Understanding Viral Clearance During Anion-Exchange Chromatography

A Novel Design of Experiments Approach

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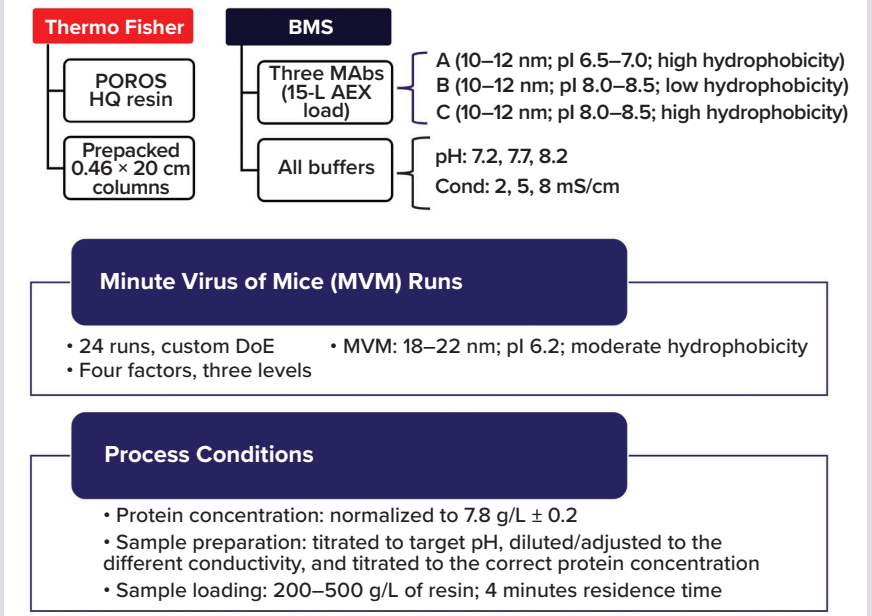
Anion-exchange (AEX) chromatography is a well-established polishing step for removal of viruses and other impurities during downstream processing of biotherapeutics. Viral clearance studies are performed during process development as necessary for investigational new drug (IND) applications, clinical trial applications (CTAs), biologics license applications (BLAs), and marketing authorization applications (MAAs). Before phase 1 clinical trials, a representative, scaled-down model of the final process must be available for use in viral clearance studies. At that point, however, scientists have no way of knowing how effective the process will be for removing viruses.

Below we describe a design of experiments (DoE) study used to define the mechanism of virus removal for a polishing step based on POROS HQ resin (Thermo Fisher Scientific), which acts as a strong anion exchanger. Conventional factors — load pH and conductivity relative to the target molecule's isoelectric point (pI) — provided insufficient information for predicting viral clearance performance. We found, however, that a virus coelution mechanism based on interactions between viruses and monoclonal antibodies (MABs) can help predict the viral clearance performance of an AEX process for MAbs with different biophysical properties.

Our DoE study used minute virus of mice (MVM) and three MAbs of different biophysical properties to examine the AEX process in general and to draw possible correlations between such properties and overall viral clearance capability. Figure 1 lists salient properties and summarizes our experimental plan.

Figure 1: Experimental plan (DoE = design of experiments, pI = isoelectric point)

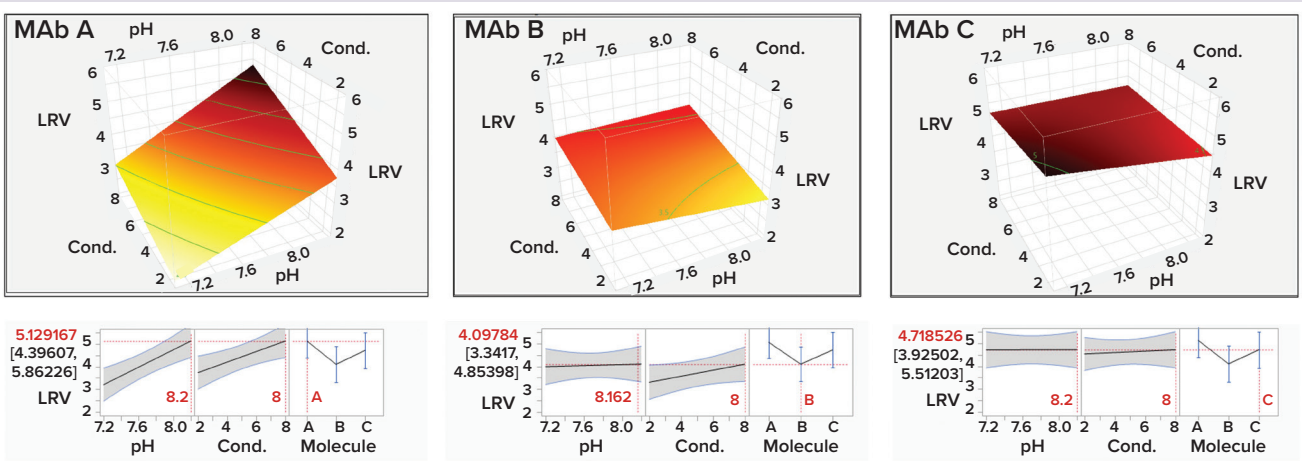


Findings

Results from the MVM experiment demonstrate a good fit between predicted and observed log reduction values (LRVs, data not shown). Model fitting achieved an $R^2 > 0.8$, and the greatest effectors were molecule properties, load pH, and conductivity. Figure 2 shows LRVs for each MAb against load pH and conductivity.

Polishing processes for MAb A, which exhibits a low pI, showed a relatively wider range of LRVs than did the other studied antibodies (2.2–4.6). As evidenced by the slope, LRVs were sensitive to pH and conductivity: Increases in those parameters resulted in significantly higher LRVs. With MAb B (high pI and low hydrophobicity), LRVs ranged from 2.7 to 4.6, with most data falling between 3.6 and 3.8. In this case, LRVs were insensitive to operating pH, but they showed slight improvements as operating conductivity increased. Processes for MAb C, a molecule with a high pI and hydrophobicity, achieved high LRVs (4.6–5.3) regardless of operating conditions.

Figure 2: The following surface plots depict minute virus of mice (MVM) clearance from three monoclonal antibody (MAb) products with different biophysical properties; MAb A exhibits a low isoelectric point (pI) and high hydrophobicity, MAb B has a high pI and low hydrophobicity, and MAb C shows a high pI and high hydrophobicity. No flow-through samples for MAb C contained detectable virus. (LRV = log reduction value; Cond. = conductivity)



LRV = 2.2–4.6 | LRV = 2.7–4.6 (most between 3.6 and 3.8) | LRV = 4.6–5.3

Results showed different responses to operating conditions than what we expected based on conventional wisdom surrounding AEX viral clearance behavior, suggesting that MAb biophysical properties might have influenced process outcomes. Subsequent experiments explored properties that could influence LRV.

Properties Influencing LRVs

In a typical AEX separation run at pH 7.0–8.0, MAb pI is higher than that of virus particles; thus, the antibodies present a positive charge, whereas the low-pI virus exhibits a negative charge. Negatively charged virus particles bind to the positively charged AEX resin while the MAbs flow through. At a pH below the MAb pI, the protein will have a larger positive net charge, causing greater repulsion between the MAb and resin. In such situations, MAbs will flow through more easily, enhancing separation from viruses and leading to a high LRV. At a pH above the MAb pI, the protein will have a net negative charge, promoting MAb–resin interaction, increasing MAb–virus competition for resin binding sites, and leading to low LRVs.

A low LRV was expected and observed for MAb A, which had a low pI and was hydrophobic. However, when pH and conductivity increased, the LRV increased unexpectedly from 2.2 to ~5.0. A high LRV was expected for MAb B because of its high pI, but a moderate LRV was achieved during our experiments. When the pH and conductivity increased, the LRV remained largely unchanged. For MAb C, we expected to find a high LRV, and experimental results confirmed that prediction. When pH and conductivity increased, LRVs largely did not change.

We posed three questions to understand the discrepancy between expected and actual results: How can a high LRV be achieved for the low pI MAb (A)? What caused the large discrepancy between the LRV values for the two high-pI MAbs (B and C) despite operation below the molecules' pI values? And what other mechanisms in addition to traditional resin–virus and resin–MAb interactions are driven by specific molecular biophysical properties?

To investigate **MAb biophysical properties** as a factor governing molecule-specific interactions, we performed surface-charge calculations using Molecular Operating Environment (MOE) software from Chemical Computing Group. Although proteins present a single net charge at a given pH, their surfaces show a heterogeneous distribution of positive and negative sites, sometimes clustered together in patches that can drive interaction with other charged species.

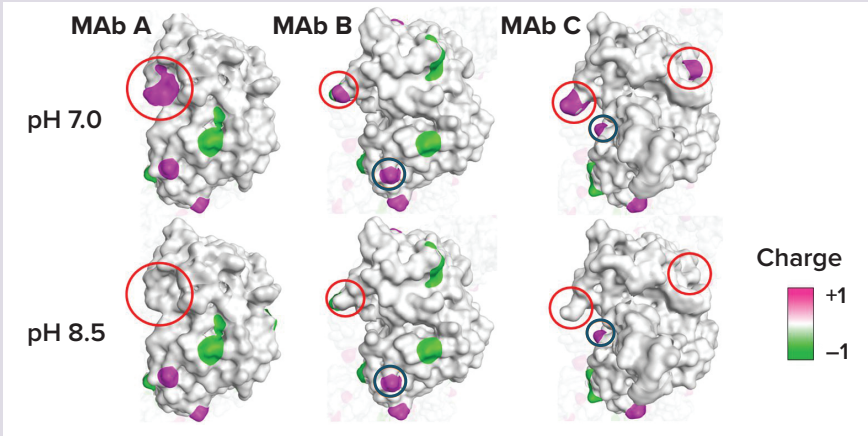
Figure 3 depicts surface-charge profiles for the three MAbs at pH 7.0 and 8.5. Positive and negative charge distribution in their complementarity-determining regions (CDRs) varied when the molecules were exposed to different pH values. At pH 7.0, MAb A showed a large patch of positive charge, whereas MAbs B and C had other positive spots. At pH 8.5, some of those patches disappeared. Hydrophobic patches on the surfaces of MAbs A and C also varied, resulting in significant hydrophobic patches in their CDRs (data not shown). Some positive-charge patches were located in hydrophobic regions; others appeared in hydrophilic regions. Given such variation, we find that biophysical properties of MAb surfaces may influence overall viral clearance LRV.

Potential for Interactions

Traditional considerations for AEX chromatographic separation of MAbs and viruses include virus–resin and MAb–resin interactions; operating conditions are modulated to maximize virus–resin bonding and minimize MAb–resin interaction. However, this mindset cannot explain our observations that MAb A, a low-pI molecule, achieved a high LRV that improved as pH and conductivity were increased and that MAbs B and C showed distinct LRVs despite having similar pI values.

We propose the possibility that attractive electrostatic interaction between the MAbs and virus particles influenced MAb–virus separation and virus removal. Such interaction might

Figure 3: Surface-charge modeling of the CDRs of MAbs A, B, and C at pH 7.0 and 8.5; large patches of positive charge at pH 7.0 that converted to neutral sites at pH 8.5 are circled in red. Positive-charge patches that differed between MAbs B and C — sections that could contribute to electrostatic interaction — are circled in blue.



not contradict the classic ion-exchange effect that leads to virus retention and MAb flowthrough; rather, the MAb–virus interaction mechanism could be used to improve overall AEX separation as a result of a MAb's biophysical properties and the influences that they may exert on viral clearance. This phenomenon could help to enhance viral clearance LRV and improve process robustness.

The coelution mechanism, by which virus particles and proteins flow through an AEX column together, is induced by MAb–virus interaction by the protein's distinctive biophysical properties. AEX resin is positively charged, whereas virus particles have negative charge; a MAb will have both negatively and positively charged patches on its surface, and those are influenced by the operating environment. Charged-patch distribution might differ by MAb biophysical properties, which also are influenced by the surrounding pH and conductivity. This coelution mechanism helps to explain the behaviors of the three tested MAbs on the AEX resin.

MAb A: When pH changed from 7.2 to 8.2, the LRV drastically improved from 2.2 to 4.6. A positive-charge cluster in the CDR region at pH 7.0 disappeared when the pH was increased to 8.5. At pH 7.0, the large positive patch might have promoted attractive MAb–virus interactions that increased the probability of coelution, resulting in a low viral LRV. Once that positive patch was neutralized through titration to pH 8.5, the possibility of such interactions was eliminated, reducing the likelihood of virus coelution. Hence, LRVs improved with increases in pH.

MAb B: We observed moderate LRVs (3.6–3.8), and pH and conductivity had relatively little effect on those values. Surface-charge maps of MAb B showed no changes in the negative patches over changes in operating conditions, and only minute changes were observed in the positive patches across the tested pH range. Little change was expected in MAb–resin and MAb–virus interactions, which may explain the relative insensitivity of the LRV to pH. However, MAb B did not realize LRVs as high as those for MAb C, which exhibits a similar pI. Virus coelution due to weak MAb–virus interaction might explain that result because MAb B features a larger number of charged patches than did the other antibodies — including more positive patches in its crystallizable fragment (Fc) region.

MAb C: Processes for MAb C achieved high LRVs (4.6–5.3) across the tested pH range. Those values were significantly higher than those for MAb B despite the two molecules having similar pI and charge distribution. For MAb C, all positively charged patches in the CDR manifest in neutral or moderately hydrophobic areas, where interactions between charged sites and virus particles are unfavorable. By contrast, all of MAb B's positively charged patches presented in hydrophilic regions, where interactions between charged sites and viruses are more favorable, promoting the coelution effect. Because MVM is generally hydrophilic, overall MAb–virus interaction would be subdued, resulting in weaker coelution.

Our results suggest that a virus coelution mechanism based on weak MAb–virus interaction can explain the behaviors observed for the tested MAbs.

Improved Prediction of Viral Clearance

The novel DoE approach described herein furthers our understanding of viral clearance during AEX chromatography. We found that the conventional factors of load pH and conductivity and their relationships to MAb net charge and pI were insufficient for predicting viral clearance behavior. To address that problem, we postulated a virus coelution mechanism based on MAb–virus interactions to explain the strong prediction of viral clearance performance trends shown in this study. The increase in LRV with pH for the low pI MAb (A) correlated with neutralization (titration) of a large positive hydrophilic patch in its CDR. For the two MAbs with similarly high pI (B and C), the MAb associated with the higher LRV contained a large hydrophobic zone near its positively charged patches.

With an improved mechanistic understanding of viral clearance in flow-through AEX chromatography, process development scientists can gain better insight into poor viral clearance results and benefit from an improved method for predicting AEX viral clearance behavior for a given MAb. Understanding a target antibody's surface charge and hydrophobicity characteristics might indicate its sensitivity to pH and conductivity changes during viral clearance.

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