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Improvement of monoclonal antibody drugs by selective removal of immunogenic contaminants like multimers and host cell proteins

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Protein purification is an important part of downstream process (DSP) in biopharmaceutical production. Process related impurities such as DNA, host cell proteins, and virus as well as product related impurities such as protein isoforms have to be removed to ensure product safety and efficacy. Liquid chromatography (LC) is often used in DSP for these purposes. While affinity chromatography, ion exchange chromatography, and hydrophobic interaction chromatography are among the very well-known LC techniques for protein purification, mixed-mode chromatography (MMC) has just begun to gain popularity in the recent decades. MMC is comprised of different modes of interactions such as hydrogen bonding, ionic and hydrophobic interactions, which are involved in the protein-ligand binding. The presence of such mixtures of interactions on one resin enables it to be used over a wide pH range and salt concentration. Up to date, the protein-ligand binding mechanism in MMC is still lacking.

In this study, two mathematical models were introduced to elucidate protein-ligand binding in MMC. Monoclonal antibody (mAb) was used as model protein. A series of mixed-mode chromatographic prototype resins with different surface modifications were tested. The influences of pH, salt, protein, and ligand on mixed-mode protein-ligand binding were investigated. Thermodynamic parameters such as Gibbs free energy, number of ionic interactions, number of hydrophobic interactions, number of water molecules released, and modulator interaction constant were determined using numerical solution. Examples were presented on how to determine these model parameters using isocratic and linear gradient elution chromatography. Results showed that the fitted model parameters from the two models were in good agreement with each other, indicating that it is possible to model protein retention in linear gradient elution chromatography simply by using fitted model parameters based on isocratic elution data and vice versa. Curve fits showed that Gibbs free energy of protein is strongly dependent on the protein and ligand type whereas Gibbs free energy of salt is strongly dependent on the salt type and stationary phase.

Besides the modeling of protein retention in MMC, attempts were carried out to separate mAb isoforms using the MMC prototypes. The mAb isoforms include fragment, monomer, and aggregate. Different chromatographic modes were tested such as linear pH gradient elution, linear salt gradient elution, dual salt-pH gradient elution, step elution and frontal analysis chromatography. Among the linear gradient elution systems, dual salt-pH gradient elution depicted the highest rate of aggregate removal and the shortest process time. Frontal analysis showed that MMC resin with the most hydrophobicity can be used to recover monomeric mAb in the flow through at elevated salt concentration. Finally, the combination of frontal analysis and dual salt-pH gradient elution showed the best monomer and aggregate recoveries in the flow through and in the eluted peak, respectively.