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Modeling of pH and solvent modulated chromatography for the purification of biopharmaceuticals

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Biopharmaceuticals are one of the strongest growing sectors in modern medicine and every state-of-the-art production process for biopharmaceuticals relies on column chromatography for protein purification to ensure a safe and effective product. Ion-exchange chromatography (IEC) is the method of choice for the removal of product related impurities such as fragments, aggregates or other undesired variants of the target protein. A detailed process understanding is desired to predict the influence of important process parameters on the performance of the chromatographic separation.
The IEC model presented in this work captures the influence of the salt concentration, the pH and the solvent composition of the mobile phase. Linear salt and pH gradient elution experiments were performed with different monoclonal antibodies (mAbs) and a bispecific antibody (bsAb) in the presence of different mobile phase modulators to characterise the influence of the mentioned process parameters. Different additives, namely polyethylene glycol (PEG), glycine and D-sorbitol were used to modulate the composition of the mobile phase. Chromatographic experiments were performed at low protein concentrations in the linear range of the adsorption isotherm to determine the fundamental parameters describing the IEC adsorption equilibrium. Static batch adsorption measurements in combination with chromatographic experiments at elevated protein concentrations were performed to investigate the influence of salt concertation, pH and solvent composition at increasing column loadings by using the steric mass action (SMA) isotherm.
The results show that the influence of the used mobile phase additives can be described by the thermodynamic model presented in this work. The model calculations confirm that the effects of the solvent modulators are related to the asymmetric activity coefficient of the protein in solution. This effect was found to be independent of the chromatographic stationary phase and is furthermore correlatable with the apparent solubility of the respective protein in solution. A beneficial effect of PEG on the separation of proteins with similar isoelectric point (pI) but difference in size was confirmed. The addition of glycine and D-sorbitol as solubility enhancers allow the addition of PEG at elevated column loadings. The determination of adsorption isotherms based on static batch adsorption measurements allows the prediction of protein elution profiles at elevated column loading also in the presence of mobile phase additives. Furthermore, pH induced elution procedures were predicted at increased column loading, which is of particular importance for a possible industrial application. The required model parameters can be established based on a manageable set of experiments and the parameters are interchangeable between pH and salt induced elution procedures. For these reasons, the presented modeling approach could be of importance for future downstream process development for biopharmaceuticals.