

Characterizing and Leveraging Protein-Surface Interactions and Protein Transport for Separations

Ohnmar Khanal

Advisor: Abraham M. Lenhoff

Committee Members: Norman J. Wagner, Kelvin H. Lee, Steven M. Cramer

Purification is the current bottleneck in the production of biologics, accounting for well over half of the total manufacturing cost. Purification productivity requires a high capacity and efficient impurity removal. Depth filtration and ion-exchange chromatography are two widely-used purification unit operations. The adsorbing protein may be an impurity originating from the host cell, as is in the case of depth filtration, or the product of interest, as in most cases of ion-exchange chromatography. The performance of these purification steps depends upon the adsorption equilibrium between the adsorbent and the adsorbate and the transport of the adsorbate through the adsorbent. This work describes characterization of depth filters to inform their function and uses fundamental aspects of protein-adsorbent affinity and protein diffusivity to develop new methodologies to overcome capacity and separation limitations of ion-exchange chromatography.

For depth filters, which are routinely used for clarification of cell-culture harvest but are not yet well understood, this work characterizes their microstructure and the mechanisms by which each element contributes to the removal of impurities through adsorption. First, the adsorption of model proteins and monoclonal antibodies (mAbs) onto depth filters and their components was correlated to measured properties such as surface area, morphology, surface charge density, and composition. The polymeric resin binder was shown to be the primary contributor to the depth filter's adsorptive functionality. Monolayer coverage can be used to estimate the capacity for proteins that adsorb.

In addition to proteins, depth filters have been shown to reduce the DNA content of the filter load by tenfold or higher. Given the lengthy and stiff nature of genomic DNA, this work evaluates exclusion, adsorption, and displacement of DNA, in its form as a process impurity, on a commercial depth filter. Through non-destructive covalent labeling and visualization of retained DNA on depth filters, retention by adsorption – driven primarily by electrostatic interactions – was distinguished from retention by size-

based filtration. Furthermore, the extent to which DNA, as a process impurity, is solubilized and removed via adsorption/exclusion was probed by labeling DNA in cells and examining its retention after cell lysis.

In ion-exchange chromatography, the impact of protein-adsorbent affinity on protein diffusivity is exploited to improve protein transport into the adsorbent. By exploiting protein surface diffusion, an original method to dramatically improve the attainable transport-limited binding capacity was devised. The method modulates the protein surface diffusivity of the load components by the transient control of buffer ionic strength to increase the rate of protein transport into the resin. The impact of enhanced protein surface diffusion on resin saturation was corroborated using mechanistic modeling, microscopy, and small-angle neutron scattering at various relevant length scales. The findings and the proposed method increased the protein uptake efficiency of a commercial resin by up to 43%, corroborating the importance of protein surface diffusion in protein transport in ion-exchange chromatography.

Compared to protein transport, the protein-adsorbent affinity impacts separation more directly. In this work, we altered the traditional process configuration and methodology to enhance the separation of proteins with similar affinities for the adsorbent. In addition to an ionic environment conducive to competitive adsorption, saturated loading was shown to promote the displacement of more weakly-binding moieties by more strongly-binding counterparts. Displacement among the mAb product and impurities result in a distribution of impurities along the length of a single- or multi-column system, with the mAb separating the relatively more basic group of impurities from those that are more acidic. The proposed displacement chromatography methods, development and optimization of which were aided by column modeling, afford efficient preparative-scale separation of product isoforms, aggregates, host-cell protein impurities, and other groups of proteins.

In general, this dissertation addresses the retention, adsorption, competitive binding, and transport of biomacromolecules in the realm of depth filtration and ion-exchange chromatography. The presented methods to characterize depth filters are invaluable in the understanding of their structure-function relationships. Furthermore, the methodologies and techniques developed here, using our fundamental understanding of protein adsorption and protein transport, can be employed to address the current challenges facing protein separation and beyond.