Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Complex binding and elution behavior of therapeutic proteins under column overloading conditions

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The binding and elution behavior of two therapeutic bispecific monoclonal antibodies (bsAbs) on the strong cation exchange resin POROS™ XS is investigated and modeled over broad ranges of pH, salt concentrations, and column loadings. One of the two bsAbs exhibits common Langmuir elution behavior under high loading and column overloading conditions, whilst the other bsAb exhibits uncommon anti-Langmuir elution behavior as a consequence of multi-layer binding on the stationary phase surface. The frequently used Steric Mass Action (SMA) model modified with an activity coefficient for the salt in solution is used to simulate the Langmuirian elution behavior. A Self-Association Steric Mass Action (SAS-SMA) model extended with two activity coefficients for the protein and salt in solution is applied to describe the anti-Langmuir elution behavior. The SAS-SMA model is able to describe self-dimerization on the resin surface and thus can predict anti-Langmuir elution behavior. The binding models are each combined with a lumped rate model to describe mass transfer inside the chromatography column.
To apply these models for describing protein elution over wide ranges of pH, the pH-dependences of all model parameters, including the linear and especially the non-linear model parameters, are investigated, described, and implemented into the binding models. Therefore, extensive data sets were generated that consist of linear gradient elution experiments comprising a pH range from pH 4.5 to 8.9 and column loadings from 0.5 to 90.0 mg_{bsAb}/mL_{resin}. The modeling results of both antibodies show that the pH of the mobile phase has a strong influence on the non-linear model parameters, thus valuable process insights can be gained by interpretation of these results. An increasing buffer pH leads to an increase in binding sites shielded by the antibodies, whilst self-dimerization on the resin surface becomes less with increasing pH. Empirical correlations describing the non-linear model parameters as functions of pH are established and implemented into the SMA and SAS-SMA formalisms. The functionality of these modified pH-dependent binding models is verified with linear salt, pH and dual gradient elution experiments using single-component simulations. Most of these experiments can be accurately predicted under high loading and overloading conditions, whereby especially the peak shapes are well-described. Slight discrepancies between the simulated and experimental data can be observed for some of these experiments, especially when they were performed under overloading conditions.
In this dissertation, it is clearly shown that these discrepancies are not primarily a consequence of limitations of the SMA and SAS-SMA models. At lower pH values (pH ≤ 5.3), overloading phenomena such as protein breakthrough during the loading phase, additional peaks, and peak-shoulders occur. The outcomes of additional experiments in which the antibodies were loaded onto the column with different counterion concentrations and loading times show that intraparticle diffusion effects and conformational changes of the bsAbs are responsible for these overloading phenomena at low pH. The applied lumped rate mass transfer model is not adequate here since it cannot describe hindered intraparticle transport and should be extended to consider these effects. Additional peaks and peak shoulders due to bsAb conformations can only be predicted by describing multi-state binding, which is shown in this dissertation for one case by a simple extension to a multi-component simulation. Furthermore, it is shown that the description of complex peak shapes arising due to competitive binding and multi-component elution of the antibodies' charge variants cannot be adequately predicted using single-component simulations. However, an extension of the model to a simple multi-component system consisting of two charge variants enables accurate prediction of some of these complex elution profiles.