Modeling of ion-exchange chromatography: Are we making the right assumptions?

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\section*{Introduction}

Efficient modeling of ion-exchange chromatography requires knowledge of the full adsorption isotherm that describes how resin properties, buffer pH and ionic strength affect protein adsorption.

Commonly, protein adsorption is described by stoichiometric displacement models, taking into account a stoichiometric exchange of counter-ions bound to the adsorber surface with charged proteins in the mobile phase, and vice versa.

These abstract models contain only empirical pH dependences and do not explicitly consider resin or protein properties. Model parameters can therefore only describe a specific adsorber-protein combination.

Non-stoichiometric adsorption models describing a colloidal interaction between protein and adsorber can provide a deeper mechanistic understanding of the adsorption process. They explicitly contain protein properties that are valid on different adsorbers.

\section*{Model Derivation}

The protein is considered to be a perfect sphere. Its surface charge density depends on the primary sequence of the protein, its size, and the buffer conditions (right). When the protein approaches the adsorber surface (below), an interaction takes place that is defined by an attractive Maxwell stress and a repulsive osmotic pressure. Due to the changing electrostatic environment, the protein regulates its charge as it approaches the surface. A kinetic formulation for the adsorption isotherm was developed assuming a short relaxation time. The final expression contains only closed analytical expressions and is therefore suitable for model-based process development.

\section*{Model Calibration}

Model parameters for a monoclonal antibody (mAb) on Fractogel EMD SE HiCap were taken from the primary sequence or determined by inverse modeling using isocratic experiments at a pH between 4.9 and 6.5. Protein parameters determined by inverse modeling were the mAb size, an equilibrium coefficient, and the pK value of the imidazole side chain of histidine. The ability of the mechanistic model to extrapolate beyond observed pH conditions is indicated by the prediction at pH 7.0 shown on the right.

\section*{Resin Transfer}

The validity of the model parameters on different adsorber systems was demonstrated by a model transfer from Fractogel EMD SE HiCap to YMC BioPro SP. Only the equilibrium coefficient is an adsorber-dependent parameter and had to be determined on the YMC adsorber at pH 5.4. Despite the new adsorber system, model predictions at pH 4.9, 5.9, and 6.5 are in good agreement with experimental data.

\section*{Simplified Parameter Estimation}

The proposed non-stoichiometric model provides deep mechanistic insights on how resin properties, protein properties, and buffer conditions affect protein adsorption on strong and weak ion-exchange resins. In contrast to traditional stoichiometric models, the pH dependence is explicitly described by the primary sequence of the protein and is therefore valid over several pH levels.

Moreover, protein parameters are not only valid for a specific adsorber system. Model complexity is limited to a small number of parameters that must be determined by inverse modeling. All model parameters are subject to physically meaningful limits which enables rapid parameter estimation and supports the use of homology models in model calibration.

\section*{Antibody Isoforms}

Efficient process modeling requires not only the description of the main component but also of impurities. Antibody isoforms often pose a difficult separation problem due to the similar physicochemical properties to the main component. Depending on the post-translational modification (PTM), they differ only slightly in the number of amino acids. Since the model is based on the primary sequence of the protein, it can predict how the PTM affects adsorption behavior.