HCP clearance strategies for Protein A chromatography

Melissa Holstein, Kristen Cotoni, Nanying Bian
EMD Millipore Corporation, Process Solutions R&D, 80 Ashby Road, Bedford, MA, USA

Introduction
Protein A chromatography continues to play a critical role in monoclonal antibody downstream purification processes due to its high specificity and efficiency. The strong and highly specific interaction between protein A and an antibody’s Fc region provides high product yield while also ensuring that more than 99% of impurities from clarified cell culture are removed in the column flow-through. The high cost of Protein A resins provides the motivation for fully utilizing these resins to achieve their greatest potential.

Protein A resins often have some degree of non-specific binding which causes host cell proteins (HCP) to co-elute with mAbs. In order to reduce non-specific binding interactions, an intermediate wash step can be employed prior to elution of the product of interest. This can improve product purity, extend column lifetime, and potentially eliminate a subsequent polishing step.

Sources of retained HCP in Protein A elution pool (Siasidya, V. et al., Biotechnol. J. 2012, 7, 1233-1241):
- HCP-base matrix
- HCP-Protein A ligand
- HCP-mAb

Methods
A wide range of intermediate wash buffer conditions were tested using a plate screening method. The plate results were confirmed using lab scale chromatography columns. A flowchart displaying the purification process is shown below:

Method details are given in Table 2. The chromatography resins used in this study were Eshmuno® A resin and ProSep® Ultra Plus resin. Two mAb feeds (mAb 1 and mAb 2) were produced and purified in-house. We determined HCP content using Cygnus Technologies FS50 CHO HCP ELISA Kit 3G immunoenzymatic assay. Yield was based on UV absorbance at 280 nm.

Results
The figure below shows a control set of data obtained by using equilibration buffer as intermediate wash buffer for Eshmuno® A resin. An intermediate wash consisting of equilibration buffer is not always optimized and cannot selectively disrupt the undesirable interactions of HCPs to Protein A resin or HCP to Protein A-bound antibody. Equilibration buffer is also unlikely to disrupt antibody-binding interactions to Protein A, providing high yields. The figure also shows a comparison with an intermediate wash consisting of 0.1 M citric acid, pH 5.5. This intermediate wash condition significantly reduced HCP levels in the final elution pool - below those obtained using an equilibration buffer wash.

By analyzing HCP levels and product yield resulting from the screening of many intermediate wash conditions, we identified several conditions that improve product purity. Four of the most promising conditions were selected for further testing in column studies. The results are shown below and indicate that arginine and GuHCl can significantly improve HCP removal. The trends for the plate screening and column tests were nearly identical. The intermediate wash plate screening and column testing was also carried out on ProSep® Ultra Plus resin. Again, arginine and GuHCl provided excellent HCP removal capabilities.

Summary
A wide range of intermediate wash conditions were tested using 96-well plate screening and evaluated based on their impact on product yield and HCP removal. Intermediate wash conditions for Eshmuno® A and ProSep® Ultra Plus resins were selected to sample a range of salt types, salt concentrations, pH, and additives. The top candidates were evaluated using lab scale column experiments. For both resins, the column results corroborated the plate screening results and indicated that arginine and GuHCl can significantly improve HCP removal. The results offer insight into optimizing the Protein A step for improved overall process performance.