Doctoral Dissertation
Doctoral Program in Chemical Engineering (34th Cycle)

Resume of the Thesis

Politecnico di Torino
January 2022

# Title: Analysis of a chromatographic purification process: an experimental and modelling combined approach

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To bring a new biopharmaceutical product to market is an expensive and time-consuming process. Traditional biopharmaceutical process development is based on experimental work by DoE, trying to explore all conditions to find an optimum and gaining a partial knowledge of the process. By traditional approach an optimal and economic process must be found exploring a large number of parameters. In the downstream processing the most used purification process is chromatography. Integrating the standard procedure with a mechanistic model can increase our knowledge, speed up the process development and the scale up/down.

The aim of this study, carried out in the GSK Vaccines laboratories in Siena and in Politecnico di Torino, is to identify the main principles guiding the chromatography behaviour and describe them with a first-principle model. The test case chosen for this study is the polishing step of a recombinant protein, that consists in a Hydrophobic Interaction Chromatography (HIC).

Since the hydrophobic adsorption behaviour is still not very clear, a thermodynamic study is performed using different commercial proteins as case study with hydrophobic resins. Static methods (high-throughput experimentations) and dynamic methods (breakthrough curves) are used to obtain proteins' adsorption isotherms and the data obtained with the two methods are compared. Two different modelling approaches are then evaluated and compared.

The predictive approach consists in finding isotherms parameters from the fitting of experimental adsorption data (the experimental data are fitted with different isotherm laws) and mass transfer parameters from literature correlations and use them with an in-house code to simulate bind-elute tests.

The in-house code can solve the system of Partial Differential Equations describing the component behaviour in the column. The simulated and experimental results of bind-elute tests are compared.

The estimative approach exploits a commercial software (DSPX from GoSilico) that performs the curve fitting using optimization algorithms that minimize the difference between experimental and simulated chromatograms, estimating the model parameters. To perform the parameter estimation, the bind-elute experimental chromatograms are used. The model found is validated simulating a run that is performed in operating conditions different from those used for the parameter estimation.

To investigate the impact of additional components on single-component adsorption behaviours, a high-throughput procedure is also applied to mixtures of commercial proteins. Binary mixtures of commercial proteins are tested in different conditions of salt and protein ratios to investigate their behaviours in hydrophobic interaction chromatography. The aim is to mimic a typical industrial chromatographic step where a target protein must be separated from mixtures of other proteins, like the industrial test case chosen for this study. The multi-component adsorption isotherms are determined with a full high-throughput procedure, exploiting microfluidic capillary electrophoresis in a high-throughput platform for the analysis. This method resulted quick and efficient, with an adequate accuracy considering the advantages of the high-throughput set up: very small amount of sample is needed and the time of test and analysis are very short.

Furthermore, to define a suitable model for the industrial chromatographic step chosen, DSPX is exploited to model the industrial process. Several runs were performed manipulating the process parameters that mainly affect the separation. Experiments are coupled by offline measurement exploiting Size Exclusion Chromatography (SEC) to determine concentrations and purities of the species involved in the purification process. *In silico* simulations are performed on these experiments to develop the model. DSPX is more accurate in describing the industrial process and requires less information and experimental work. The lesser need of experimental work to develop a model of an industrial process is crucial because it saves time and materials, that often are expensive and not available in large amount.

*In silico* models appear to be able to provide sufficiently accurate information about the process and can help the scale up/down and process development with a low workload.