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A Review on Chromatography-based purification of monoclonal antibody

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Abstract - *The foundation of downstream processing remains chromatography, especially at the process scale. There are several causes for this, but crucial ones include the superior scalability, robustness, and selectivity that process chromatography provides in comparison to its competitors. For the purpose of removing contaminants and capturing antibodies, chromatography selection is crucial. In this mini-review, we attempt to summarize different types of chromatography for monoclonal antibody purification.*

***Key Words*: Downstream process, purification, chromatography, mAbs, biopharmaceuticals.**

1. 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] 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. Following a depth filter, a filter with an absolute pore size rating (usually 0.45 m or 0.2 m) is used to assure the removal of solid particles (and bacteria in the case of the 0.2 m filter) from the cell culture harvest supernatant. 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. Ion exchange, hydrophobic interaction, and multimodal chromatography are frequently utilized as polishing processes for the monoclonal antibody purification after capturing with Protein A. [6]

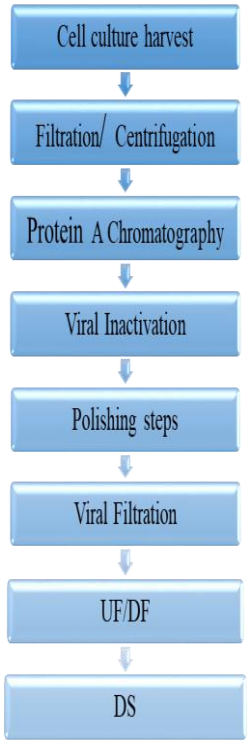

Fig -1: Process flow for mAb purification

2. CHROMATOGRAPHIC PROCESSES

2.1 Protein 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. [7] [8]

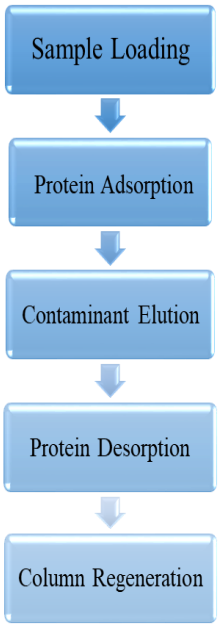

Fig -2: Process flow for protein A chromatography

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. [9] [10] [11] [12]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.[13] [14] 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. [15][16] [17]

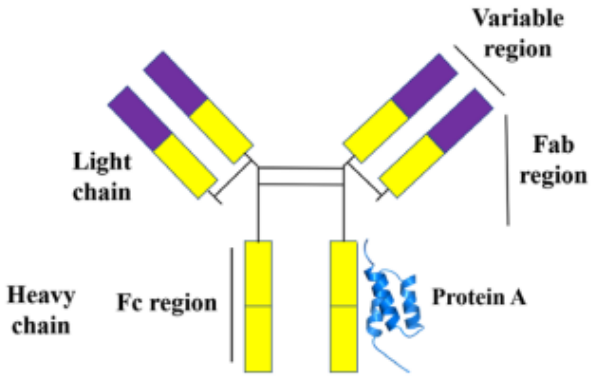

Fig -3: Antibody and Protein A interaction

2.2 Ion Exchange chromatography

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][18]. 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. [11] [19] [20] [21] [22] 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. [23]

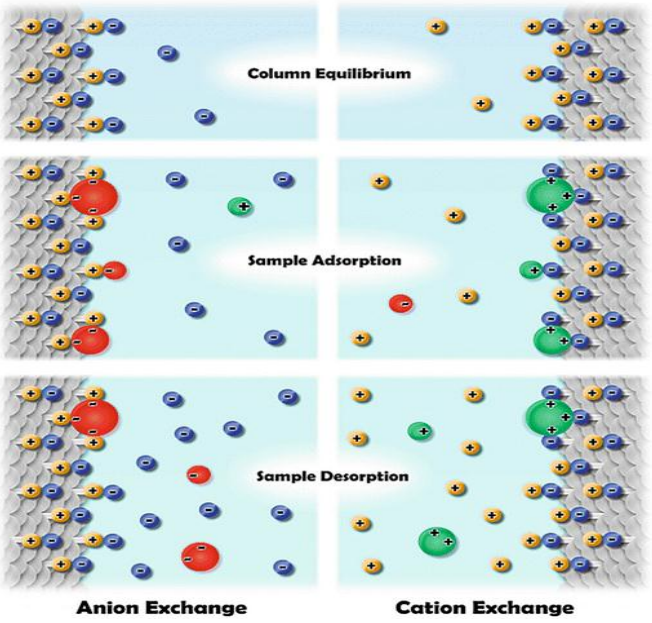

Fig -4: Ion exchange chromatography

2.3 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. [24] 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. [18] [25]. 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. [26] [27].

3. CONCLUSIONS

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 chromatographic processes is a crucial step in the manufacturing of

biopharmaceuticals. It usually requires a lot of resources and time, which affects how long it takes to launch a product.

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