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Separation and characterization of antibody-based protein variants in column chromatography for the publication of biopharmaceuticals

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The purification of a biopharmaceutical is not a simple task, and to ensure the quality and consistency of a product, a deep understanding of the unit operations must be achieved. Currently, the method of choice to tackle this problem is mainly focused on chromatographic techniques. For instance, ion exchange chromatography (IEC) is often used as a polishing step on the purification train of biopharmaceuticals because it provides effective removal of product-related impurities and contaminants. IEC is a powerful and ubiquitous unit operation in the purification of therapeutic proteins. However, an ion exchange process's performance depends on a complex interrelationship between several parameters, such as protein properties, mobile phase conditions, and chromatographic resin characteristics. Consequently, batch variations of IEC resins play a significant role in the robustness of these downstream processing steps. Ligand density is known to be one of the main lot-to-lot variations, affecting protein adsorption and separation performance. The use of a model-based approach can be an effective tool for comprehending the impact of parameter variations (e.g., ligand density) and their influence on the process.

The objective of this work was to apply mechanistic modeling to gain a deeper understanding of the influence of ligand density variations in ion exchange chromatography. First, the stoichiometric displacement model was applied to describe the retention of mAb and BSA at different ligand densities, but a dependency on the thermodynamic parameters $\Delta\hat{G}_p^0/RT$, $\Delta\hat{G}_s^0/RT$ and ν_i was observed. To overcome this hurdle and be able to describe the observed dependencies on the ligand density variation, some assumptions in the SD model were reconsidered. Such as the introduction of the activity coefficient of the protein in the adsorbate state into the adsorption isotherm. By using the description of ν_i and the model parameters ($\Delta G_i^0/RT$, $\Delta G_s^0/RT$, $A_{1/2}$, and $A_{2/1}$) the whole range of ligand density variation can be predicted. Thereafter, the model proved to be transferable to cation exchange resins, and the prediction of monomer isoforms of monoclonal antibody retention was achieved. The ligand density SD thermodynamic model that was introduced in this investigation can describe the influence of governing IEC process parameters, such as the salt concentration, the pH of the mobile phase, the variation of ligand density on the stationary phase, and protein variations. Moreover, the use of a modified protein net charge model proved to describe the dependence of pH and salt concentration in the adsorbate phase on the characteristic charge, showing that the influence of ligand density on the retention of the protein can be explained by the intraparticle conditions at which the protein is exposed in the pore volume of the matrix.

Lastly, it is noteworthy to mention that the ligand density SD model has a significant impact not only on the area of modeling and simulation of chromatography but, more importantly, on the process development in the biopharmaceutical industry. The influence of raw material variability is still a blind spot in the manufacturing of biopharmaceuticals (GE-Healthcare, 2019). Novel molecular formats display a pattern of similar product-related impurities and have become a real challenge to obtain a reliable separation method. These new modalities have not only the intrinsic heterogeneity of the host mAb (i.e., glycosylation, aggregation, etc.) but are also very susceptible to production process modifications and storage conditions, leading to a complex purification process. New high-resolution methods can be applied, but this comes with their limitations, such as high sensitivity (small variations will lead to different attributes in the final product). Therefore, to ensure the quality and consistency of a product, the variability of process parameters and raw material attributes must be addressed. To the extent of the author's knowledge, no ligand density dependency has been modeled before with such an extensive range. Therefore, the model presented in this work will help to elucidate the influence of raw material variability on the robustness of the process and assist in the development of strategies to mitigate these influences in the process.