SPEEDING-UP PROCESS DEVELOPMENT OF MIXED-MODE CHROMATOGRAPHY WITH MECHANISTIC MODELING

Application to the purification of a recombinant protein

Lucrèce Nicoud[A], David Pfister[A], Célia Valentim[B]

A Ypso-Facto, Nancy, France.
B Sanofi, Vitry sur Seine, France.

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INTRODUCTION

- **Mixed-mode chromatography** is widely used for **protein purification** in the biopharmaceutical industry.
- **Mechanistic modeling** is increasingly recognized as a valuable tool to speed up **process development** and **process optimization**.
- In this poster, we show how mechanistic modeling can be used to **predict the impact of critical process parameters** on key performance indicators (KPIs) like purity, yield, productivity and Process Mass Intensity (PMI).
- This work was performed in the frame of the **CALIPSO** project, a 5-year program led by Sanofi and co-financed by the French state.
- All experimental data shown in this poster were generated by Sanofi, while the model was developed by Ypso-Facto.

PRESENTATION OF THE CASE STUDY

The product

- The product to be purified was a 111 kDa antibody.
- The main impurity to be removed was a 99 kDa fragment of the antibody, noted LMW (Low Molecular Weight).
- The separation was challenging due to the high similarities between the product and impurity.

The process

- The purification was performed on Capto™ MMC ImpRes resin from Cytiva, whose ligand exhibits both **ionic** and **hydrophobic** interactions with the target molecule *(Fig. 1).*
- The column volume was 15.7 mL.
- The purification protocol was as follows:

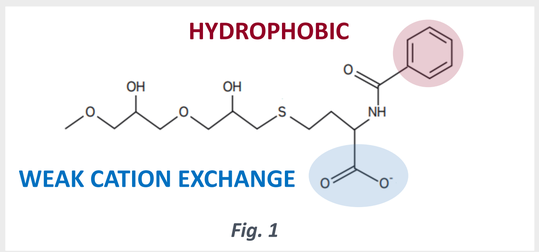

Fig. 1

1. Equilibration with 50 mM Acetate pH 5 + NaCl 20 mM
2. Load of post VI material adjusted at pH 5
3. Wash with 50 mM Tris pH 8, with or without NaCl
4. Wash with 50 mM Tris pH 7
5. Elution with 50 mM Tris pH 7 + 150 mM NaCl
6. Strip with 50 mM Tris pH 7.5 + 1 M NaCl
7. Equilibration with 50 mM Acetate pH 5 + NaCl 20 mM

PRESENTATION OF THE MODEL

- The model developed in this work derives from the ion exchange (IEX) model embedded in Ionic and presented in Kobl et al, OPRD, 2024, 28, 7, 2569–2589.

- The IEX model in particular accounts for:
  - The dissociation equilibria of buffer species (here Tris and Acetate)
  - The evolution of the protein net charge with pH, which can be calculated theoretically from the amino acid sequence *(Fig. 2).*
  - The competition of ions at the surface of the chromatographic medium (here the product, the LMW impurity, but also Na+ and Tris+)

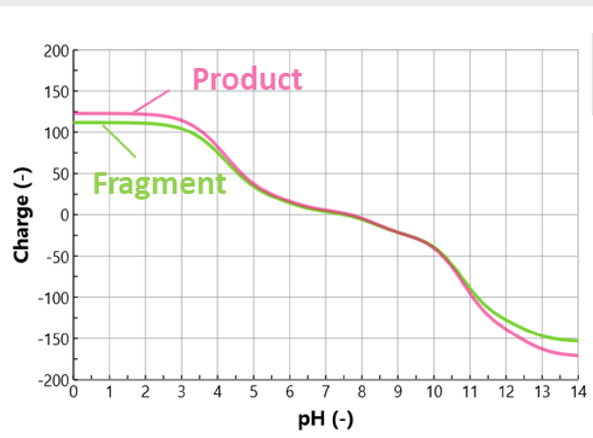

Fig. 2

- This IEX model was coupled with the Hydrophobic Interaction Chromatography (HIC) model which was presented previously in Nicoud et al, Biopharm Int., 2023, 36, 04, 22–29. Briefly, the HIC model relies on an activity coefficient calculated in solution based on the Truesdell-Jones equation.

CONCLUSION

- A mechanistic model for **mixed-mode chromatography** has been developed. The new model combines **ion exchange** and **hydrophobic interaction chromatography** models presented previously.
- The model has been shown to describe well a set of experimental data generated by Sanofi.
- Only ONE purification experiment has been used to estimate model parameters.
- The model has been used to predict the impact of two **critical process parameters**, namely loading and salt concentration in the wash, on the performances of the purification process.
- This illustrates how mechanistic modeling can be used to speed up process development.

RESULTS

Fitting of model parameters

- The model parameters were fitted using a series of pulse experiments performed at different NaCl concentrations and two pH values *(Fig. 3)* together with a reference experiment with fraction analysis *(Fig. 4 and Fig. 5).*

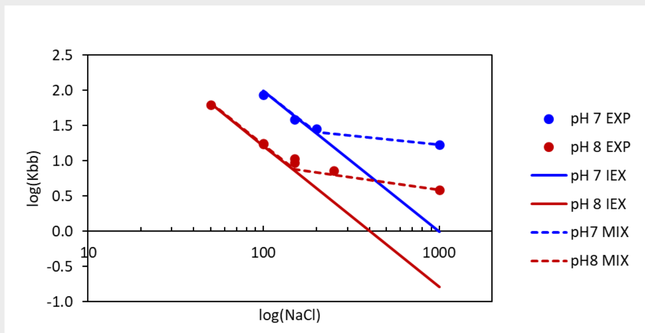

Fig. 3

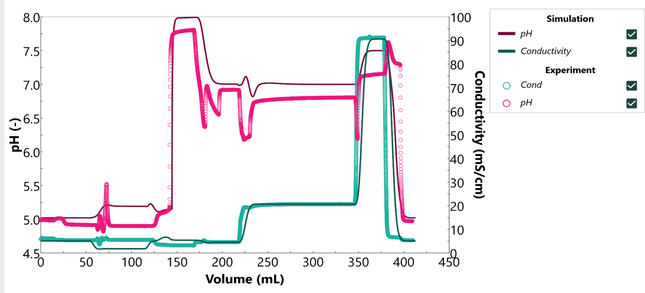

Fig. 4

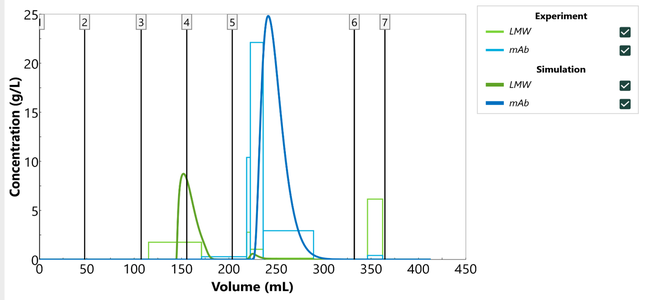

Fig. 5

- In the case of a pure IEX behavior, the retention of the protein in pulse experiments is expected to vary linearly with the salt concentration when plotted in a log-log scale (solid lines in *Fig. 3*). It is seen that the experimental data show a deviation with respect to the IEX behavior (symbols) and that the developed mixed-mode model is capable of describing the experimental data (dashed lines).

- The model parameters were fine tuned using one reference purification experiment. The model predicts both the pH and conductivity profiles *(Fig. 4)* as well as the product and impurity concentrations in collected fractions *(Fig. 5).*

Illustration of model use in process development: prediction of the impact of critical process parameters

- The model was then used to predict the impact of 2 critical process parameters, loading and the concentration of NaCl in the wash, on 4 KPIs, namely purity, yield, productivity and PMI *(Fig. 5).*

- It is observed that a low concentration of NaCl in the wash (around 25 mM) helps improving purity. However, the presence of NaCl in the wash buffer has a drastic negative impact on the yield.

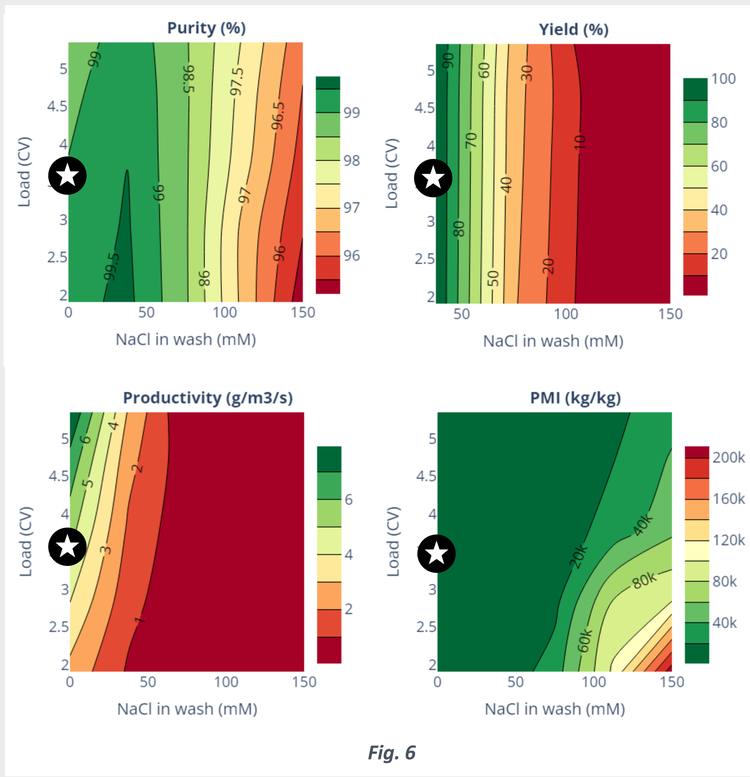

Fig. 6

- Loading has been found to have a low impact of purity and yield. But an increase in loading was shown to improve productivity and PMI, which is directly related to eluent consumption.

- The operating conditions that were selected independently by Sanofi during process development with the more classical “DoE” approach are indicated by stars on contour plots. It is seen that the model supports the choice of these conditions, leading to a sufficient purity, with a satisfactory yield and acceptable values of productivity and PMI.

- It worth highlighting that the results of the mechanistic model were obtained using a series of pulse experiments (that do not require fraction analysis) and ONE purification experiment only (requiring fraction analysis). As a comparison, the classical “DoE” approach requires tens of purification experiments. This study therefore illustrate how mechanistic modeling can be used to speed up process development.

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