ABSTRACT

Chemical engineering seeks to connect molecular structure to physico-chemical properties at the continuum scale so as to design effective manufacturing processes. In the biotherapeutics industry, the large size and complexity of the key molecules is an obstacle for this paradigm to be realized. This is exemplified by protein A chromatography, a ubiquitously used platform technology that, by reducing the overall impurity content by $> 99\%$ in a single high-yield step, enables the manufacture of monoclonal antibodies (mAbs) and related classes of biopharmaceuticals. Despite its preeminent role in mAb manufacture, the design of this unit operation is carried out predominantly using heuristics and empiricisms, due to the absence of a suitable *in silico* model. This points to the underlying phenomena being incompletely characterized, which this thesis seeks to address.

As a starting point, a mechanistic chromatography model was developed, under simplifying assumptions to make model development tractable. This single-component model was parameterized using pure mAb feeds at various pH values, and was subsequently able to predict mAb chromatographic behavior during loading, wash and low-pH elution stages of protein A chromatography. Predictions were accurate for process conditions beyond the training set, including larger column volumes, thus demonstrating its potential utility in scale-up and process design. The model idealizations included use of a specific buffer system with high buffer capacity, and high as well as pH-invariant ionic strength, allowing possible effects of conductivity and protein titration to be ignored. The behavior and persistence of impurities, an important process attribute, was also largely ignored. In the subsequent chapters, the model idealizations were individually tested, with the aim of increasing model utility to typical industrial cases.

First, the application of the model to various buffer systems showed that mAb binding isotherms at acidic pH (but not neutral) were affected by ionic strength and, to a lesser extent, the identity of the ionic species in the mobile phase, explaining the modulation of elution behavior by various mobile-phase additives. The transferability of model parameters to various mAb molecules and subclasses was also evaluated, with results suggesting that the model, as parameterized for a specific IgG1, may well be generalizable to other IgG1s, and even IgG4s in some cases. Such studies were facilitated by the chromatography modeling scheme developed in this work, which enabled deconvolution of equilibrium effects from transient ones. An example of the latter are the conductivity spikes and delayed pH transitions observed upon switching from wash to elution buffers on a mAb-free protein A column. These phenomena resulted from titration of the protein A ligands, and were predicted using a separate chromatography modeling scheme that included pH equilibria, electroneutrality and convective transport.

The presence of persistent impurities in the protein A eluate is a critical process consideration, hence the model was extended to include major impurity classes, informing chromatography and electron microscopy experiments, and providing a mechanistic explanation for impurity persistence. The combined modeling and experimental results suggest that host-cell proteins (HCPs) and high-molecular-weight species (HMWs) can exist as mixed protein moieties, described here as 'mAb-HCP heteroaggregates'. These species behave similarly to the mAb monomer, likely due to the presence of exposed Fc fragments capable of binding to protein A ligands and of low-pH elution, hence they function as HCP vectors during the protein A step, leading to their persistence in eluate. The multicomponent modeling framework provides a quantitation of the relative binding strengths of monomer and aggregate species, and enables modeling of harvested cell-culture fluid (HCCF) feeds.

Finally, a molecular-scale investigation was carried out on the protein A ligand using a rational site-specific mutagenesis strategy. By identifying and manipulating biophysical interactions at the molecular scale, a novel protein A ligand was developed

that was more resistant to mAb unbinding at alkaline conditions. This mitigated the problem of yield loss observed when bound mAb on the protein A resin was subjected to alkaline washes, and is an instance of protein-protein interactions studied under non-physiological conditions.