Chemometric Investigations into Ion Exchange, Multi-modal, Protein A and Hydroxyapatite Chromatographic Systems: Understanding Selectivity and Implications for Quality by Design

by

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ABSTRACT

While recent advances in multi-modal chromatographic systems have shown significant potential for protein purification, there is a significant need to establish a deeper understanding of the nature of selectivity in these systems. Further, implementing the Food and Drug Administration (FDA) recommended concept of "Quality by Design" (QbD) into chromatography processes in biopharmaceutical industry requires a thorough understanding of these processes. To this end, this work seeks to evaluate protein binding affinity in various chromatographic systems and to apply chemometric approaches to develop predictive tools for these systems.

Predictive quantitative structure-property relationship (QSPR) models using homologous protein libraries were developed to gain insights into protein binding affinity in ion exchange (IEX) and multi-modal chromatographic systems. Chromatographic and spectroscopic studies were carried out to elucidate the effects of protein unfolding in chromatographic systems. The results indicated that chromatographic retention times were well correlated with structural changes and that they were more sensitive to tertiary structural change. Steric mass action (SMA) isotherm parameters were also examined and the results indicated that urea induced protein conformational changes could affect both the characteristic charge and equilibrium constants in these systems. These differences in protein behavior may provide insight into how these partially unfolded proteins are interacting with the resin material.

A novel principle component analysis (PCA) approach was developed to improve QbD in large-scale Protein A manufacturing processes. A method is developed to carry out detection of column integrity failures before their occurrence without the need for a separate integrity test. In addition, analysis of various transitions in the chromatograms was also employed to develop PCA based models to predict both subtle and general trends in real-time protein A column yield decay. The developed approach has significant potential for facilitating timely and improved decisions in large scale chromatographic operations in line with the process analytical technology (PAT) guidance from the FDA.

QSPR classification and prediction models were generated to evaluate complex protein retention behavior in hydroxyapatite chromatographic (CHT) systems. New molecular descriptors were developed which focused on possible interactions between proteins and CHT, distances between potential interaction groups on the protein surface, clusters/densities of these interaction groups and possible synergistic binding between the protein and CHT surfaces. Interestingly, a class of descriptors which describe synergistic binding with both metal chelation and cation exchange interactions on the angstrom length scale was found to play a vital role for protein binding in all of the models developed for CHT. The importance of this descriptor suggests the importance of synergistic binding in CHT, which has not been previously described in the literature. This study provides a deeper understanding into the mechanisms and selectivity of protein adsorption in CHT and will help to create predictive models which could be used for methods development in bioseparation processes.

Finally, evaluation and QSPR prediction of protein retention in multi-modal anion exchange chromatography using a commercial protein library was carried out. Experiments were carried out under linear sodium chloride gradient conditions. This retention data was then analyzed and compared to the traditional anion exchange Q to determine selectivity trends. Proteins were more strongly retained on the multi-modal anion exchange resin and were observed a different elution order as compared to the traditional anion exchanger Q. In addition, mobile phase modifiers such as ethylene glycol, urea and arginine were added into the mobile phase and unique selectivity trends were achieved in multi-modal chromatography.

This work will improve our understanding of these complex chromatographic systems and will be helpful to develop new chemometric modeling approaches and classes of molecular descriptors for biopharmaceutical applications.