purity (DNA, process impurities, leached protein A, product variants such as aggregates and fragments (McDonald et al., 2009) were equally removed). High molecular polyelectrolytes that coped with high ionic strength reduced clearance of HCP and formed gel-like precipitates (McDonald et al, 2009). According to [5] the scale-up is subjected to variation in process parameters such as mixing speed, time, temperature, pH, the results of which are better controlled when CEX conditions are optimised for differential binding, washing and elution of mAb.

Although crystallisation offers high purity, it cannot be used for antibody purification for two main reasons: First mAbs are produced and packaged as liquid formulations. Hence there it is futile crystallising it. Secondly the activation energy barrier of crystallising proteins and mAb, assuming solid formulations were necessary, is tremendous and the process might take up to months to crystallise making it unfeasible.

2. 3. 2. 2. Ultrafiltration

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Diafiltration is a mode of ultrafiltration which is similar to dialysis technique. Intermediate diafiltration steps used to adjust process conditions by changing buffers prior and following ion-exchange chromatography or concentrate product stream prior to size exclusion chromatography (Shukla et al., 2007), tremendously increase both cost and purification cycle. Diafiltration tangential flow filtration (TFF) when used to adjust process conditions also achieves partial clearance of process-derived DNA [5].

Batch diafiltration and continuous single-stage ultrafiltration currently used for protein concentration and desalting is not suitable for protein-protein fractionation encountered in downstream processing. It often pose the issue of trade-offs between recovery of mAb and purity. Multiply-stage purification schemes which have the pedigree to meet both have been encouraging, but being run on batch scale limits its success. Mohanty and Ghosh (2008) showed that high recovery can be achieved without compromising purity (maximum of 64% recovery at 85% purity) by using a continuous three-staged cascade TFF ultrafiltration based on membrane module. This led to high-resolution protein-protein fractionation and suggestion that membrane module per se would give poor mAb/impurity selectivity. Following successful simulation, the cascade system was proposed as initial step in purifying mAb (Mohanty and Ghosh, 2008).

Expanded bed chromatography and precipitation can be used as alternatives to it

2. 3. 2. 3. Aqueous Two-Phase Extraction

When two structurally different aqueous solutions such as two polymers, or a polymer and a salt are mixed above a certain critical concentration, an aqueous two-phase system (ATPSs) results [6]. The quest for alternatives to high cost protein A chromatography has stimulated variety of research revolving around the chances of ATPS in mAb purification.

Figure 1. Schematic diagram illustrating the behaviour of antibodies (IgG) and contaminant proteins (impurities) in a PEG-salt ATPS and the strategies used to enhance the

partitioning of antibodies towards the PEG-rich phase: addition of NaCl (right) or decreasing the molecular weight (MW) of PEG (left) (6).

Single-step ATPE used to effectuate simultaneous clarification, concentration and partial purification by combining high selectivity and biocompatibility has been promising (Rosa P. A. J et al. 2009). The process is easy to scale up using a continuous operation mode.

A major concern in ATPSs, however, is the cost of polymers used, with the cost of fractionated dextran close to US\$500/kg. Although there are cheaper polymer alternatives such as waxy starch, hydroxypropyl starch, the use of a cost-effective polymer/salt ATPS is gaining prominence recently. However, this is not without hindrance as it also massive use of saline water leads to proportionate amount of waste water, which require treatment. In response to this non-toxic and biodegradable PEG has been attempted but disposal of phosphate salt makes the process no different from the latter. Recycling of phase component is a possible remedy. [6] reviewed a chromatography-free

recovery of mAb through ATPS using polymer- salt and functionalised

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polymer-polymer systems. In the former, low molecular weight PEG and high salt concentration was used to target most of the mAb in the upper phase (PEG rich) by promoting salting-out and minimizing the polymer exclusion effects [6]. In reaction to environmental impacts of PEG-Phosphate high phosphate salt concentration waste water stream, [6] tested the replacement of phosphate with biodegradable citrate salt. The PEG-Citrate system achieved a final IgG yield of 99% and 96% final protein purity.

In the polymer-polymer system (e. g. PEG/Dextran), the PEG is functionalised with protein A to increase yield and selectivity. This makes the process less economical feasible on large-scale. The best performing ligand for purifying CHO cell culture supernatant according to Rosa P. A. J et al was found to be glutaric acid, with the best purification of mAb accomplished using PEG/dextran ATPSs containing at least 1% (w/w) tri-ethylene glycol diglutaric acid (TEG-COOH). Upon evaluation of multiple-stage and single stage ATPE, high recovery and purities can be achieved by finding a compromise between TEG-COOH concentration, number of stages and volume ratio. PEG-rich top phase with a final recovery yield of 95%, a final concentration of 1.04mg/mL and a protein purity of 93% was obtained, using a PEG/dextran ATPS containing 1.3% (w/w) TEG-COOH, 5 stages and volume ratio of 0.4 (Rosa et al., 2009).

2. 3. 3. Purification and Polishing

During antibody production, for instance using CHO cell culture in a bioreactor, and posterior purification stages unwanted and sometimes lethal materials are generated with the product of interest. Some of them include:

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Small chemicals from cell culture such as methotrexate and antifoaming agents [8];

Buffers used to condition feed stream prior ion-exchange chromatography;

Enzymes used to cleave of fusion protein introduced to facilitate capture of mAb from protein melange;

Salts used to elute mAb in size exclusion chromatography;

Additives used to precipitate HCP prior to ultrafiltration or chromatography;

mAb variants and aggregates resulting from process conditions.

With the cost of resin being a major contributing factor to chromatography's lion share of purification cost, Tugcu et al.(2008) designed an experimental screening approach for identifying the best most suitable resins for primary capture and polishing steps used for the purification of monoclonal antibodies based on productivity [11].

The use of continuous chromatographic technique warrants cleaning-in-place procedures to maintain sanitization and observe good manufacturing practices (GMP) effectively. The effect of irreversible adsorption frequently encountered and CIP on the physical properties of the system and purity of mAb in chromatography has been investigated by Muller-Spath, T. et al, (2009).

The following steps are frequently employed to eliminate the above constraints.

2. 3. 3. 1. Gel Permeation (or Size Exclusion or Molecular Sieve) Chromatography

Typically used as a final step to expel transferring, albumin or aggregates (Schmidt, C., 1989) from the final product, it separates proteins based on their differences in molecular size. It has low productivity and partition coefficient ranges from zero to one. This limits the range of resolution that can be reached and as such delimits the types of protein that it can separate. As Schmidt puts it, “ the degree of selectivity of the chromatographic media will strongly affect the resolution of the separation, which is of greatest importance for the resulting purity.” Consequently it is no surprise that it is losing its place for the much more robust hydrophobic interaction chromatography and the newly developed hydroxyapatite chromatography

2. 3. 3. 2. Ion-Exchange Chromatography

They are frequently used as polishing steps in blind-and-elute or flow-through modes, to capture trace amounts of contaminants in later stages of downstream processing. The yield and purity of mAb, as reported in literature, are 80-90% and 95% respectively [5...2].

Except for clearance of leached protein A and aggregates, the use of anion exchange chromatography as a polishing step has witness massive success in reducing endotoxin, host cell proteins, retrovirus and residual nucleic acid [Arunakumari et al, Gagnon et al). However cation-exchange

chromatography can override these bottlenecks. A versatile chromatographic

set was developed using Calcium Hydroxyapatite (CHT) that is theoretically
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capable of retaining solutes via three distinct modes namely cation-exchange, anion exchange and metal affinity chelate formation. Gagnon et al. (2006) holds that anion exchange between CHT calcium and carboxyl groups of protein does not make significant contribution to protein binding, and protein binds exclusively CHT phosphate groups using their amino groups (cation-exchange), which can be eluted using salts. CHT binds proteins with calcium metal affinity and phosphoryl cation exchange, each providing varying contribution to the overall binding (Gagnon et al., 2006). Phosphoryl cation exchange is the major contributor to IgG binding although the weak affinity from calcium must be suppressed to achieve elution. Elution with phosphate gradient can reduce aggregate formation and leached protein-A levels in purified mAb, at the expense of control of two dominant retention mechanisms (Gagnon et al., 2006) posing difficulties in adapting the process to various mAb. This was resolved by eluting with NaCl gradient while maintaining phosphate concentration at calcium affinity-weakening levels. DNA binds very strongly but 0.5M phosphate buffer can elute all sizes. Endotoxin also binds since they are phosphorylated and may require up to 1.0M phosphate buffer for complete elution.

It was found that retention characteristics of individual components are additive: aggregates have more charge and bind more strongly than native proteins hence they were eluted later. It was also proven that protein-A leachate levels can be reduced to undetectable levels using NaCl gradient and 5mM phosphate buffer. IgG-protein-A complex remained bound to resin until during cleaning when phosphate concentration was raised to 0.5M.

Elution of DNA requires at least 0.5M phosphate buffer and DNA does not elute in NaCl gradient until the cleaning step during which conc. of phosphate buffer is increased to 0.5M.

Table 1. Contamination removal efficiency in sodium chloride gradient as a function of phosphate concentration^{1, 2} (Gagnon et al., 2006)

Concentration

Phosphate in buffers (mM)

5 10 15

Contaminants

Protein A, IgG pool (ng/mL)

0.033

0.03

0.01

DNA, IgG pool (ng/mL)

1.0

1.0

3.9

Endotoxin, IgG pool (EU/mL)

0.05

1.0

1.6

1Sample: protein-A- purified IgG; 22ng/mL leached protein A, 2.3×10^3 ng/mL DNA, 1.9×10^4 EU/mL endotoxin.

2All results for a NaCl gradient to 1.5M at pH 6.5 with phosphate.

30. 2ng/mL is the lower linear sensitivity limit of the assay

According to Gagnon et al. (2006, 2007, 2009) optimisation can be achieved by eluting with NaCl gradient over a baseline phosphate concentration of 5mM. Native IgG is eluted within gradient of 1.5M NaCl and phosphate concentration can be increased to 10mM to completely elute the IgG. Any further increase proved counterproductive as it decreased the efficiency of removal of aggregates, endotoxin, leached protein A and nucleic acids (table 1).

Gagnon et al. (2006) examined the inclusion of this process in a protein A purification platform. The CHT was successfully integrated with protein-A chromatography in a two-step purification platform, in the absence of citrate or chelating agents. Similarly, a three-step platform involving the interspersed anion-exchange chromatography between protein-A affinity and CHT also proved promising with undetectable levels of leached protein-A, less than 1ng/mL DNA, and less than 0.05Eu/mL endotoxin.

CHT solves two most challenging problems facing the use of protein-A as capture step: aggregates and leached protein-A. It also has the additional ability to reduce levels of endotoxin and DNA hence the likelihood of a two step purification platform which is very economical. Cation or anion exchange or hydrophobic interaction chromatography can be interspersed between protein-A affinity and CHT in the event of contamination or variation in mAb property. Finally CHT simultaneously removes endotoxin and DNA and this makes it suitable replacement for anion-exchange chromatography especially when the sample stream contains wide variety of unwanted solutes from previous steps such as high salt concentration.

2. 3. 3. 3. Hydrophobic Interaction Chromatography (HIC)

At a time when already existing purification techniques are under high scrutiny and evaluation to meet current demands of high titre, HIC has emerged as a potentially powerful and indispensable polishing step (Kuczewski et al., 2010). In HIC high ionic strength in leading buffer initiates and sustains attractive interactions between exposed hydrophobic patches on mAb surfaces and hydrophobic adsorbent. The salt disrupts the ordered structure of water surrounding the hydrophobic pockets, which then interact with each other and the adsorbent. HCP and mAb binds based on their hydrophobicity. This makes the process quite flexible in that different mAb with different flexibility can be separated. Hydrophobicity is a function of proportion of hydrophobic side chain in protein structure; hence there is a wider range of range of partition coefficient that allows for greater resolution. After washing the bound proteins are eluted by decreasing the ionic strength.

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Conventional HIC uses resin in a flow through mode. The use of membrane in HIC in a bind and elute mode provides higher binding capacity. It is advantageous because there is no diffusion limitation; binding is mainly by convection (not influenced by flow rate) and hence reduced purification cycle. Consequently these devices are comparatively smaller with respect to column of similar throughput. In of the most recent publications, Kuczewski et al., 2010 demonstrated the ability of phenyl membrane adsorber to purify mAb with a high dynamic binding capacity (order of 20mg mAb/mL-membrane) and excellent resolution and impurity reduction. With a binding capacity of 16mg/mL, a yield of 90%, reduction in HCP up to 80%, final aggregates level below 1% and a 50-fold scalable range was reached.

2. 3. 3. 4. Affinity Chromatography

The use of protein as a capture step for mAb has been widely studied [Gagnon et al., 2006, Arunakumari et al., 2009, Zhou et al., 2008, Shukla et al., 2007]. Protein A affinity chromatography is well established, traditional method of preference and most widely used with over 98% of mAb purity and 95% yield can be accomplished [Zhou et al., 2008, Arunakumari et al., 2009]. Its high selectivity can be demonstrated using complex cell culture media in single step purification; more than 95% purity is attainable (Shukla et al., 2007). Thus it can handle unconditioned feed directly from bioreactors, its scale-up is robust, and purity of mAb is extremely high (Arunakumari et al., 2009, Gagnon et al., 2006).

Leaching of protein A from chromatographic column during elution, these constitute the major challenge in using the method of affinity

chromatography. However this is more than compensated for: it can remove endotoxins, host cell proteins, and nucleic acids from the mAb stream.

Supplement to this is its ability to tolerate varieties of product streams with little or no modification of process conditions such as pH and salt concentration. It is unarguably the method of choice of mAb capture and initial purification (Gagnon et al., 2006).

Low pH elution instigates soluble aggregate formation which in turn reduce yield and complicates polishing. Figure 1 below depicts some proposed measures to reduce the impact of aggregation.

Fig1. Strategies adopted for addressing aggregation/precipitation during Protein A chromatography (Shukla et al., 2007).

Protein A is predominantly used a second step in purification to prevent fouling from cell culture media. However effective column regeneration schemes that can enable up to 100 cycles of runs with direct loads from cell culture supernatant has been developed (Shukla et al., 2007). This potentially eliminates the preconcentration or buffer exchange of feed stream.

2. 3. 3. 5. Non-Protein-A Capture and Polishing

Despite the hallmarks of protA chromatography, there are still tangible reasons to seek for alternative methods. Fragments from cleavage-proned protein A ligand are capable of coupling unto mAb leaving behind another purification hurdle. Moreover alkaline conditions which are cheap and frequently use to sanitize column are not applicable to protA

chromatography since the ligand is prohibited from alkaline exposure (Shukla et al., 2007). The use of concentrated chaotropes poses is costly and warrants proper disposal. Furthermore, low pH elution enhances aggregation and most crucially, cost of protein A ligand is overwhelmingly high. This has stimulated research into small ligands that can match protein A's capture efficiency (Shulka et al., 2007) or alternative non-protein A methods (Arunakumari et al., 2009, Bilgicer et al) .

Arunakumari et al compared three non-protein-A techniques. In the first process, buffer-exchanged clarified cell culture supernatant from ultrafiltration tangential flow filtration (TFF) which has been conditioned to permit mAb capture on CEX resin [Arunakumari et al., 2009] was used. AEX constituted the polishing step in a two-step mode since it clears the major process contaminants and also provide a mechanism for viral clearance. In spite the efficient capture step, this process is limited by the low loading capacity of AEX membrane resulting from upstream impurities such as HCP. This reduces the no of purification cycles performed.

The second process attempts to counteract the previous obstacle of HCP in feed stream reducing membrane loading capacity. The technique used is usually referred to in literature as " HCP exclusion technique" because it is based on the isolation of HCP by precipitation (or filtration) . The remnant is a less contaminated feed stream which required less downstream processing. Here an unnamed additive was add to clarified cell culture and pH adjusted to selectively precipitate HCPs (Arunakumari et al., 2009) w