WHEN THE DOE FAILS

In this case study, a high-capacity cation-exchange resin was used. The target component was an intermediately eluting antibody. One low molecular weight (LMW) and two high molecular weight (HMW) species were to be removed. All simulations, parameter estimation, and process optimization were performed with GoSilico’s software ChromX. While the impurities are hardly visible in the chromatogram (right), an SEC fraction analysis (bottom) shows that the LMW impurity strongly overlaps with the target antibody such that LMW removal would result in less than 70% yield.

FROM BATCH-WISE TO CONTINUOUS PRODUCTION

As fully integrated continuous processing is being adopted by the biopharmaceutical industry, the individual batch-wise processes have to be replaced by continuous equivalents.

Periodic counter-current chromatography (PCCC) systems are able to cope with ternary mixtures. PCCC has been successfully employed as first capture step and design charts have been developed for such cases by Carta and Perez-Almazor. However, intermediate IEX purification steps are much more sensitive to small changes in the process parameters.

MEMBRANE CAPTURE FOR A VLP

VLPs are composed of multiple copies of recombinantly produced viral structural proteins which assemble into empty particles. They can either mimic the structure of viruses or present epitopes of foreign pathogens or tumor cells on their surface. Process development for VLPs is a complicated task, as there are no platform process available, yet. Mechanistic modeling can be used to efficiently identify optimal process conditions. In a case study, we used only three gradient runs on a Sartobind AEX membrane capsule to design a capture step for the purification of Human B19 Parvo-virus-like particles (30nm) derived from insect cell culture.

The model was calibrated using two tracer runs with and without the column connected and three gradient elutions in non-linear mode. Additional information was obtained by offline SEC fraction analyses. ChromX automatically generated pseudo chromatograms from the relative fractions content determined by SEC, shown as dashed lines in the plots on the right.

All unknown model parameters were determined by curve fitting. The plots show the target protein and all impurities. Once the model is calibrated, subsequent process development and optimization can be done solely in the computer.

Compared to the DoE experiments the load volume was reduced and the column length was increased. Together with optimized salt elution steps, a high yield and purity could be achieved.

Because of the many degrees of freedoms of PCCC set-ups, such as flow rates, buffer concentrations, cycle duration, etc. model-based process development is the method of choice to identify the design space and optimal conditions. As the protein-parameters can be taken directly from the batch-mode model, only the flow sheet is substituted.

The optimal process conditions for single-column batch and 3-column (3C) PCCC processing were determined with an evolutionary multi-objective optimization regarding yield, purity and production rate. All optimization variables are listed on the right together with the Pareto surface for 3C-PCCC.

GoSilico is a spin-off company of Karlsruhe Institute of Technology (KIT) that develops software and methods for computer-aided – in silico – bioprocess development.

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