Experimental planning of protein runs for mechanistic modeling of ion exchange chromatography in bind-elute mode

A mechanistic chromatography modeling workflow typically requires a model calibration step which includes system and column characterization, protein experiments, and parameter estimation (Fig 1).

This document provides you with the expertise to choose the protein experiments to model ion exchange chromatography (IEX) process in bind elute (B/E) mode.

Although it is impractical to provide a precise guide on the type of experiments to conduct, some useful experiments are provided.

The experiments described are the standard operating protocol (SOP) that may vary based on your type of modeling project. This document should be treated only as a high-level guideline to plan the calibration experiments.

Experiments to characterize protein-adsorber interaction

The goal of the experimental plan is to need as few experiments as possible to gain knowledge on as many physical aspects as necessary. In general, a set of about 5 experiments is needed to acquire suitable information about the protein-adsorber interaction. The exact number of experiments depends on the complexity of the process (e.g., including various pH values) and also on the desired model application. As an example, a model that shall be used to determine first process conditions in early phase requires less accuracy than a model that shall be applied for process characterization in late phase. For model calibration different experimental scopes can be used from robotic columns scale to pilot, or even manufacturing scale. As data quality is more important than data quantity, lab-scale experiments provide the best suited data. In contrast to smaller or bigger scales, bench top experiments enable better system and column characterization.

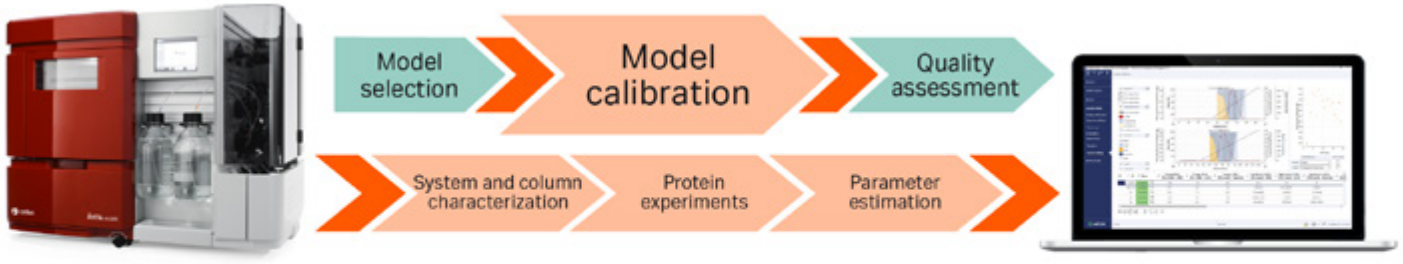

Fig 1. Outline of a mechanistic chromatography modeling workflow, with a focus on model calibration.

Here is a list of the generally useful experiments for working with a constant pH:

- Three experiments using linear gradient elutions with different gradient slopes at low load density (e.g., 1 g/L) in the linear isotherm range. The experiments can be performed at different flow rates and allow to obtain information about the protein charge and the equilibrium constant of the isotherm. To apply it, the Yamamoto method is integrated in GoSilico™ Chromatography Modeling Software.
- One experiment featuring a step elution (be careful to perform a pump wash previous to the step). This experiment gives information about the time dependent parameters such as mass transfer/ pore diffusion parameters and binding kinetics

As a general guideline you can set the salt step concentration to the approximate salt concentration where the proteins are eluting in the initial salt gradient elutions, i.e. at the peak maximum. If the goal of the modeling project is to obtain an optimized step elution, one or two additional step elutions at different salt concentrations should be added to the calibration space. The other salt step concentrations could be distributed below and/or above the concentration of the first step, e.g., +/- 0.05 mol/L.

- One experiment at high load density of 80% of DBC (at 10% breakthrough) to obtain information about protein interactions and ligand shielding.

For more complex models (e.g., including pH dependencies), additional experiments need to be perfomed. In the case of pH variations, at least two linear gradient elutions should be conducted at a different pH. Currently, the simpler pH dependent isotherms are only suitable for narrow pH ranges. Therefore, those additional experiments should use a pH not exceeding +/- 0.5 pH units. In the case of a broader pH range, you may require a more sophisticated pH model.

Offline analytics

In the fraction analysis, a variety of different analytical methods can be performed and implemented in GoSilico™ Chromatography Modeling Software. Frequently used analytical methods are size exclusion chromatography (SEC) and IEX if there are critical charge variants of the product. Sometimes it is also possible to explain non-symmetric peak shapes considering charge variants. It is preferable to conduct the offline SEC and IEX analytics at the same wavelength as the recording of the chromatogram. Apart from the offline analysis of the mAb variants, additional components such as host cell proteins (HCP) or DNA can be analyzed to be modeled in GoSilico™ Chromatography Modeling Software.

To obtain an accurate model, the resolution of the different peaks needs to be fine. As the resolution depends on the fraction size and the number of analyzed fractions, we recommend a fraction size of 0.5 CV if possible (for lab-scale columns of 5 to 10 mL).

It is strongly dependent on the individual process to choose where it is well suited to take the fractions, and the number of fractions to be analyzed. We suggest taking fractions throughout the process and selecting the fractions to analyze later. Because of the trade-off between resolution and effort, a “rule of thumb” for B/E chromatography would be to analyze some fractions wherever some peaks are visible in the UV signal. This could include:

- 1 to 3 fractions distributed through your potentially occurring load breakthrough
- ~ 3 fractions of your wash
- ~ 3 fractions of your strip
- About 5 to 10 fractions (depending on the peak width) of the elution peak chosen at the positions where the impurities of your process are expected to elute. If too many fractions are collected, you can analyze every second.

To calculate the mass balances of the proteins, we recommend analyzing the feed material loaded onto the column.

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