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A Review on Downstream Processing of Monoclonal Antibodies (mAbs)

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Abstract: ***The creation of therapeutic monoclonal antibodies has increased recently. mAbs have grown significantly in relevance, and these compounds are now more crucial than ever in the global biotechnological drug industry. For the treatment of various diseases, a lot of biotechnology companies are investing in the manufacture of monoclonal antibodies. The two main processes in the synthesis of these antibodies are bioreactor production and purification. This article goes into great detail on the current purification techniques utilized for these compounds. The fundamental monoclonal antibody recovery and purification procedures, including cell harvesting, Protein A affinity chromatography, and further polishing steps, are all enumerated.***
Keywords: Biotechnology, downstream processing, monoclonal antibodies, purification, chromatography.

I. INTRODUCTION

There are hundreds of monoclonal antibodies (mAbs) either in use or being developed. Due to the popularity of mAbs as therapeutics, several businesses have many antibodies. Today, monoclonal antibodies are recognized as a critical treatment approach for a variety of disorders. Companies have steadily increased the total number of mAbs under clinical development throughout time. An appealing strategy for solving the diseases is to use monoclonal antibodies (mAbs), which may be made to target cells only when they are specifically desired and cause a wide range of reactions when bound. These substances have the ability to either directly kill cells by delivering poisonous substances to the target or to orchestrate cell death in other ways, such as by activating immune system components, inhibiting receptors, or scavenging growth factors. [1] The most common category of recombinant protein treatments is established to be monoclonal antibodies (mAbs). They are often very soluble, exhibit high levels of expression in cell culture, and are comparatively stable after processing. It is generally known that mammalian cell cultures can produce antibodies. Currently, mammalian cells make up around 70% of recombinant therapeutic proteins, with Chinese hamster ovary (CHO) cells being the most common expression host. In addition to purity and process capacity, downstream process development places a strong emphasis on yield and productivity [2].The most important step in the development of biopharmaceuticals is the efficient recovery and purification of mAbs from the cell culture medium. Product stability is one of the most crucial characteristics that must be preserved throughout the process. The highest priority should be given to maintaining product quality and boosting purity while overcoming multiple obstacles. To minimize material loss and to reduce the potential of contamination, each step of downstream processing should be handled carefully. [3] Based on a typical flow of unit operations, numerous organizations have defined platform purification methods

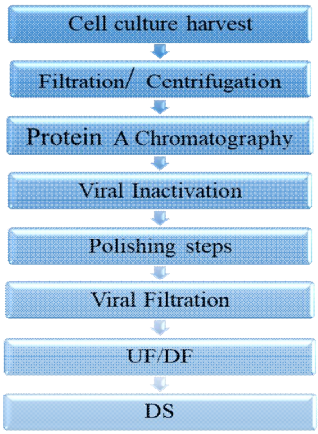


Figure 1. Process flow for mAb purification

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Figure displays an example of a popular mAb purification platform. Removing the majority of the cell culture's insoluble components from the product stream is the first step in recovering secreted antibodies. Whole cells, cell fragments, colloids, and other kinds of contaminants make up these components. Harvests from bioreactors are usually clarified by centrifugation and/or filtering through a number of depth filters Utilizing continuous disc-stack centrifugation along with depth filtering is one approach that the industry prefers to use for completing this initial separation. These initial recovery methods are designed to eliminate the majority of particles from cell broth in order to reduce the workload for the following purification steps. [4][5]Due to its great resolution, chromatography is a crucial and often used separation and purification method in the biopharmaceutical sector. Chromatography separates biomolecules by making use of their physical and chemical distinctions. After that, a succession of chromatography procedures, beginning with the capture stage, are applied to the clarified crop. Capture chromatography, a technique that involves binding and eluting, guarantees both a decrease in harvest volume and the safety of the final product by eliminating the majority of contaminants, including potentially harmful proteases [6] Protein A chromatography enables direct product capture from the centrate and delivers excellent product purification and a sizable product concentration. Additionally, virus inactivation is achieved by the low-pH elution from the Protein A step. To decrease contaminants coming from host cells/medium and those connected to the purification process, two chromatographic polishing processes are performed. These polishing stages frequently result in additional virus elimination. Anion-exchange (AEX) chromatography, frequently in flow-through mode, is one of the polishing processes virtually always used. Though occasionally ceramic hydroxyapatite chromatography and hydrophobic interaction chromatography (HIC) are utilized, cation-exchange (CEX) chromatography is commonly used as the another polishing stage. The result, which is now the bulk drug material, must go through two more steps of the manufacturing process: virus removal filtration (VRF) and ultrafiltration/diafiltration (UF/DF). [7] [8]. It might only be necessary to perform one additional phase of chromatography polishing if the affinity pool is relatively pure. Some platforms just employ the two chromatography processes[9]. The nature of the finished product and any contaminants that are process-related will dictate the number and kind of polishing processes. Protein A pools are typically purer than non-affinity pools. The selection of an affinity or non-affinity procedure, or the necessity of extra polishing stages, may be influenced by cell culture circumstances that promote expression while also producing higher quantities of aggregate or charge variations.[10] Processes may be made simpler, expenses could be cut, and processing big batches might be made easier with alternatives to chromatography. Adsorptive membranes are currently less capable of binding impurities than resins, which makes them best suited for flow-through chromatographic procedures where contaminants are bound in relatively small amounts. As an alternative to resin column chromatography, membrane adsorbers could take the place of an IEX polishing step and provide smaller pools and less buffer use. [11] One of the major obstacles encountered during the development of a protein purification process is low feed concentration. Other significant obstacles include complicated and poorly defined raw and feed ingredients and product instability. [12] HTS techniques are primarily used in the downstream process development to screen filters and resins and optimise process parameters like binding and elution conditions (pH and conductivity) for chromatographic steps and the best membrane type, transmembrane pressure, and cross flow rates for membrane filtration unit operations. [13]

II. CENTRIFUGATION/DEPTH FILTRATION

In recent years, the predominant set of unit operations used for the primary recovery of therapeutic proteins has been centrifugation combined with depth filtration. [14] Harvest characteristics such as centrifuge feed rate, G-force, bowl shape, operating pressures, discharge frequency, and auxiliary equipment utilized in transferring cell culture fluid to the centrifuge have an impact on the centrifugation process' ability to clarify materials. Performance of separations will also depend on the features of the cell culture process, such as peak cell density, total cell density, and culture viability during the culture phase and at harvest. [15] [16]

Cell culture broth is supplied into a rotating bowl during disc-stack centrifugation, and centrifugal force causes particles to separate in a confined channel between the discs. The separated solids are discharged on a regular basis from a solid holding area that slides along the underside of the discs. The protein-containing clarified liquid keeps moving up the disc stack and out of the bowl. If the centrifuge conditions do not result in shear-induced cell destruction, this approach works incredibly effectively for extracting entire mammalian cells. [17]

K. Jin et al. have used a quick and easy spectrophotometric technique to measure the amounts of recombinant inclusion bodies in the presence of Escherichia coli cell debris to evaluate the operation of an industrial disc stack centrifuge.[18] R. Kempken et al. used a prototype disc stack centrifuge to remove animal cells from 80-L and 2000-L fed-batch fermentations in order to quickly and accurately separate solids from vast amounts of cell culture fluids. Animal cells were very well clarified, however particles persisted in the liquid phase and interfered with the cell-free harvest fluid's ability to be processed further down the line.

No appreciable product loss was noticed. In order to improve the procedure conditions for use with animal cells, a variety of parameters were tracked. [19].It is important to note that earlier research by Tebbe et al. also demonstrated the important impact of equipment design on the separation performance of a disk stack centrifuge. Specifically, Tebbe et al. demonstrated that the use of a CSA-1 centrifuge equipped with a hydrohermetic entry zone, a feature which significantly reduces the shear forces in the inlet to the centrifuge by removing the air/liquid interface, results in minimal cell lysis. [20]

Further clarification by depth filtering is often employed to remove smaller solid particulates that still remain in the centrifuge product since there is a limit to the particle size that can be removed by a disc-stack centrifuge. Following a depth filter, a filter with an absolute pore size rating (usually 0.45 µm or 0.2 µm) is used to assure the removal of solid particles (and bacteria in the case of the 0.2 µm filter) from the cell culture harvest supernatant [17]. Inorganic filter aids are attached to a thick, porous matrix of cellulose fibres in commonly used depth filters by a positively charged resin. The positive charge endows the filter with adsorptive capabilities, while the thick matrix offers a winding path to retain a variety of particle sizes. [21] [22] [23].When the particle load of our liquid phase was significantly larger than it was in a filtrate of 0.2 µm tangential flow filtration (TFF) procedures, the capacity of the majority of depth filters—which have huge dead volumes—was quickly depleted. It was discovered that the latter fluid could benefit from additional downstream processing. [18] [24] Depth filters have been used for the removal of endotoxin from water and DNA from cell culture supernatant The binding capacities of depth filters for DNA and CHO proteins (CHOP) have also been measured directly using cell culture fluid post microfiltration clarification [25]

III. PRO A CHROMATOGRAPHY

A native or recombinant proteic ligand derived from Staphyloccocus aureus or Escherichia coli, respectively, is combined with a natural or artificial base matrix in Protein A affinity chromatography. Five homologous domains of Protein A E, D, A, B, and C can bind to the Fc moiety of immunoglobulin G. (IgG). Because of its strong binding affinity and the high levels of purity that may be attained, Protein A affinity chromatography is a well-known procedure in the pharmaceutical sector. The target antibodies are loaded onto the immobilized Protein A support at neutral pH as part of the purification process, facilitating the interaction of the ligands. Following the separation of the protein from contaminants like host cell proteins (HCP), the mobile phase's pH is lowered to facilitate the product's desorption. The resin is then regenerated and put through a clean-in-place technique. [26] [27]

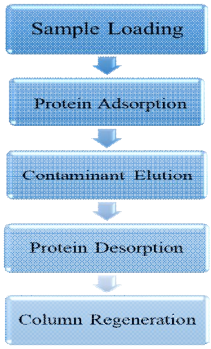


Figure 2. Process flow for protein A chromatography

Protein A chromatography is the most expensive stage, making it a suitable place to start. The monoclonal antibody's interaction with immobilized Protein A is the foundation of this affinity chromatography technique. Hydrophobic interactions are predominantly responsible for the binding but ionic and hydrogen bonds also play a role. Depending on the antibody subclass, the pH range of 6–9 is where the antibody binds to Protein A, and the salt content of the binding buffer can affect this. A pH buffer with a low range between 2 and 4.5 is chosen to elute the binding. Since the antibody elution occurs at a low pH level, this method is also utilized to inactivate viruses. [28] [29] [30] [31]Protein A purification is focused and effective. However, there are still a number of problems that need to be resolved, such as the inadequate removal of contaminants including host cell proteins, DNA, aggregates, etc. Moreover, the impact of wash buffers on protein. The physicochemical properties of antibodies are still not well understood after purification. A new monoclonal antibody purification method that enhances the physicochemical characteristics of the antibodies by simply adding a basic buffer wash step following the traditional protein A purification's capture stage .[32] [33]

In comparison to packed columns, Protein A membrane adsorbers and monoliths are more productive because of their low bed heights and high operational flow rates. These gadgets might serve as a model for future increases in protein A productivity even though they are not now practicable for large-scale production. [34][35][36]. One of the long-recognized difficulties with the Protein A ligand has been its inadequate stability under the very alkaline conditions that are frequently utilised in chromatography column clean-in-place (CIP) procedures [37] . As a result, older generations of Protein A chromatography resin were frequently processed using high doses of chaotropic chemicals such urea or guanidine hydrochloride, occasionally at an acidic pH [38] [39] More recently, resin producers have tried to modify the Protein A ligand such that it is more alkaline tolerant, leading to the introduction of products like MabSelect SuRe [40] Julia Scheffel et.al discovered that selective protein A resin permits the moderate elution of antibodies by sodium chloride. Aggregates, which made up to to 34% of all eluted antibodies from MabSelect SuRe at pH 3, were prevented from forming by the mild elution of an IgG4 antibody. [41]

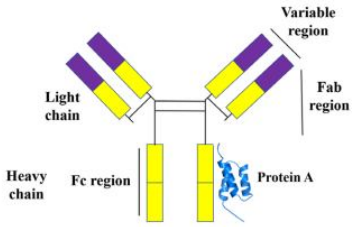


Figure 3. Antibody and Protein A interaction [42]

IV. VIRAL INACTIVATION

A vital element in ensuring the security of biotechnology goods is the demonstration of viral clearance. A low pH viral inactivation step is usually included between the Protein A capture and the subsequent chromatographic polishing steps in a conventional mAb manufacturing process. This procedure serves to inactivate any possible enclosed viruses (such as retroviruses), and it makes a substantial orthogonal contribution to the assurance of viral safety in biological products. Christopher Gillespie and colleagues describe a continuous in-line viral inactivation process that overcomes many of the bottlenecks present in the conventional batch viral inactivation process. [43]Protein Since the majority of mAbs are stable in solution at low pH levels and a column eluate is at this pH level, it is quite simple to incorporate a low pH incubation step to inactivate viruses. For a number of biotechnology products, it has been demonstrated that low pH treatment effectively inactivates retroviruses. More than 4 log10 of large enveloped viruses, including endogenous retrovirus, have been consistently cleared by some commercial purification modules, incorporating nanofiltration and low-pH inactivation. When the protein content of any of the four model proteins is greater than 40 mg/mL and the NaCl concentration is greater than 500 mM, Kurt Brorson et al. discovered that a 30-min incubation of X-MLV at pH 3.8 in either citrate or acetate buffer results in robust inactivation ($\geq$ 4.6 log10).[44] In their study of the mAb aggregation during low pH viral inactivation and subsequent neutralisation, Ruben Wälchli et al. discovered that limiting denaturation at low pH by adding d-sorbitol or lowering the temperature had a significant positive impact on monomer recovery. [45]

Proteins can be denatured by solvents and detergents, and they can be exposed to low concentrations of these substances for brief periods of time without suffering severe, irreversible changes to their structure or function. Horowitz and coworkers used this observation as justification for creating a virus inactivation procedure that involves the use of both a solvent and a detergent.[46] Yi-Chin Fan et.al studied the antigenic structure of the JEV (Japanese encephalitis virus) E protein can be maintained during virus inactivation by H2O2, but not by UV or by short-duration, higher temperature formalin treatment. Therefore, a different inactivation technique, such H2O2, that can preserve the integrity of the E protein may be necessary to increase the effectiveness of inactivated JEV vaccines.[47]. Peter L. Roberts employed a solvent/detergent step based on 1% triton X-100 rapidly inactivates a variety of enveloped viruses by >6 log inactivation within 1 minute of a 60 minute treatment duration [48]According to research by Maria del Rosario et al., 20% ethanol inactivated 3.7 Logs of the enveloped viruses HSV-1 and HIV-1 but was unable to inactivate the nonenveloped viruses HPV-1 and CPV. However, 0.1N HCl is a powerful chemical agent capable of inactivating >6.13 Logs of high resistance nonenveloped viruses[49]. It has long been recognised that the best way to create these plasma derivatives in a noninfectious form is to inactivate hepatitis viruses in preparations of human serum albumin and stable plasma protein solutions by pasteurisation at 60 C for 10 hr. The relative effectiveness of this approach and its practical applicability depend on the employment of plasma fractionation techniques that eliminate the majority of infectiousness beforehand, it is now evident. Shikata and co.[50]

Numerous attempts to adapt batch low-pH hold to continuous processing have been made as interest in the conversion of biopharmaceutical manufacturing from batch to continuous operation has increased.

A low-cost, column-based continuous viral inactivation was described by Hong M. S. et al. Their study offers resources for continuous viral inactivation system design and operation in support of raising output, upgrading product quality, and boosting patient safety [51]In both continuous and batch modes, the inactivation of the xMuLV virus was examined by Laura David et al. The idea for a virus study for the ongoing viral inactivation at low pH in a CFI was created and successfully tested[52] When traditional methods of virus inactivation, such as low pH and/or solvent/detergent treatments, are either impractical or undesirable due to protein stability restrictions, Justin T. McCue et al. examined the use of pH-neutral arginine-containing buffer solutions as an alternate method. [53]

V. ION EXCHANGE CHROMATOGRAPHY

When the majority of an ion exchanger's functional groups are charged, it is typically used in those situations. At pH ≤ pKa, weak anion exchangers are typically utilized, whereas at pH ≥ pKa, weak cation exchangers. Strong ion exchangers have pKa values outside of the pH range at which it is typical to interact with proteins, therefore pH variations won't affect the ion exchanger's charge. [54] The protein's pI value is used in ion-exchange chromatography to achieve separation. A positively charged protein is drawn to a negatively charged solid support in cation-exchange chromatography, whereas a negatively charged protein is drawn to a positively charged support in anion-exchange chromatography. Elution is often accomplished by changing the buffer's salt or pH content in a stepwise or gradient fashion. Ion-exchange is a quick and affordable approach for purifying antibodies, and it's frequently employed as a polishing step after Protein-A affinity chromatography.[3][55]. For the purification of monoclonal antibodies, cation exchange chromatography is commonly used in bind-and-elute mode. However, it was discovered that bind-and-elute conditions were insufficient for eliminating appreciable amounts of antibody aggregate. The desired product flows through a stationary phase and is subsequently retained by a medium in positive purification, also known as the retention mode. Protein A affinity and cation exchange chromatography is employed in retention mode in a broad platform mAb purification procedure. On the other hand, the flow-through mode is frequently utilized with anion exchange chromatography. Given that the flow is continuous, the operation technique known as flow-through chromatography (FTC) is regarded as an effective way to separate two components. The desired product is flowed through a medium in the negative purification process, also known as the flow-through mode, while contaminants are trapped by a stationary phase. Target proteins are removed from the chromatography column in FTC without being adsorbed, whereas contaminants are tightly attached. In contrast, to bind and elute, higher column loadings are often attainable in the flow-through mode. [56] [57] [58], [59] ,[60] If applicable, purification using the flow-through mode provides many benefits over positive purification in terms of the quantity and size of buffers needed, workload, and processing time. [61] When overexpressed proteins are being purified, tiny impurities with different net surface charges from the protein of interest are frequently simple to separate. On the other hand, two proteins that have very similar net surface charges may elute together. In this situation, size exclusion chromatography or some alternate technique may be used to separate the proteins. [62] The particles must be mechanically robust for chromatography at high flow velocities. Fast mass transfer is made possible by large pores, but the available surface area and resulting equilibrium capacity are reduced.Chromatography matrices have been created using a range of materials. Polysaccharides (such as cellulose, dextran, and agarose), synthetic organic polymers (such as polyacrylamide, polymethacrylate, and polystyrene), and inorganic substances are the most prevalent types (silica, hydroxyapatite). The materials are chemically cross-linked and given a functional ligand to create a mechanically stable and useful matrix.A hydrophilic layer is coated with ion exchange groups on a mechanically robust support, like a polystyrene bead, to promote crosslinking, or a hydrophilic layer is placed on top of a support and crosslinked more.[63]

Jan Schwellenbach et.al investigated fiber-based cation-exchange stationary phase for the purification of monoclonal antibodies. Convection, axial dispersion, boundary layer mass-transfer, and the salt-dependent binding behaviour in the fibre bed were all relevant mass-transport phenomena during a bind and elute chromatographic cycle that were detailed. The combination of model adaptation, simulation, and experimental parameter determination using distinct measurements, correlations, or geometric factors that are not dependent on the chromatographic cycle is highlighted in the work. [64] whereas monoclonal antibody capture and viral clearance by cation exchange chromatography were examined by G.R. Miesegaes et al.[65]

VI. MULTIMODAL CHROMATOGRAPHY

Multimodal or mixed-mode chromatographic media (MMC) that demonstrate several binding interactions are more tasteful substitutes for a sequence of processes that each involve a single interaction. MMC is a chromatographic technique that utilizes many sorts of interactions between the stationary phase and the mobile phase, where various solutes are present. [66] In multimodal or mixed mode chromatography, the chromatography ligand interacts selectively with the analyte molecule through a variety of interactions. These contacts can be ionic hydrophobic, hydrogen bonding-based, or even Vander Waals interactions.

In the development of mixed-mode chromatography, ligands are crucial. The adsorbent can offer salt tolerant qualities, improved separation, and high binding capabilities with which a wide range of medicines can be purified if the hydrophobic and ionic moieties in the mixed mode ligand are balanced properly. Electrostatic repulsion between the analyte and resin ligand can result in selective elution in the case of hydrophobic charge induction chromatography with the choice of an adequate pKa value. [55] [67]. MMC has selectivities and specificities that are distinct from those of conventional ligands, giving it a versatility that makes it possible to address a variety of difficult purification issues. [68] [69].The efficiency of arginine as an eluent in multimodal chromatography systems has been reported, and molecular dynamics (MD) simulations have been used to examine its interactions with the ligands for Capto MMC (N-benzoylhomocysteine), 4-MEP (4-mercaptoethylpyridine), and Capto Adhere (N-benzyl-N-methyl ethanolamine) [70] [71] [72]. A mechanistic knowledge of the impact of the cosolvents on protein retention is crucial for the improvement of multimodal chromatography methods combining arginine and guanidine. In order to determine the binding areas of these modifiers on the three proteins in this study (ubiquitin, horse cytochrome C, and -chymotrypsinogen A), MD simulations are run with each cosolvent independently. The electrostatic and hydrophobic areas on the surfaces of the proteins are then characterised for a larger group of proteins using quicker, coarser-grained methods. The density profiles of multimodal cation-exchange ligands (Capto MMC and Nuvia cPrime) around proteins are calculated in the work using a spherical harmonics technique. Both the Capto MMC and Nuvia cPrime ligands have a carboxylate, a benzene, and an amide group; the Capto MMC ligand also has an additional aliphatic group, whilst the Nuvia c Prime ligand has an additional amine group. These ligands were discovered to bind with hydrophobic and positive protein areas. A mechanism is put forth to explain the effects of arginine and guanidine in multimodal cation exchange chromatography using these findings from the prior work, in addition to MD simulations of protein-cosolvent systems, protein surface characterization tools, and the observed trends in protein retention behavior. [73] [74] [75]. humanised monoclonal antibodies (mAbs) were chromatographed using ceramic hydroxyapatite (cHA) by David L. Wensel et al. [76]. Joshua Osuofa et.al studied High-capacity multimodal anion-exchange membranes using Purexa™-MQ for polishing of therapeutic proteins [77].A hydrophobic ligand with cation-exchange capability was included in a mixed-mode chromatographic resin, which Leslie S. Wolfe and colleagues explored to characterise protein interactions with. [78] In order to eliminate heterogeneous aggregates, Rebecca A. Chmielowski et al. compared the effectiveness of multimodal chromatography with that of traditional cation exchange or hydrophobic chromatography [79].

VII. VIRUS FILTRATION

Through the use of genetically modified cell lines, tainted raw materials (such as serum), operator interaction, process gases, and other means, viruses can be introduced.[80] The production of monoclonal antibodies (MAbs) made from mammalian cell lines involves a number of complex steps, one of which is the control of viral contamination. Endogenous retroviruses are produced by mammalian cells utilised in the production of therapeutic recombinant proteins like mAbs, and they can also get accidentally infected with viruses throughout processing [81] [82] .The log reduction value (LRV), which is the logarithm (base 10) of the ratio of the microbiological concentration before and after processing, is used to describe clearance rate. [80] In addition to other phases in the virus clearance process, virus filtration can offer a size-based viral clearance mechanism. Viral filters must be created to eliminate these huge porous defects since the presence of even a few unusually large pores will allow for substantial virus leakage. [83]For the purpose of eliminating viruses from serum-free cell culture media, Shengjiang Liu et al. designed a unique virus barrier filter. The 20 nm mouse minute virus is eliminated by this filter by >3 log reduction value (LRV), the 28 nm bacteriophage is eliminated by >4.5 LRV, the mycoplasma Acholeplasma laidlawii is eliminated by ≥ 8.8 LRV, and the bacteria Brevundimonas diminuta is eliminated by ≥ 9.2 LRV.[84] Since the filter is developed to achieve extremely high levels of removal of possible virus contamination, viral filtration differs from conventional pressure-driven membrane filtration procedures. Due to the fact that normal flow (dead end) mode is less difficult and only needs a single pump, virus filters are often operated in this mode as opposed to the tangential flow mode utilised for protein ultrafiltration.[85] High permeability, no reduction in protein production, and consistent performance across various media lots, device lots, and device scales are all characteristics of the Viresolve™ Prefilter. Filter capacity can be reduced by plugging brought on by the trapping of protein aggregates, denatured proteins, and other contaminants. In the current investigation by Glen R. Bolton et.al, different prefilters were examined to see if they could get rid of the species that clog Viresolve™ NFP (normal flow parvovirus) filters. [86] Via the use of model feedstreams and both commercial process fluids, Glen Bolton et al. examined the bacteriophage ΦX-174 through normal-flow virus filters. The focus of the research was on normal-flow viral filters and the phenomena of virus breakthrough, which is seen when particular operating parameters are met.

Virus filter membranes are thought to have a specified porosity shape that functions as a sieving barrier, trapping big virus particles (20-100 nm) while allowing protein molecules (10 nm) to flow through. [87]

In other cases, viral filtration membranes are built with a reverse asymmetric structure to filter out contaminants and foulants .The barrier layer in this instance faces away (downstream), whereas the more open support layer faces the feed stream. A tight barrier layer can be protected by the support layer, which can serve as an inline prefilter to catch bigger foulants . Membranes that are basically symmetric are nevertheless employed in industry. In contrast to standard pressure-driven membrane separation procedures like ultrafiltration, viral filtration has very specific needs. It can be particularly difficult to choose and size an effective virus filter. [88] [89] [90] [91] The tiny aggregates, which have sizes of less than 50 nm, can obstruct the pores of viral filters but cannot be eliminated by exclusion filters of 0.1 or 0.22 microns in size. Typically, virus filtration membranes have pores at the separation-active layer that are around 20 nm in size. Dissolved aggregates can be eliminated by adsorbent prefilters to avoid clogging virus filters. It has been demonstrated that adsorbent prefilters bind aggregates, decreasing subsequent viral filter clogging as a result. Adsorptive prefilters are effective at product oligomers between 600 and 1500 kDa that cannot be eliminated by 0.22 µm prefilters with size exclusion. Ion exchange prefilters have demonstrated excellent promise for removing agglomerates for efficient downstream processing [92]. Anion exchange prefilters can capture the polysaccharide moiety whereas hydrophobic prefilters can bind the phosphorylated lipid moiety to remove endotoxins Because the positively-charged ligands connect with the negatively-charged endotoxin (isoelectric point = 1-4), anion exchange membranes are effective at removing endotoxins. [93] [94] [95]

VIII. ULTRAFILTRATION/DIAFILTRATION

To concentrate and exchange buffers with the product, ultrafiltration/diafiltration (UFDF) is frequently used in the purification of recombinant proteins. Putting the protein in its final formulation and removing small molecules introduced in earlier purification steps, it is frequently the last step in the purification process. The buffer exchange is carried out using a diafiltration (DF) mode, in which minor contaminants and buffer components are removed from the product by adding a fresh buffer with the right ingredients for the following procedure or the finished product.[96][97]The final pH and concentration values are not necessarily identical to those in the diafiltration buffer because of electrostatic interactions between the charged proteins, solute ions, and uncharged excipients. This impact is more pronounced at high protein concentrations, which are typical for industrial formulations. Rok Ambroiet al. present a solid mathematical framework that allows the prediction of pH and concentration profiles throughout the UF/DF process while taking into account difficulties that may arise in practical settings. [98] The target product profile can be improved, the drug substance storage and supply chain can be made more efficient, and dosing methods can be more flexible with the formulation of protein biopharmaceuticals as highly concentrated liquids. There can be a significant pH imbalance due to the Donnan effect between the objective set using the diafiltration buffer during the concentration and diafiltration of charged proteins using the ultrafiltration membrane. In order to dilute and concentrate proteins in liquids containing monovalent and divalent ions, Glen R. Bolton and colleagues looked at novel equations for the Donnan effect. [99]

In a variety of buffers (a citrate/phosphate and a histidine buffer) and over a broad range of protein concentrations, Youngbin Baek et al. investigated the ultrafiltration behaviour of three therapeutic proteins (an Fc-fusion protein and two mAbs). Because the protein diffusion coefficients in the histidine buffer have a lower value due to its higher viscosity, the filtrate flux in the histidine buffer is uniformly lower than the values in the citrate/phosphate buffer (due to the high sucrose concentration). However, the trend was reversed at high protein concentrations, with the Fc-fusion protein having a lower flux than either of the mAbs in the histidine buffer. The Fc-fusion protein had the highest filtrate flow at low protein concentrations in the histidine buffer.[100]Even after a full buffer exchange at 10 diafiltration volumes, the excipient concentrations in the product retentate are lower than those in the diafiltration buffer at the final UF/DF stage of many protein synthesis methods. By using mathematical modelling and experimental UF/DF runs, Fudu Miao et al. concluded that reduced concentrations of charged excipients are predominantly brought about by excipient-protein charge interactions. In contrast to [101], Vishwanath Hebbi et al. established a model for the drift in the excipient concentration in the ultrafiltration/diafiltration (UF/DF) operation by taking into account the flux of each species across the system's membrane module. [102]Even though tangential flow filtration (TFF) is frequently employed to produce concentrated proteins, only a small number of studies have examined the effects of concentrations greater than 150 mg/mL. Here, protein gelation, membrane fouling, and protein aggregation may result from low fluxes in TFF and concentration polarisation brought on by high viscosities. Therefore, fresh ideas would greatly advance TFF ultrafiltration and subcutaneous injection of large concentrations by decreasing protein interactions to reduce viscosity and aggregation. [103] [104] [105] [106] By using a suitable wall shear stress or shear rate to sweep protein molecules close to the wall back into the bulk flow in TFF, fouling and concentration polarisation can be reduced Particularly in the presence of air-solution and solution-solid interfaces excessive shear stress may result in protein denaturation and aggregation [107] [108] [109] [110] [111]

IX. CONCLUSION

Some of the crucial components of a platform downstream process for mAbs were discussed in this review. In addition to this, several other technologies are still utilized during mAb purification. The development of cell lines with higher cell-specific productivity, particularly mammalian cells, has made downstream processing a bottleneck. In response, there have been a number of advancements in chromatographic processes, including improvements in the resin matrix, ligand chemistry, modalities, high throughput process development, process modeling, and approaches for control.

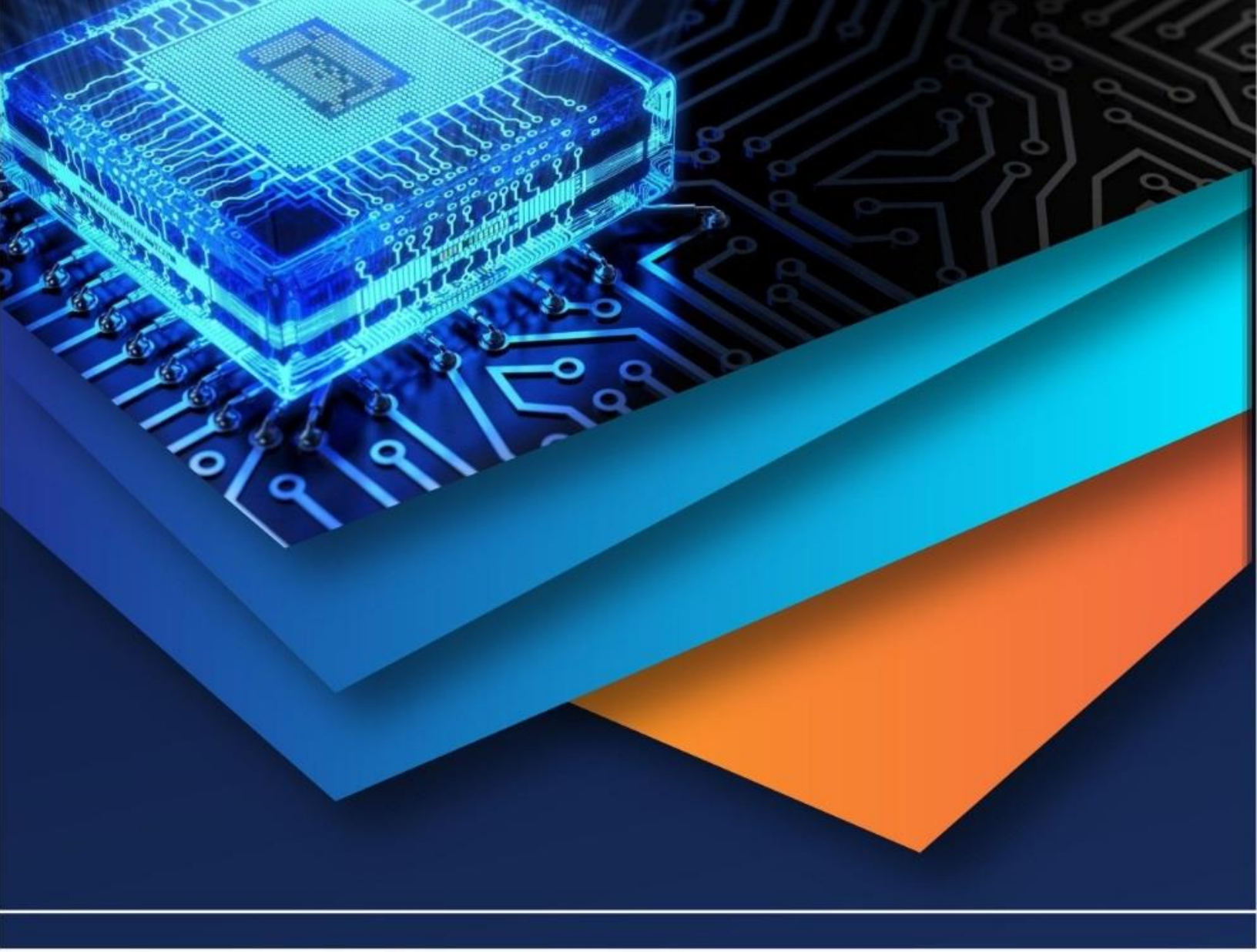
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