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Monoklonal Antikor Üretimleri İçin Alt Akım Prosesleri

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Özet

Son yıllarda, terapötik monoklonal antikorların (mAb'lar) geliştirilmesi büyük önem kazanmıştır ve bu moleküller dünya biyoteknolojik ilaç pazarında giderek daha önemli bir hale gelmiştir. Birçok biyoteknoloji şirketi monoklonal antikor üretimine yatırım yapmaktadır. Bu derlemede ilgili moleküllerin üretimi için kullanılan mevcut saflaştırma yöntemleri tartışılmaktadır. Bu kapsamda hücre hasadı, Protein A afinite kromatografisi ve ileri saflaştırma adımları gibi monoklonal antikorlar için temel geri kazanım ve saflaştırma işlemleri özetlenmiştir.

Downstream Processes for Monoclonal Antibody Production

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Abstract

In recent years, the development of therapeutic monoclonal antibodies (mAbs) has gained considerable importance, and these molecules have become increasingly important in the worldwide biotechnological drug market. Many biotechnology companies are investing in monoclonal antibody production for treatment of various diseases. Bioreactor production and purification are the two major steps in the manufacturing of these antibodies. Current purification methods used for these molecules are extensively discussed in this review. The basic recovery and purification processes for monoclonal antibodies such as cell harvesting, Protein A affinity chromatography, and additional polishing steps are all summarized.

Introduction

A variety of monoclonal antibodies (mAbs) are currently on the market or under development to deliver potential therapies to the patients. Many companies are investing as mAbs have become prominent in the biotechnological pharmaceutical industry as therapeutic agents.

Efficient recovery and purification of mAbs from the cell culture medium at the end of the biopharmaceutical manufacturing processes is the most critical part of the production. One of the most important product properties that must be maintained throughout the process is product stability. At the same time, maximum attention should be paid to maintaining product quality and increasing purity while dealing with numerous challenges. Each step of downstream processing should be handled with care to avoid the risk of contamination and to ensure that material loss is kept to a minimum.

Process related impurities (e.g. host cell proteins, DNA, endotoxin, leached Protein A and some cell culture media additives) and product related impurities (e.g. high molecular weight and low molecular weight species) need to be removed during purification. In addition, potential viruses must be removed from the system to ensure complete safety of the product.

The first step is clarification, which provides the removal of cells from supernatant and the remaining is named as harvested cell culture fluid (HCCF). This operation is generally performed by centrifugation or filtration but methods may vary depending on scale and facility capacity.

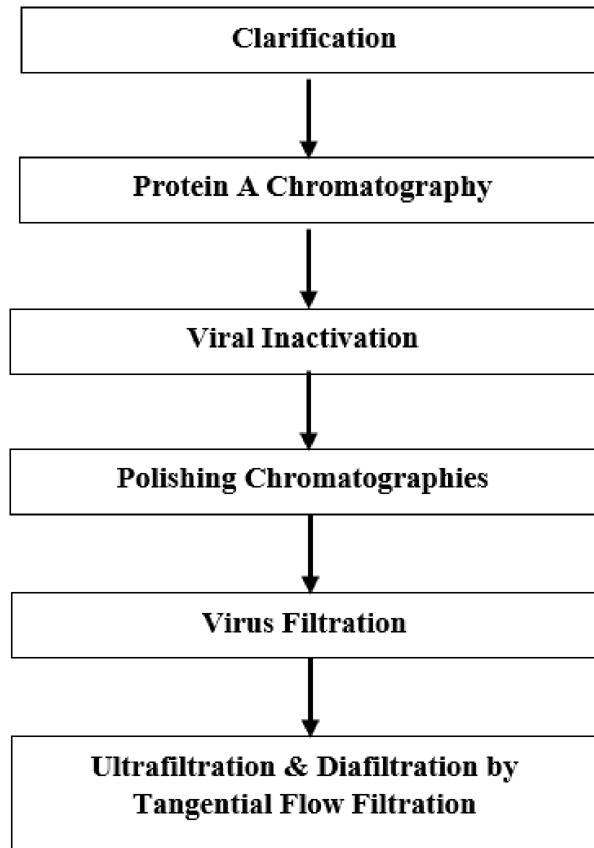
Purification for mAbs usually begins with Protein A chromatography, which can provide a high degree of purity in a single step. Since high purity levels are required at the end of the purification process, other chromatography techniques are coupled with Protein A chromatography. Following capture with Protein A, ion exchange chromatography, hydrophobic interaction chromatography and multimodal chromatography are generally used as polishing steps for the monoclonal antibody purification. Ceramic hydroxyapatite chromatography can be also be preferred in some cases.

In this review, the basic recovery and purification processes for monoclonal antibodies such as cell harvesting, Protein A affinity chromatography, and additional polishing steps are summarized.

Platform Processes for mAbs Purification

Since mAbs have similar physico-chemical properties, a platform process can be integrated for a variety of mAbs by making small changes like choosing different chromatography techniques as a polishing steps. Figure 1 summarizes a platform process for mAb purification.

Figure 1. Platform Process for mAb Purification



Clarification Step

Since monoclonal antibodies are secreted proteins, they can easily be removed from cells and cell debris by clarification processes. Depth filtration or centrifugation combined with depth filtration are generally used for clarification.

Large-scale centrifugation can be used as the primary harvesting step, but it cannot achieve the desired level of separation of cells and cell debris that needs to be removed before chromatography. Centrifugation can also cause the burst of the cells which can increase the amount of host cell proteins in the supernatant, for this reason depth-filtration is the generally preferred methodology.

Depth filters are preferred in certain scales due to easy scale-up and handling. A depth filter is generally followed by a filter having an absolute degree of pore size (typically 0.45µm or 0.2µm) which allows for the removal of solid particles (and bacteria in the case of 0.2 µm filter) from the cell culture medium. Surface area, pressure and average loading capacity are process conditions for the clarification step.

Protein A Chromatography

Although the variable specific regions and some sequences for mAbs have differences, the purification steps are essentially similar because of the common fragment crystallizable (Fc) region. This region shows high specificity to Protein A, Protein G or Protein A/G and constitutes the first step of purification as affinity chromatography. Protein G is expressed from Streptococcus spp. with lacking the albumin binding site. It is very useful to prevent albumin contamination of the purified IgG. Protein A/G chromatography is combined with binding domains of both Protein A and Protein G and it is useful for polyclonal IgG or unknown antibodies of subclasses. Currently, Protein

A chromatography is known to be the most commonly used method for the purification of almost all IgG therapeutic products due to its simple mechanism. Impurities do not bind to the column while IgG is binding. Protein A is highly sensitive and selective to the Fc region of IgG type antibodies.

Protein A is a type of cell wall protein isolated from S. aureus. When several studies were examined, it was found that Protein A affinity chromatography was highly selective for mAbs and was able to yield approximately 95% purity from the complex cell culture medium in a single step.

The Protein A step also serves as a concentration step because it captures only the desired molecule at a high selectivity from HCCF. The cell culture supernatant can be loaded directly onto the column (at pH 6-8) and the product eluted from the column at pH values between 2.5 and 4. A washing step between column load and elution is generally performed for removing non-specific interactions of host cell proteins and other impurities with mAbs and/or protein A ligands.

The binding capacity of the protein A chromatography is also highly variable between different mAbs. Shukla et al. have shown differences in operational loading capacity ranging from 10 to 40 g/L resin for fourteen different mAb molecules, indicating that loading capacity requires an experimental determination for each molecule. 10 to 20 cm column bed height is generally packed during scale-up and large scale production. The linear velocity used for Protein A chromatography is between 300 and 500 cm/h. The dynamic binding capacity ranges between 15-50 g of antibody per liter of resin and depends on the flow rate with 3 to 6 minute residence time. Fahrner et al. have developed a method for determining the dynamic binding capacity of Protein A resins. According to these studies, at a lower loading flow rate, antibody residence time and binding capacity are increased. At the same time, the low flow rate increases the cycle time, resulting in fewer cycles and thus less buffer consumption.

While Protein A chromatography is highly selective for mAbs, immobilized ligand can cause a number of difficulties. The ligand is prone to proteolysis and may adhere to product of interest. Most importantly, the cost of Protein A resins is much higher than conventional chromatographic resins. Another important limitation of protein A chromatography is the need to elute the product at low pH. Under low pH conditions, soluble high molecular weight aggregates (as can be determined by analytical size exclusion chromatography) may be increased.

Shukla et al. have developed various strategies to address the problem of aggregation/precipitation during Protein A elution. Modification of Protein A elution buffer is generally the most common solution to keep product stability. In the study of Arakawa et al., stabilizers such as arginine were added to the Protein A elution buffer to reduce aggregate formation.

On the other hand, next step after protein A chromatography is viral inactivation which is performed around pH 3.5. A low pH elution is also an advantage in order to perform viral inactivation by adding less acidic buffer to the product.

Viral Inactivation

According to the Food and Drug Administration (FDA) Q5A guideline, there are specific procedures that should be performed for viral reduction as well as chromatographic steps to ensure the safety of biotechnological products produced by mammalian cell culture. At low pH values, retroviruses can be successfully inactivated for various biotechnology products. The pH of the protein A elution pool can be adjusted by adding weak acid at high concentrations. After inactivation, the solution is neutralized to bring the product to a more stable pH range for the next chromatography step.

Chromatographic Polishing Steps

Commercial mAb purification is usually carried out by Protein A chromatography followed by one or two chromatographic polishing steps. The polishing steps aim to reduce host cell protein impurities, high molecular weight aggregates, low molecular weight species, DNA and leached Protein A remained after the Protein A chromatographic step. The choice of chromatography methods for the polishing steps depends on the nature of the impurities that must be removed.

Ion Exchange Chromatography

Most mAb purification processes include at least one ion exchange chromatography step. Separation by this technique is highly selective and the resins used are relatively inexpensive. Ion exchange chromatography is performed as a polishing step after the Protein A step in monoclonal antibody purification. Ion exchange chromatography is ideal for reducing high molecular weight aggregates, charge-variants, residual DNA and host cell protein, leached Protein A and viral particles.

Generally, ion exchange resins can be cleaned with high concentration of sodium hydroxide. Applicable flow rates range from 100 to 500 cm/h. Existing ligands are strong and weak anion and cation exchangers. The choice of the resin depends on the resolution required for the process, the binding capacity and the feasibility of use on the production scale.

Anion Exchange Chromatography (AEX)

Anion exchange resins have positively charged groups as diethyl-aminoethyl (DEAE), or diethylaminoethyl. This technique is generally used to remove process-related impurities such as host cell proteins, DNA, endotoxins and leached Protein A. It can also provide viral clearance. AEX chromatography is preferred to be used in flow-through mode in which impurities bind to the resin and the product of interest flows through the column.

Cation Exchange Chromatography

In cation exchange chromatography, negatively charged functional groups (strong acidic ligands such as sulphopropyl, sulfoethyl and sulfoisobutyl groups or weak acidic ligand such as carboxyl group) are immobilized to the resin. Cation exchange chromatography is suitable for many mAb purification processes with pI values ranging from neutral to basic. The antibody is bound to the resin during the loading step and eluted with increased conductivity or increased pH in the elution buffer. Impurities related to the most negatively charged process, such as DNA, host cell proteins, leached Protein A are removed in the load and wash fraction. It can also successfully provide high molecular weight (HMW) aggregate removal.

Dynamic binding capacity depends on the loading conditions, ligand and density of the resin. Removal of the impurities is related with loading capacity. High loading on the resin causes more impurity in the elution pool, but different ligands and resin bead sizes can have significant effects on impurities. Therefore, a resin screening study should be performed to select the optimum resin in terms of binding capacity and impurity removal. Elution conditions are also important for impurity clearance.

Hydrophobic interaction chromatography

Hydrophobic interaction chromatography (HIC) is a useful tool for separating proteins according to their hydrophobicity and is complementary to other techniques that separate proteins by charge, size or affinity. It has hydrophobic ligands such as phenyl, butyl and hexyl. The sample is loaded onto the HIC column in a high salt buffer. The salt in the buffer interacts with the water molecules to reduce the dissolution of the protein

molecules in the solution, resulting in hydrophobic regions of the protein molecules that bind to the HIC resin.

HIC is often used as an intermediate purification step after Protein A chromatography or as a polishing step after ion exchange chromatography. In flow- through mode, the HIC allows for the separation of large quantities of aggregates with relatively high efficiency. In bind-and-elute mode, most of the host cell protein, DNA and aggregates can be separated from the antibody by selecting a suitable salt concentration in the elution buffer or using a gradient elution method.

Multimodal chromatography

Multimodal also called as mixed-mode chromatography (MMC) involves different interactions between ligand and mAbs. It contains different ligands of different techniques together such as anion exchange and hydrophobic interaction. MMC can also be used as a polishing step to remove aggregates and HCP. The key benefits of MMC are high binding capacity and productivity and also saves time for purification since it can be operated with high flow rates. On the other hand, during development period, a number of studies should be performed to find the optimum conditions.

Ceramic hydroxyapatite chromatography

Ceramic hydroxyapatite (CHT) is a form of calcium phosphate that can be used for the separation and purification of proteins, enzymes, nucleic acids, viruses and other macromolecules. Hydroxyapatite has unique separation properties in terms of selectivity and solubility. It usually separates proteins that appear homogeneous by other chromatographic and electrophoretic techniques.

Gagnon et al. has successfully used ceramic hydroxyapatite (CHT) chromatography by sodium phosphate gradient elution to separate dimers, aggregates and leached Protein A for human antibody IgG4. This provides insight that CHT chromatography can be a robust polishing step in the mAb purification process.

Viral Filtration

After chromatographic techniques in downstream processes, viral filtration is used for potential viral clearance. Virus filters are selected based on the volume of solution that can be arranged per unit surface area of the membrane. The viral filters are operated at constant pressure. Due to their small pore size, they can be easily clogged, especially in the presence of aggregates. To prevent this, a prefilter is generally used before virus filter.

Ultrafiltration/Diafiltration

Ultrafiltration is a pressure-operated membrane process commonly used for protein concentration and buffer exchange. Ultrafiltration is a separation in which molecules larger than membrane pores are retained and smaller ones pass freely. Separation in ultrafiltration is achieved by transmembrane pressure. The buffer exchange is carried out using a diafiltration mode in which the buffer of the final desired composition is added to the retentate at the same rate at which the filtrate is removed, thereby maintaining a constant retentate volume.

Ultrafiltration membranes can be in different polymer structures, including polysulfone, polyethersulfone, polyvinylidene fluoride, and regenerated cellulose. Synthetic polymers can be reused because of their strong resistance to acids, bases, alcohols and high temperatures for effective membrane cleaning. Although they can provide chemical and thermal stability, they tend to contaminate protein. In contrast, cellulose membranes have low protein binding, but can be damaged by harsh cleaning methods. The new compound regenerated cellulose membranes have significantly less protein binding, are easier to clean and have excellent mechanical strength. Because

of these properties, cellulose membranes are more advantageous in terms of process permeability and retention properties for ultrafiltration and diafiltration than other membranes.

Ultrafiltration is performed in tangential flow filtration (TFF) mode, in which the fluid passes through the filter (cross flow) tangential to the filter surface. The advantage of the TFF is that the cross-flow continuously sweeps the filter surface which reduces the deposition of materials on the filter surface and increases the filtration efficiency.

Membrane type, the transmembrane pressure, flow rate and final concentration at which the diafiltration is performed can be determined for all mAbs. When very high protein concentrations are used, problems with viscosity and product aggregation may occur for certain molecules, and this should be taken into account according to the process.

Future Prospects for mAb Purification

Downstream processes will need to progress to keep pace with the increase in cell culture titers and to control market changes and costs. In the near future, downstream processes should be adapted to cope with the rise in more cell culture titers in the existing facility infrastructure. An excellent prospect of future mAb purification has been published.

As the titers increased, it created difficulties for the existing infrastructure to save the whole party. These limitations are due to the available column size of the chromatography, buffer and holding tank volumes in the production facility. The use of high capacity resins may allow the use of existing chromatography columns to process larger quantities.

Future advances in purification technology, different techniques such as high flow and high capacity chromatography resins and membrane adsorbers may be used. Moreover, continuous chromatography will most probably be the choice as a purification technique in the near future since it significantly reduces the costs and improves productivity. These systems are designed to work with perfusion systems on bioreactor that provides a constant product flow through chromatography systems.

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