Application of mechanistic modeling to speed up purification process development of novel formats: A virus-like particle case study

Tobias Hahn[1], Maria Casals[1], Thiemo Huuk[1], Jens Wölfel[2], Christian Niehus[2], Nikola Strempel[2] ([1] GoSilico, Karlsruhe, Germany, [2] Cevec Pharmaceuticals, Cologne, Germany)

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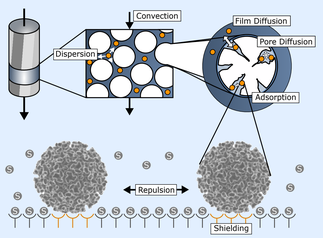
INTRODUCTION

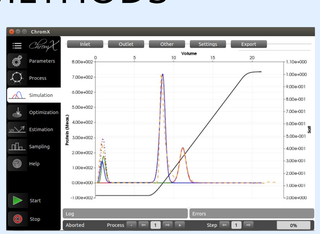
Virus-like Particles (VLPs) are a promising complex molecular format for the development of vaccines. Their size and shape enable an easy uptake by antigen-presenting cells (APCs) resulting in an effective induction of an adaptive immune response. A very time and labor consuming part in developing a VLP production process is the downstream process development. Conventionally, the operational parameters are optimized by Design-of-Experiments studies (DoE). Dozens of experiments are needed to screen a process design space.



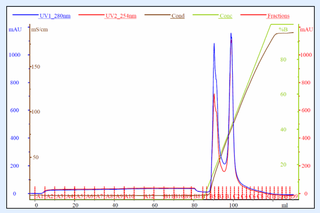
In contrast, mechanistic models describe the transport of sample components using fluid dynamic and thermodynamic principles. Further, they allow for efficient process optimization in silico.

MATERIALS AND METHODS

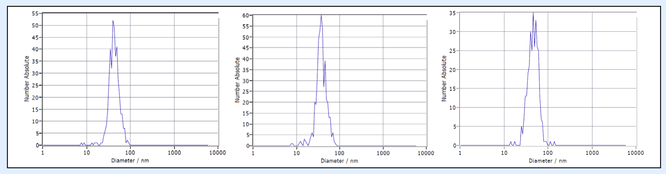
VLPs are assembled by recombinant expression of viral structural proteins. A method for the large-scale production of VLPs is the expression in suspension cell culture. CEVEC's CAP®Go platform was employed to produce a flavivirus VLP of ~ 40 nm size. As a first purification step, tangential flow filtration was used.
The selected chromatography medium was a CIMmultus QA 1 mL monolithic adsorber (BIA Separations). Chromatography experiments were conducted with an Äkta Explorer (GE Healthcare). Chromatograms and additional fraction analysis were imported to GoSilico's ChromX software for modeling and optimization.



The ChromX user interface with an exemplary model.



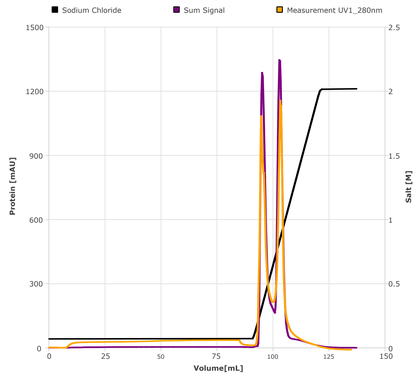
Initial reference chromatogram exported from GE Unicorn.



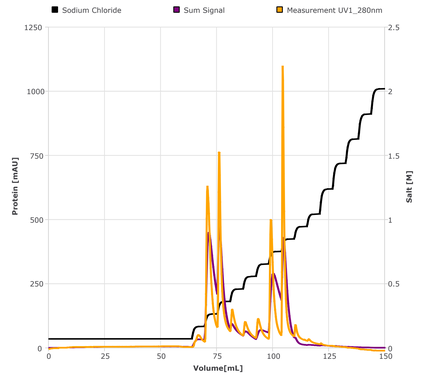
Nanoparticle tracking analysis of feed (left), first elution peak (center) and second elution peak (right), showing two VLP variants.

1 MODEL CALIBRATION

Initially only two chromatograms, one gradient and one step-wise elution, were recorded. Using only spectral analysis, 6 different feed components were identified. A radial-flow column model was chosen together with the SMA model for ion-exchange chromatography. Model calibration was accomplished using the built-in peak detection and parameter estimation methods of ChromX. After uncertainty quantification (see Model Quality), a first in silico optimization was done using a combination of a step and a gradient.



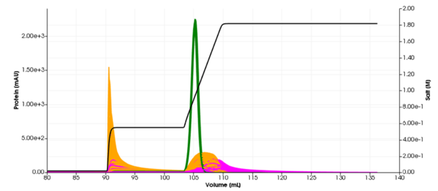
The first experiment using a gradient elution shows two VLP peaks.



A step-wise elution was performed to identify a suitable level for peak separation.

2 MODEL QUALITY

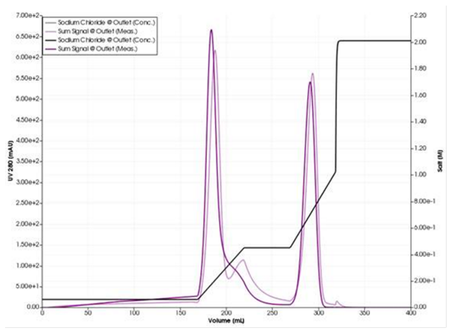
While the visual model fits are very good, only the calculated 95% confidence intervals provide information, if the parameters are well identified. The uncertainty of the estimation of components 4 (orange) and 6 (pink), which elute close to the product (green), leads to a possible co-elution. The resulting chromatograms and yield distribution for a 1000-point Latin Hypercube Sampling is shown below.



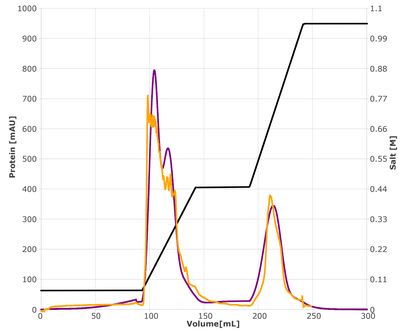
Uncertainty in the model parameters of component 4 (orange) and 6 (pink) leads to a variety of chromatograms at optimized process conditions (first VLP peak not shown).

3 MODEL-BASED PROCESS OPTIMIZATION

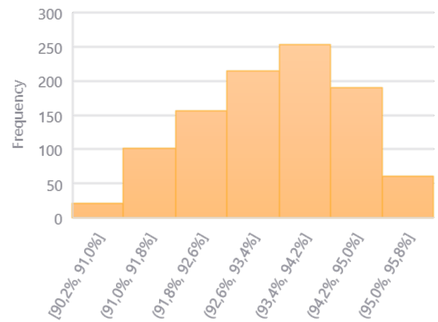
Robust elution conditions were developed based on the preliminary model. To improve model quality while aiming for base-line separation, the first step was replaced by a gradient. Most of the host cell proteins could be removed and > 95 % of the VLPs could be recovered. Furthermore, the elution conditions achieved the desired base-line separation. Alternatively, a flow-through of the first peak could be considered.



A robust optimization was performed that shifts component 4 in the first peak.



The validation run is in good agreement with the model-based prediction.



The predictivity of the initial model was evaluated in a 1000-point LHS sampling. The average yield is predicted to be 93.3% with 95% confidence interval of ± 0.1%

IMPROVED PROCESS UNDERSTANDING

Based on the finalized model, understanding of product quality attributes and their relation to process parameters could be shown. This is a requirement of the Quality-by-Design guideline set forth by EMA and FDA. Especially valuable in this case is the mechanistic model's ability to predict the outcome of a scaled-up process, as the employed annulus shaped monolith is only available in a limited set of sizes. In summary, the combination of a limited set of experiments and a mechanistic model enabled a very efficient process optimization and design space characterization. This novel approach could be used whenever no downstream platform process exists yet.

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