Poster #324

# Using mechanistic models to design a platform process for the separation of full and empty AAV capsids

J Senaratne, A Olivares-Molina, M Jenny, B Leewis

Manufacturing Sciences and Technology, MeiraGTx, London

## 1. Introduction

The separation of full and empty AAV capsids presents a challenge due to the similarity in their properties. Anion exchange chromatography is commonly used to achieve this separation by exploiting the difference in charge between empty and filled capsids to achieve this separation.

However, developing these processes requires the optimization of multiple parameters which can require extensive experimentation. Defining a platform process can be further complicated as the developed process needs to work across multiple products with minimal optimization.

In this study a mechanistic model was developed for the enrichment of full capsids for an AAV2 product using Sartobind Q membranes. The model used for this study consisted of the equilibrium-dispersive model of chromatography (Eq. 1) coupled to a steric mass action isotherm (Eq. 2), and this model was solved using GoSilico™ (Cytiva).

The developed model was used to explore the design space of the process with a view to establishing a process that would deliver consistent performance across multiple products. This included:

- Evaluating the bind and elute vs. weak partitioning operating modes.
- Identify strategies for improving robustness of process.
- Understanding the impact of variables such as load ratio and feed full capsid ratio that are likely to differ from product to product.

$$\frac{\delta c}{\delta t}(x,t) = -\frac{u(t)}{\epsilon_{tot}}\frac{\delta c}{\delta x} + D_{app}\frac{\delta^2 c}{\delta x^2}(x,t) - \frac{1-\epsilon_{tot}}{\epsilon_{tot}}\frac{\delta q}{\delta t}(x,t)$$

Equation 1: Equilibrium-dispersive model

$$K'_{kin,i}\frac{\delta q_i}{\delta t} = k_{eq,L,i}\cdot\bar{q}_{salt}^{v_i}\cdot c_{p,i} - q_i c_{p,salt}^{v_i}$$

Equation 2: Steric mass action isotherm

## 2. Data Pre-processing

The mechanistic model was trained on data produced using a qPCR assay and a Gyrolab-based immunoassay to measure the full and total capsid concentrations, respectively. The empty capsid concentrations were then calculated from the difference of these two values.

Consistent full and empty capsid losses were observed during the training experiments which prevented the back fit routine used to train the model from achieving good fit. This discrepancy was corrected for by adjusting feed concentration by a “loss factor” which was calculated from the observed total recovery across multiple experiments.

A discrepancy between the full capsid ratio calculated from the qPCR and Gyrolab results, and AUC results was identified. This was dealt with by calculating the average ratio between these results and applying this as a correction factor to the Gyrolab results.

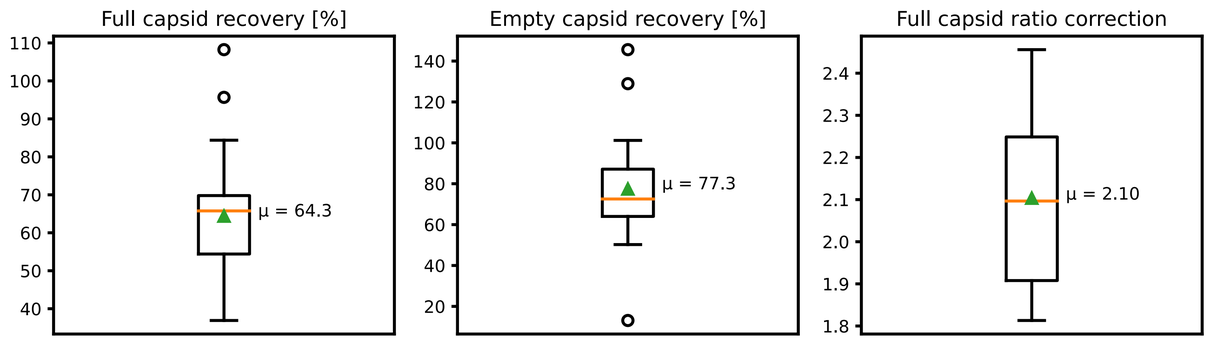


Figure 1: Box and whisker plots showing distributions of points that were used to calculate the loss factor and the full capsid ratio correction.

## 3. Model Training

Three gradient experiments were performed to create the training dataset for this model. The gradient experiments were performed at three different gradient lengths (50, 75 and 100 MV) and the Yamamoto method was used to produce an initial estimate of the isotherm parameters.

The steric mass action isotherm parameters were fine-tuned by backfitting to the results of the three gradients as well as an additional experiment performed under isocratic elution conditions. The isocratic experiment was added to improve the estimation of the kinetic parameter of the isotherm.

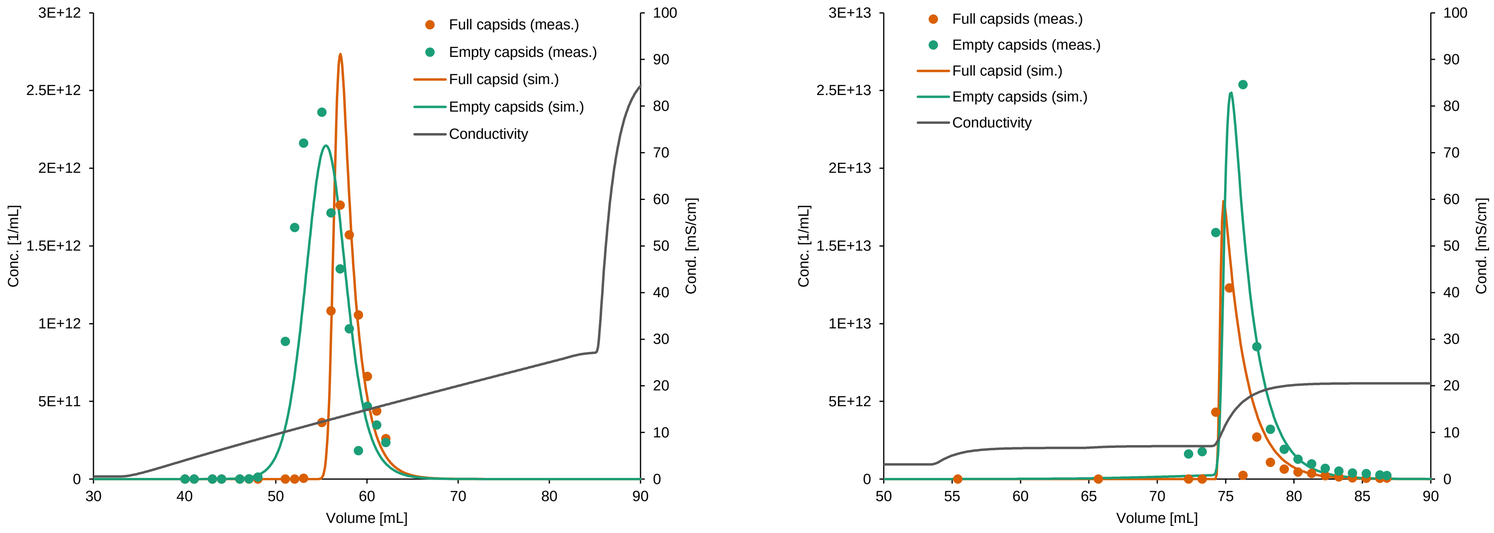


Figure 2: Results of backfitting that was performed to fine-tune isotherm parameters. The figures show the experimental measurements and the simulated results produced using the mechanistic model.

## 4. Model Validation

The accuracy of the model was assessed by simulating the process at the conditions currently used to manufacture this AAV2 product in development.

The model was able to accurately predict the full capsid ratio and the recovery for this process as shown in Figure 3.

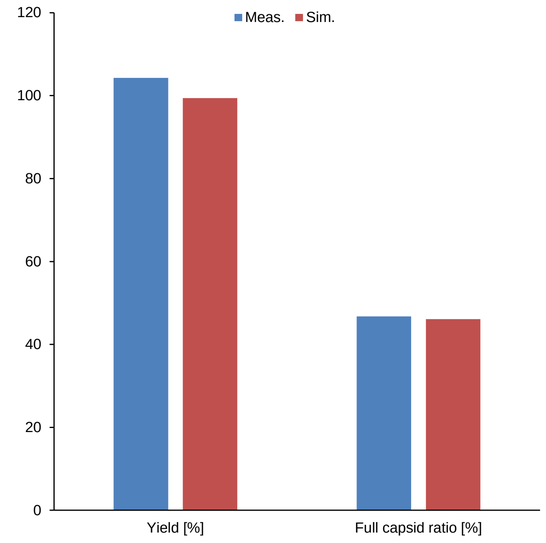


Figure 3: Comparison of the measured and simulated results for the process conditions of the existing process.

## 5. Insights Derived from Model

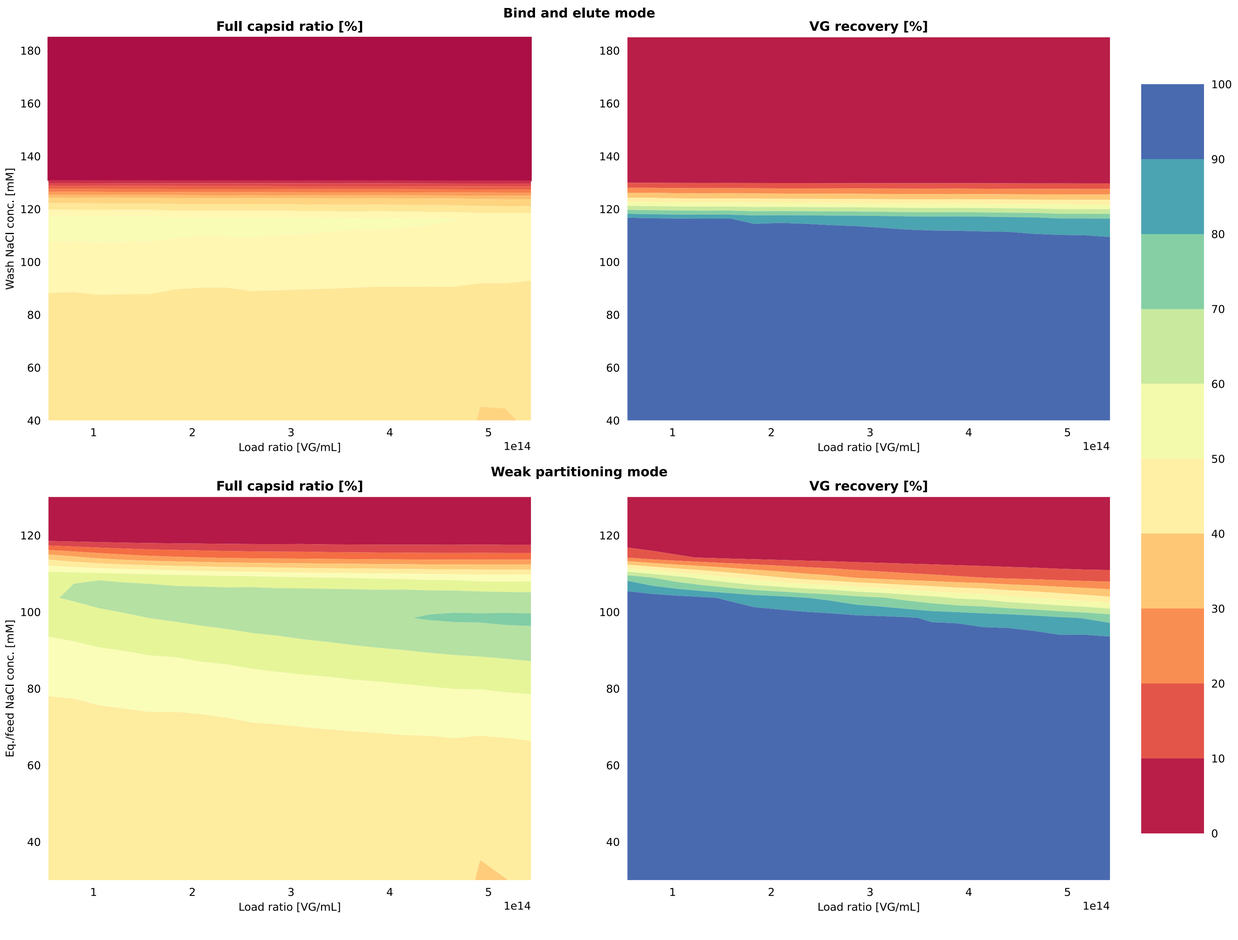


Figure 4: Results of simulations performed to compare performance of Sartobind Q operated in bind and elute (top) and weak partitioning (bottom) modes.

Extensive simulations were performed to understand which operating mode could deliver the best full capsid ratio and recovery and the results are shown in Figure 4.

These results show that weak partitioning mode can deliver significantly higher full capsid enrichment. However, while higher load ratios improve the robustness of this operation, bind and elute mode is not affected by the load ratio.

Products in the early stages of development can suffer from low titers and poor encapsidation which a platform process would need to process robustly. Figure 5 shows that the load ratio does not have a large impact, but the feed full capsid ratio is strongly correlated to the product full capsid ratio.

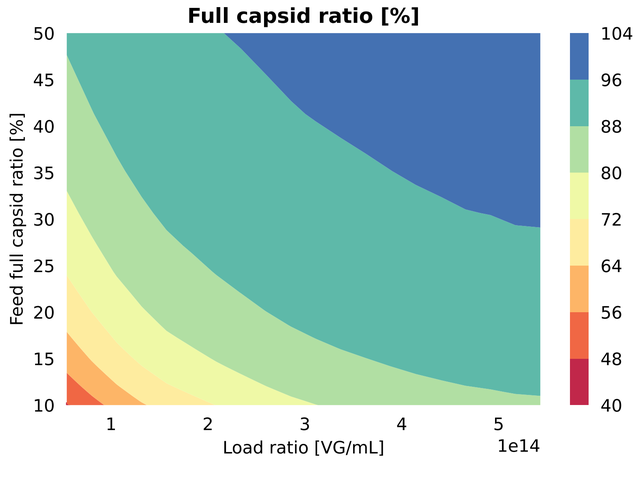


Figure 5: Results of the weak partitioning simulations performed to evaluate the impact of feed full capsid ratio and load ratio on eluate full capsid ratio when operating at an eq./load NaCl conc of 100 mM.

## Conclusions

This study shows that a mechanistic model can be trained and fine-tuned using a relatively small set of input data. However, some pre-processing of the input data was required to achieve a good model fit.

The resulting model was used to produce detailed characterizations of the process design space which in turn were used to optimise the platform process.

Using the model to evaluate the platform process showed that bind and elute mode was more robust but weak partitioning mode could deliver greater full capsid enrichment.

## Acknowledgements

Pia Graf and Nicholas Whitelock from Cytiva for support with developing and optimizing the mechanistic model.