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PURIFICATION OF THERAPEUTIC MONOCLONAL ANTIBODIES (mABs) – A REVIEW

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ABSTRACT

In this review, several conventional techniques have been described in order to purify monoclonal antibodies which include Bead-based chromatography, affinity chromatography with protein A to several alternative technologies including ion exchange chromatography, affinity chromatography and cation exchange chromatography. However, the demand of mABs in pharmaceutical industries is enhancing which requires high efficiency of the product which will render different polishing techniques to be encountered in such processes. These trends in advancement of the purification of mABs will lead to increased efficacy and fulfill the requirements of mABs in industry.

Key words: Monoclonal Antibodies, Therapeutic proteins, Ion exchange chromatography, Purification Techniques.

INTRODUCTION

One of the most prevalent groups of recombinant protein therapeutics that has been established is Monoclonal antibodies (mAbs). Monoclonal antibodies (mAbs) can be indicated at elevated ranges in the culture of cell, are generally extremely solvable and are comparably firm at time of processing (Zhou and Tressel, 2006). Additionally, by using a platform procedure, industries having equipment planned for single mAb manufacturing can be promptly modified for the production of others (Tao *et al.,* 2014). For the monoclonal antibodies (mAbs) process scale purification there is excess utilization of a range of methods that are of preparative chromatography. Protein A affinity chromatography which has the physicochemical constancy, the easiness and effortlessness of process improvement and have specificity for the antibodies fragment crystallizable (Fc) region because of these properties this method is utilized by many of organizations as a capture phase in purification (Luciani *et al.,* 2015).

There is quite variation between the procedure developed for the manufacturing of recombinant mAbs and the procedure utilized for the manufacture of immunoglobulin (IVIG) which intravenous derived from the plasma of human vary from those employed for recombinant mAbs. There is a handling framework for the purification of IV mAbs, which is based on many publically issued process flow sheets. (Siegel, 2005). Approximately newly prepared procedures are still in the association with the upstream stages which are based on the procedure of Cohon, contain chromatography, nevertheless this is utilized to a confined level (Lebing *et al.*, 2003).

MAbs recovery and harvest

Among the numerous methods which are biopharmaceutical tonic a prominent place is occupied by the monoclonal antibodies (Walsh, 2004). For the treatment of the broad scope of signs which are clinical an important figure of these mAbs is nowadays commercially available. Additionally, the mAbs are the important percentage of the drug nominees in the clinical development, expressing the significance of this healing method will keep on develop in the prospect (Reichert, 2008). The adaptability of mAbs, to either antagonize or agonize functions of them or by triggering the cascade of the complement, and for provoking their deterioration through cytotoxicity which is cell mediated antibody-depended (ADCC), in the effectively pointing a broad array of the cytokines and the receptors which are cellular causes the prevalent use of them (Zhang and Van Cott, 2007). When the production of hybridoma was done by merging the B-lymphocyte with eternal cell line of mammal, the style traces back to the early improvement of the mAbs by the Kohler and Milstein (Zhang and Van Cott, 2007). Even though the hybridomas are seldom employed for manufacturing of maAb which commercial, other cell culture organizations of mammals are broadly used, such as Chinese hamster ovary (CHO), SP 2/0, and NS0 cells. A rationale for this choice is intricacy and huge mAbs size, along with 2-heavy and the 2-light chains combined by non-covalent or covalent combinations for obtaining the renowned Y-shaped configuration. Another fact is that mAbs are sugar coated, and this glycosylation performs an important function in their biological activity (May and Pohlmeyer, 2011).

Technologies for sample Clarification

In the biopharmaceutical industry monoclonal antibodies (mAbs) are now known as the most notable and flourishing therapeutic proteins due to their positive result in the treatment of indications like autoimmune disorders

and cancer (Tran *et al.,* 2014). Biotechnology corporations are urging for significant process enhancements to increase yield and assure sustainability with the constant rise in bio-similar approaching to the marketplace and promptly increasing need for the proficient and cost-effective monoclonal antibodies analyses (Castro Forero *et al.,* 2015). Current progress of the development in cell culture, greater invention titers which are up to 10 g/L has been significantly generated, in comparison to the initial titers which are of 0.5–3 g/L. Although, due to this there is an elevated stage in the biomass of cell and course related contaminants like host cell protein (HCP) and the DNA, that imparts major load on the downstream clarification and on the stages of purification. Lowering the stages of these insoluble & soluble contaminants formerly in the procedure is thus a central demand for the effective refinement. Biomass removal from cell culture broths is a primary stage in restoration & in the target mAbs purification. This is commonly attained by the process of centrifugation, the depth filtration, microfiltration, & mixture of all these technics. While the process of centrifugation manages bulky capacities and allows prompt disposal of the items, it can also instigate greater shear pressure onto the cells, leading to destruction of cell and also undesirable insoluble discharge and solvable contaminants into the centrate. Additionally, in the elimination of the solid particles rise in biomass of the culture broths of cell also decreases the effectiveness of separators. In contrast, for making it appropriate for the clarification of mAbs the technique of microfiltration can functioned at small shear pace (Singh *et al.,* 2016).

Affinity chromatography based on protein-A strategies

Aimed at the process scale refinement of monoclonal antibodies (mAbs), a diversity of preparative chromatography methods have been utilized. Protein A affinity chromatography which has the physicochemical constancy, the easiness and effortlessness of process improvement and have specificity for the antibodies fragment of crystallization (Fc) region because these properties this method is utilized by many of organizations as a capture phase in purification. For the simplification of the whole downstream purification procedure huge aspect of purification helps a lot. Overall, after this process of unit there will be only the exclusion of trace rudiments left and in one or two succeeding chromatography phases this step of polishing can typically be accomplished (Shukla *et al.,* 2007). US Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA) approved 47 mAbs in the timely 2016, although there are more than 500 antibodies which are in progress encompassed by the global antibody pipeline. There is a petition of a number of hundred kilograms or more of product annually due to the naturally recurrent high doses involved in antibody therapy, which then, for some of the indications, united with the huge population of patients. Consequently, for the triumph of the industry there is a need for the development of cost-effective mAbs (Walsh, 2007). In fedbatch process of 12-day the level of expression 5–10 g/L have been achieved due to the recent advances in production and growth media, cell line assortment, feeding approaches, process controller, and process policy. Subsequently, the cell culture budgets are disturbed by the costs of purification of mAbs and the procedure holdups have progressed downstream. In a downstream procedure for mAb construction roughly one-quarter of the whole rate, is constituted only by the Protein A resin, of consumables (Bisschops and Biosystems, 2007).

Non-Protein strategies

The derivatives of the mAbs and mAbs themselves are the major profit producers in the worldwide market of biotechnology, with approximately more than 40 antibodies have been approved as the therapeutics already & strong nominee duct at various steps of clinical advancement consisting of antibodies greater than 350. Utilization of the podium technics has been extremely fruitful at time of development of procedures for the production of commercial scale antibodies, particularly employing Chinese hamster ovary (CHO) cells as system for expression and chief downstream procedure like affinity refinement. Methodologies, equipment, and the techniques for extensive mAb production have adapted to fit in equally present demands & forthcoming solicitations. Incorporation of skills in technical zones of expansion of cell line and manufacturing, molecular biology & the cell culture optimization has considerably improved crops of antibody. Conversely, the advantages resulted by such outstanding upstream advantages can be understood completely when downstream yield is correspondingly improved. Influence of the great-titer procedures incorporated by extremely effective refinement outlines become obvious not solitary regarding creation economics however also in terms of the entire approach of procedure and product development (Low *et al.,* 2007). These greater productivity procedures provide a chance to concurrently lower equally the period of marketplace & the products price for the recombinant mAbs. Unified procedure strategies with inclusive switch approaches can be also produce efficient the regulatory schemes. Thus, entire improvement of drug timeframe & the prices has been decreased considerably (Gottschalk, 2009).

Membrane and Filtration Technologies

- **Charged Ultrafiltration membranes**

The use of Ultrafiltration membranes has been done from the start of the development of industrial biotechnology and are used in combination with microfiltration for the production of monoclonal antibodies. The mAb process-scale decontamination is exceedingly lucrative, and corporations are giving great interest and devotion for the development of apparatus for the separation of surfaces and layers in conversant staffs which required desirable skills for enterprising, activating, or troubleshoot separation of sheath. It is possibly innocuous to approximately imagine separation of membranes will constantly be a giant fragment for good mAB good production (Etzel and Arunkumar, 2013).

The partition of proteins through charged UF membranes remained originally testified in a revolutionary journal, that exhibited the separation of cytochrome C and myoglobin is made possible by the addition of either negative or positive charge to ultrafiltration membrane via adjusting pH of buffer keeping it nearby the isoelectric point to one of those proteins, by giving it consent to permeate through the membrane, then in that way rebuffing similar charged proteins through repulsion caused by electrostatic forces present among. Therefore, writers established that it stood conceivable to distinct proteins with diverse pI values by means of charged UF membranes, uniform nevertheless the proteins remained closely to the identical extent (Etzel and Arunkumar, 2013).

- **Charged microfilter membranes**

Working of Charged microfilter (MF) membranes depends on the protein adsorption which has contrasting charge to ultrafilter membranes in which the charge works in the reduction of protein permeation into the membrane. Presentation of charge Microfiltration technique was done in 1988 for overawing the restrictions caused by column chromatography technique. The yield obtained from charged microfiltration is also referred to as chromatographic membranes, membrane adsorber and product of adsorptive membrane. Charged MF membrane yields are also acknowledged as membrane chromatography, membrane adsorber and adsorptive membrane, products. Immobilized ligand is present in all belongings of chromatographic media that consists of membranes having micropores. Pressure drop confinities are not substantial as the membranes are reedy when the membrane is utmost thin of ~100µm then pressure drop for the membrane is not limited. Transportation of solutes is done through convection but not by diffusion which consequently diminish the limitations of diffusion (Ghosh, 2002).

Industrial scale purification of mAbs

- **Affinity Purification Platform**

The physicochemical resemblances among mAbs, and the necessity to grow industrial procedures for a wide-ranging mAb-built irrefutable pipeline, have directed the key biopharmaceutical producers to approve a stage line to purification. Protein A affinity chromatography is highly discriminate for mAbs therefore, this notion was probably made. Protein A is characteristically the first phase owing to its capability to apprehend the product from the undefined harvested cell culture fluid (HCCF) with concentrations beyond 99%. (Shukla *et al.,* 2007).

- **Advances in the purification of mABs by CEX chromatography**

For developing marketing for cleansing of mAbs, the technique which is frequently used is CEX chromatography, attaining leached Protein A, the subtraction of HCP, product variants, DNA, and viruses (Harinarayan *et al.,* 2006). Commercially accessible cation exchangers may be isolated into low and high-volume resins, interpreting to their DBC directed at mAbs decontamination. Supreme DBCs remained in a variety of 50 g/L during scientifically appropriate load dwelling times which were designed at low-capacity pitches for example SP Sepharose. For high-capacity resins, fast Flow includes (SP FF) and POROS 50HS, kept at ~100 g/L such as Capto S, SP Sepharose XL (SP XL), and Fractogel SE Hicap. Low-capacity resins have widely been used for developing uses. These resins are also inclined to show higher purification narration.

- **Advances in CEX process development for mABs purification**

For industrial IEX process development the idea of midway grave conductivity for supreme DBC is of great importance. It may more be presumed that the chief volume will be achieved at a minimum pH and ionic asset. As with CEX course growth the revisions of DBC must have to done as purpose of residence time, conductivity, load pH, and to recognize the critical conductivity rate for a given illness. It must also have to be exposed that critical conductivity is a function of load residence time. Usually, the optimization of load pH and conductivity has been focused at an approachable short load residence time, to decrease the amount of exploration runs. The result of load residence time observed on DBC has been independently resoluted at a random conductivity and load pH. It is only adjacent to the inference of course improvement actions that the load flow rate is symptomatically accustomed

rendering to mass quantity rations and plant restraints. This refers to a suboptimal alignment of time, applied load residence time, conductivity, pH and load residence. Subsequently, one critical first catalogues the longest after mass quantity supplies and plant restraints, and then character progression advancement trainings at the following load flow rate, resulting in underutilization of CEX (Gottschalk, 2009).

- **High Performance Tangential Flow Filtration (HPTFF)**

The manufacture of mAb goods classically trails a stage procedure connecting preliminary detention by chromatography of Protein A affinity which is tracked by several phases of polishing (For example, ion exchange and HIC hydrophobic interaction chromatography), with concluding construction by ultrafiltration (UF) and diafiltration (DF) by means of tangential flow filtration (TFF)

- Diafiltration is being used to confiscate small molecular mass contaminations and accomplish the anticipated buffer for the framed drug merchandise although UF delivers the beleaguered mAb absorption for packing and distribution
- High concentration provisions (well above 100 g/L) are characteristically essential to transport the anticipated therapeutic quantity in a sole hypodermic injection.
- Profitable UFDF schemes for mAb dispensation can engage numerous hundred rectangular beats of film part to procedure a single invention batch.
- The cost-effective submission of huge TFF classifications stereotypically entails expansion of the suitable membrane scrubbing rules for lessening the cost allied with the membranes /components. In account, the practice of clean-in-place diminishes the employment obligatory fixing of TFF cartridges and reduces the risk of letdown owing to inappropriate installation (Baek *et al.,* 2018).
- To get the substantial for ultrafiltration researches, the yield cell values liquid was administered. The yield was softened from -20 °C at 4 °C and formerly carried to room temperature beforehand 0.2 μm filtration.
- Anion exchange chromatography was used to manage the material in house protocols. Tris buffer was maintained at pH 7 by keeping the concentration at 8.5 g/L 2L so that the final drug volume obtained could be (Thakur et al., 2020).

Rayat *et al.,* (2014) made a corresponding length of hydraulic for its interpretation of flow disturbance properties of network with variations of flow path so that effectively imitate compression and clip charges existing under full-scale processing. TFF arrangements which falling membrane zone used at 10 cm2. Stirred-well systems was in rummage-sale, wherever the purpose was the preservation of clear exterior of membrane, to investigate environmental properties on protein dispensation like salt and pH attention keeping film areas of 0.25 cm2 in multi-well dishes (Kazemi and Latulippe, 2014) or the membrane possessions of hole size and configuration explicated for transmission of certain proteins by means of 1.5 cm2 membrane discs (Kallberg *et al*., 2012).

Polishing Steps for purification of mAbs

After the completion of Protein A step, some process linked impurities still occur in trace amounts, these include DNA, leached Protein A, host cell protein (HCP), endotoxins, and even some additives of culture media (Flatman *et al.,* 2007). Additionally, some product associated impurities such as aggregates and degradation products of varying molecular weights are also present. The succeeding chromatographic processes are termed as polishing steps as they lower the contaminants to ascertain safety of the product (Gottschalk, 2009). A variety of chromatographic techniques can be employed for polishing of mAbs. An account of these is described here:

- **Ion Exchange Chromatography**

Ion Exchange Chromatography (IEX) is one of the most commonly used chromatographic processes used in the purification of various therapeutic proteins including mAbs. The principle of IEX is primarily based on the electrostatic interactions between proteins and oppositely charged functional groups in the resin (Kumar *et al.,* 2017). The relative difference in charges causes adsorption, leading to separation of existing impurities (Gottschalk, 2009). Ion exchange matrices bear either positive charge, in which case they are called anion exchanger; or a negative charge called cation exchanger (Gottschalk, 2009; Kumar *et al.,* 2017). Ion exchange chromatography is considered as a fairly selective technique and is usually very cost efficient. It has been found to be effective in lowering contaminants such as leached Protein A, HCP, charge variants, DNA, viral particles and aggregates of high molecular weight (Liu *et al.,* 2010).

Anion Exchange (AEX) Chromatography is one of the well-known and frequently used strategies in the polishing of mAbs. It utilizes a positively charged group (such as DEAE, TMAE and QAE) immobilized to a resin (Liu *et al.,* 2010). Some impurities associated with antibody manufacture such as HCP, endotoxins and DNA are generally negatively charged which causes their strong binding to anion exchange columns (Gottschalk, 2009).

Other than these, it has also shown to be a powerful resource in removal of virus (Curtis *et al.,* 2003). Depending on the type of suspected impurities, AEX can be operated in both flow- through as well as bind and elute mode (Liu *et al.,* 2010). Low conductivity and alkaline conditions (pH 8-8.2) are characteristic of AEX in flow through mode, which is extensively used and is designed with two to three unit operations (Weaver *et al.,* 2013). In case of bind and elute mode, the AEX column is firstly loaded with antibody product pool, followed by loading of target product. Elution is then carried out using a higher salt concentration either stepwise or as a linear gradient, leading to binding of bulk of impurities to the column (Liu *et al.,* 2010). However, a drawback of anion exchange chromatography is that it is found to be of little or no use in the removal of some major impurities such as leached Protein A and some dimers and exerts a burden onto the succeeding polishing steps (Gottschalk, 2009). Owing to this, membrane chromatography is emerging as an alternative to AEX chromatography over the past few years (Zhou and Tressel, 2005; Shukla *et al.,* 2017).

Cation Exchange (CEX) Chromatography is also one of the frequently employed chromatographic processes used for the purification of monoclonal antibodies of varying pH ranges spanning from neutral to basic (Liu *et al.,* 2010). A resin modified by negatively charged functional groups is used in CEX. These may include both strong (e.g., sulfoethyl) and weak acidic ligands (e.g., carboxyl group). CEX has been found effective in the clearance of major impurities such as HCP, HMW aggregates and leached Protein A (Gottschalk, 2009). In some instances, CEX has seen to be a more efficient in removal of leached Protein A in comparison to AEX because Protein A possesses an acidic nature and for this reason it may remain retained weakly, unlike antibodies, causing its subsequent removal in the flow through process or via an intermediate pH wash (Gottschalk, 2009). CEX is deemed an ideal technique for polishing of most humanized IgG1 and IgG2 subclasses. During the loading step, antibody is bound to the resin, after which, increasing conductivity or increasing pH of the elution buffer results in easy elution (Liu *et al.,* 2010). CEX ensures efficient removal of almost all process-related impurities in the load and wash fraction. In addition, it also provides separation power for reduction in antibody variants from the target product like oxidized and high molecular weight species. The conductivity and pH are the two major factors that capacitate the mAbs to bind so strongly with the cation exchange resins (Liu *et al.,* 2010). Multiple factors like removal of other impurities, product pool and binding capacity are taken into account for finally selecting a strong or weak cation exchanger. Usually, if anion exchange chromatography is to be followed by CEX then a smaller pool volume having lower conductivity and greater product concentration is preferred (Liu *et al.,* 2010). Lately, due to the increasing concentration of aggregates, the burden on polishing during downstream processing of monoclonal antibodies in increased that is driving innovations into existing polishing approaches. In a recently conducted study, polymer grafted cation exchanger was used for the polishing of mAbs and results obtained showed it to be an efficient prototype (Akerblom *et al.,* 2014). In another study, CEX with pH conductivity using the hybrid elution method was observed to be a powerful technique for the separation of mAb1 monomer as well as its aggregates (Xu *et al.,* 2012).

- **Hydrophobic Interaction Chromatography (HIC)**

Hydrophobic Interaction Chromatography (HIC) is a powerful technique that separates proteins on the basis of their hydrophobicity and is being utilized from 1973 onwards (Tonini *et al.,* 2013). The principle of HIC is based upon the interaction between hydrophobic patches on the protein and the hydrophobic ligands which may be either aliphatic or aromatic (Gottschalk, 2009). HIC is frequently used as intermediate purification step after Protein A chromatography. However, it is applied as a polishing step when removal of HCP and HMW aggregates are the main aim (Gottschalk, 2009). Being orthogonal to ion exchange chromatography, HIC proves to be successful in removing HCP that remain present even after IEX. The procedure of HIC involves loading sample on the HIC column with a buffer having high salt concentration. This salt interacts with water molecules leading to lowering of solvation energy of the protein in solution, which causes their hydrophobic patches to be exposed and eventually their binding to the HIC resin. Higher the hydrophobicity of the protein, lesser the salt is required for encouraging binding. Elution of samples is carried out using a gradient of decreasing salt concentrations. The exposure of the hydrophilic regions with the decreasing ionic strength decreases, which leads to elution of molecules in ascending order of hydrophobicity (Porath, 1986). A study was conducted where HIC was tested for polishing of antibodies under no salt conditions. Required selectivity was attained by opting for very hydrophobic stationary phase resin and adjusting pH to an optimum for the mobile phase. Interestingly, the results showed a similar performance as is in the case of traditional high salt utilizing HIC (Ghose *et al.,* 2013). Efficient lowering of HMW aggregates is possible with HIC as the aggregates have a hydrophobic nature and can retain well on HIC resin (Liu *et al.,* 2010). However, HIC is not an ideal approach for removing some major impurities such as DNA and leached Protein A and, as the former may not adsorb onto the resin in presence of high salt conditions that are used in HIC (Gottschalk, 2009). Depending on the kind of impurities that are to be eradicated, HIC can be operated in either flow

through or bind. In general, flow through mode is preferable as low loading capacities exist in bind and elute mode (Tonini *et al.,* 2013).

- **Multi modal chromatography (MMC)**

As it is evident from the name, Multi modal chromatography (MMC) also known as mixed mode chromatography is a technique that exploits and integrates multiple interaction mechanisms in a combination to achieve separation (Gottschalk, 2009). It primarily involves interplay of various mechanisms simultaneously, such as CEX and AEX and IEX and hydrophobic interactions.

As a mix of interactions is used here MMC can provide a distinctive selectivity in the manufacturing process. Capto Adhere and Capto MMC (by GE Healthcare) are two commercial resins having multimodal ligands and are found useful for polishing during antibody purification. These resins mix mechanisms of CEX with hydrophobic moieties laid on an agrose backbone, allowing loading onto IEX chromatography with elevated salt concentration, leading to simplification of process by removing the need for diafiltration or dilution (Gottschalk, 2009). Successful removal of impurities like leached Protein A and HMW aggregates has been achieved using MMC. If IEX is the main mechanism then procedure for development of resins for MMC is similar to CEX and AEX (Liu *et al.,* 2010). It is important to note that due to secondary hydrophobic interactions the operation of MMC resins with high salt concentrations in bind and elute mode, might not prove efficacious for elution of protein. Complete recovery must be aided by addition of chaotropic reagents and modulating the pH (Yang *et al.,* 2007). The results of a study aimed to test MMC for purification of mAbs showed that yield of the process was 88% and the final purity of the mAb was 99.9%, values which are comparable to those obtained from a traditional affinity process. Significant reduction in level of impurities was observed to levels that fulfill the purity standards set in place for therapeutic products (Maria et al., 2015). Further, a two-step non- affinity platform using void-exclusion AEX for purification and electropositive MMC aided by chromatin removal for polishing revealed promising results with recovery rate of mAbs around 90.4% showing that it is a useful alternative to conventional protein A chromatography (Liu *et al.,* 2019).

Other Chromatographic processes

The aforementioned chromatographic techniques are the most frequently used approaches in the polishing of mAbs. However, a few other chromatographic processes are also employed successfully, although less frequently. Ceramic hydroxyapatite (CHT) chromatography is one of such techniques. Separation and purification of proteins, enzymes, nucleic acids, viruses etc. can be carried out with Ceramic hydroxyapatite $(Ca_5(PO_4)_3OH)_2$ which is a form of calcium phosphate (Liu *et al.,* 2010). This process is known to have a unique selectivity and separates proteins that appear to be homogeneous by other electrophoretic and chromatographic techniques. CHT chromatography with a sodium phosphate gradient elution has been applied as a powerful resource for polishing of mAbs to get rid of leached Protein A, aggregates and dimers (Gagnon *et al.,* 2006). In another study human antibody IgG4 was polished by CHT chromatography using sodium chloride linear gradient for elution (Franklin, 2003). Some considerations associated with the use of CHT chromatography include resin life, viral clearance potential and resin lot-to-lot variability (Liu *et al.,* 2010). Another such technique is dye ligand affinity chromatography which has also been used for the purification of monoclonal antibodies from cell culture. The specific resin for this process called Cibacron Blue resin comprises a synthetic polycyclic dye ligand which enables it to bind to a wide array of proteins by various interactions such as hydrogen bonding, hydrophobic and ionic interaction, and in some instances by bio-affinity (Gottschalk, 2009). Thyroid stimulating hormone (TSH) and albumin commercial manufacturing processes have already utilized this technique which is now emerging as a possible mAb polishing step. The same resin has been used for polishing step of monoclonal IgG4 and proved to have great loading capacity as well as efficient in eradication of impurities. Further, numerous Protein A mimetic ligands based on triazine dye chemistry have been developed and many are still in the pipeline, which could function as possible polishing approaches (Food and Drug Authority, 1995).

Virus Filtration

The manufacturing of monoclonal antibodies and other therapeutic proteins requires mammalian cells which often get infected with adventitious viruses during the course of production (Liu *et al.,* 2010). In addition, the cells may also produce some endogenous retroviruses which make virus filtration an essential step in the manufacturing of mAbs (Gottschalk, 2009). In general, orthogonal mechanisms for virus clearance are utilized which are complementary to one another in order to ensure that a virus not cleared by one mechanism gets cleared by the next (Food and Drug Authority, 1995). Filtration allows viral clearance based on the size of viruses via passage through a membrane containing a small sized pore. As virus leakage is allowed only via a few abnormally large pores, the

manufacture of virus filters should ensure design for removing these faulty pores (van Reis and Zydney, 2007). The use of composite membranes that offer both virus retention and mechanical stability simultaneously is preferable to achieve this (van Reis and Zydney, 2007; Liu *et al.,* 2010). Two type of virus filters are available which are differentiated on the basis of their pore size. These include retroviral filters with pore size<50 nm and parvoviral filters with pore size <20 nm (Gottschalk, 2009). These filters are made of hydrophilic polyethersulfone (PES), regenerated cellulose and hydrophilic polyvinylidene (PVDF) (Liu *et al.,* 2010; Junter and Lebrun, 2017). Lately, parvoviral filters are becoming the preferable industrial choice as they have found to meet the regulatory compliances set in place. However, larger surface area is required by parvoviral filters as they may clog easily even when the process stream consists of relatively lower amounts of aggregates (Liu *et al.,* 2010). In general, fouling of virus filters usually occurs due to presence of debris such as DNA and protein dimers. This can be taken care of using prefilters (Bolton *et al.,* 2006). Additionally, prefiltration of the feed solution can drastically influence virus filtration performance. Impurities with a larger size can be easily removed using 0.1–0.2 µm microfilters, but those that are only slightly larger than the protein may not allow convenient removal through size-based methods (Bolton *et al.,* 2006). Prefiltration using charged membranes adsorptive depth filters and prove to be successful in safeguarding the virus filter. Pre- and post- use integrity testing of virus filters is done as a routinely exercise in order to attain desired performance level (Kuriyel and Zydney, 2000). Followed by Protein A chromatography virus filtration may be the second most expensive step in a typical set up for purification of therapeutic monoclonal antibodies. Therefore, optimization of this step is a major concern in the biopharmaceutical industry (Gottschalk, 2009).

Conclusion

Monoclonal antibodies (mAbs) are considered a prime product of biotechnology and they are widely recognized as a major therapeutic modality. Currently, the purification of mAbs is dominated by Protein A chromatography. However, the global market for mAbs has been increasing over the years, and their demand is greater in comparison to many therapeutic proteins. These factors present a challenge in the industrial manufacture of mAbs to produce high quality product through utilizing innovative low-cost approaches. Platform processes have been under examination in order to investigate the potential alternatives in purification and polishing steps of mAbs for cost cutting of the downstream processing. In future, more robust combination of techniques must be explored. In this regard, an interesting approach is to link the purification and polishing to produce one or two step simple purification procedures.

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