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Exploration of overloaded cation exchange chromatography for monoclonal antibody purification

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ABSTRACT

Cation exchange chromatography using conventional resins, having either diffusive or perfusive flow paths, operated in bind-elute mode has been commonly employed in monoclonal antibody (MAb) purification processes. In this study, the performance of diffusive and perfusive cation exchange resins (SP-Sepharose FF (SPSFF) and Poros 50HS) and a convective cation exchange membrane (Mustang S) and monolith (SO_3 Monolith) were compared. All matrices were utilized in an isocratic state under typical binding conditions with an antibody load of up to 1000 g/L of chromatographic matrix. The dynamic binding capacity of the cation exchange resins is typically below 100 g/L resin, so they were loaded beyond the point of anticipated MAb break through. All of the matrices performed similarly in that they effectively retained host cell protein and DNA during the loading and wash steps, while antibody flowed through each matrix after its dynamic binding capacity was reached. The matrices differed, though, in that conventional diffusive and perfusive chromatographic resins (SPSFF and Poros 50HS) demonstrated a higher binding capacity for high molecular weight species (HMW) than convective flow matrices (membrane and monolith); Poros 50HS displayed the highest HMW binding capacity. Further exploration of the conventional chromatographic resins in an isocratic overloaded mode demonstrated that the impurity binding capacity was well maintained on Poros 50HS, but not on SPSFF, when the operating flow rate was as high as 36 column volumes per hour. Host cell protein and HMW removal by Poros 50HS was affected by altering the loading conductivity. A higher percentage of host cell protein removal was achieved at a low conductivity of 3 mS/cm. HMW binding capacity was optimized at 5 mS/cm. Our data from runs on Poros 50HS resin also showed that leached protein A and cell culture additive such as gentamicin were able to be removed under the isocratic overloaded condition. Lastly, a MAb purification process employing protein A affinity chromatography, isocratic overloaded cation exchange chromatography using Poros 50HS and anion exchange chromatography using QSFF in flow through mode was compared with the MAb's commercial manufacturing process, which consisted of protein A affinity chromatography, cation exchange chromatography using SPSFF in bind-elute mode and anion exchange chromatography using QSFF in flow through mode. Comparable step yield and impurity clearance were obtained by the two processes.

1. Introduction

At present, there are hundreds of monoclonal antibodies (MAbs) that are either currently on the market or are under development [1]. MAbs are derived from a common framework and have a high degree of homology, which results in similar properties. These similarities are being exploited as companies establish platform processes that allow for the purification of similar molecules with minimal development and optimization, especially for early clinical stage development. As a project moves to late clinical development and commercial stages, however, further development is typically required to address cell culture process changes, increased antibody titer, and facility fit, as well as to optimize the yield and reduce the cost for the commercial process.

Downstream processing of MAbs is increasingly centering on a platform process that includes Protein A affinity

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chromatography as a capture step, followed by one or two polishing steps such as ion exchange, hydrophobic interaction, or mixed mode chromatography. Host cell protein, host cell DNA, high molecular weight species, and other process-related impurities can usually be reduced to levels low enough to be considered acceptable by the FDA by this standard approach. Reduction of endogenous and adventitious viruses can also be achieved [2–6].

These classic purification processes have typically been operated in bind-elute mode for protein A, cation exchange, hydrophobic interaction and mixed mode chromatography, while anion exchange has mostly been employed in flow through mode. One approach to reducing the downstream cost of purifying monoclonal antibodies from CHO derived cell culture fluid has been to leverage recent developments in anion exchange chromatography, including weak partitioning or partial binding chromatography, to enable a two column purification process (using protein A and anion exchange, followed by a viral filtration step) [7,8]. Throughput with anion exchange chromatography can reach >250 g/L of column volume; however, success with the two column process is limited when faced with a higher level of high molecular weight species in the feedstock or an antibody with a high pI value ($\geq$9). Removing residual process related impurities that are added or generated during cell culture processes can also be a challenge for the two column process since these impurities can be more effectively removed by cation exchange or other types of chromatography. Therefore, a three column process is still widely used to purify monoclonal antibodies.

In the search for ways to simplify and reduce the cost of the traditional three column approach, ion exchange membranes and monoliths have also been explored [9–11]. Recent discoveries with anion and cation exchange membranes used as the second or third purification step to effectively remove product and process related impurities have been reported [9,12,13]. Membrane chromatography provides an advantage over traditional column chromatography because convective mass transfer dominates in the flow path. Monolithic matrices may also enable a fluid flow that is dominated by convective mass transfer with a minor component of diffusion. Convective mass transport is more efficient, resulting in a dynamic binding capacity (DBC) that is mostly independent of flow rate. Effective resolution and separation power across a broad range of flow rates is maintained, allowing for considerable process efficiency improvements [10,14–16]. Membranes and monoliths can be used as single- or multi-use, disposable matrices. Lower buffer volume consumption, eliminating the need for packing and unpacking large columns, and reducing the complexity and size of a plant footprint are also advantages with membrane and monolith matrices.

The original intent of this work was to further explore the use of cationic monolithic matrices and membranes as an alternative to the traditional cation exchange chromatography step. Overloading of a traditional cationic chromatography column was incorporated into the experimental design for the purpose of understanding and comparing the binding of antibodies and impurities to various matrices. To that end, four cation exchange chromatographic matrices having diffusive, perfusive, and convective flow properties were selected for comparison. An isocratic running condition was used under typical binding conditions for monoclonal antibodies with an antibody loading amount of up to 1000 g/L of chromatographic matrix. This loading density clearly exceeded the point of MAb breakthrough, but was used to fully explore the binding of impurities, which may reach breakthrough at a higher loading value. The most intriguing result of this work was the performance of the traditional cationic chromatography column. Operating these columns in an isocratic overloaded mode demonstrated promise to achieve a more efficient and/or cost effective approach compared to standard bind-elute cationic chromatography, while still providing acceptable levels of impurity removal.

Based on this observation, further studies were performed with overloaded conditions to explore the influence of processing parameters, such as flow rate and conductivity, on the removal of impurities. Finally, isocratic overloaded cation exchange chromatography was incorporated into a MAb purification process, where the performance was compared to the traditional bind-elute process. Superior processing performance was demonstrated with isocratic overloaded mode, even under heavily overloaded conditions. While this process used standard cation exchange resin, performing the process in an isocratic overloaded mode provided a means of reducing the resin volume, equipment size, processing time and process complexity required for a given amount of product, thus offering the potential to lower the cost of the purification process.

2. Materials and methods

2.1. Materials

Two types of small scale cation exchange membranes were tested, the Mustang S and the Sartobind S. The Mustang S is a PES membrane coupled with sulfonyl ligand sold by Pall Corporation (Port Washington, NY). Two different sizes of this filter were used in the study: the Acrodisc S is their syringe scale filter (0.18 mL of the membrane bed volume, 6 layers) and the Mustang Coin unit is their coin filter S (0.35 mL of the membrane bed volume, 16 layers). A 13 mm O.D. (10 mm I.D.) stainless steel membrane holder from Pall Corporation was used with the coin filter. Sartobind S is another PES membrane coupled with sulfonyl ligand (1 mL of the membrane volume, 15 layers), and it was purchased from Sartorius Corporation (Goettingen, Germany). The monolithic SO_3 disks tested were coupled with sulfonyl ligand (0.34 mL of monolith volume per disk, 13 mm I.D. $\times$ 2 mm thickness). The monolithic discs and disk holder were purchased from BIA Separation (Villach, Austria). SP-Sepharose Fast Flow (SPSFF) was bought from G.E. Healthcare (Uppsala, Sweden). SPSFF is a cation exchange resin coupled with sulphopropyl ligand; the resin has a particle size range of 80–100 μm. Poros 50HS is a cation exchange resin coupled with sulphopropyl ligand and was purchased from Applied Biosystems (Foster City, CA). The average particle size of Poros 50HS resin is 50 μm. Small scale glass column hardware with 6.6 mm I.D., obtained from Omnifit (Diba Industries, Mahopac, NY), was used to conduct the SPSFF and Poros 50HS column chromatography experiments.

The load material for this study was Chinese hamster ovary (CHO) cell derived cell culture fluid that was generated and subsequently purified through protein A chromatography at Genentech's Oceanside facility (Oceanside, CA, USA). The cell culture fluids containing each monoclonal antibody were harvested by continuous centrifuge to remove cells and cellular debris, and further clarified through filtration with depth filters and a 0.2 μm filter. For the protein A chromatography step, the loading and washing conditions were at neutral pH, and elution was performed at low pH. The protein A protein pools were adjusted to the appropriate pH and conductivity for loading onto cation exchange chromatography, followed by 0.22 μm filtration. The same pH and conductivity were used for the cation exchange chromatography loading, equilibration, and post-load wash conditions. Concentration of product (g/L) in the protein A and cation exchange pools was determined by absorbance (278 nm) on a UV–Vis spectrophotometer. All chromatography runs performed with the membrane, monolith, and resin particle packed columns were run on an ÄKTA Explorer 100 system from G.E. Healthcare.