SIMULATION-AIDED PROCESS DEVELOPMENT

Application to peptide purification by RP chromatography

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INTRODUCTION

- **Reversed-phase chromatography (RPC)** is popular for **peptide** purification
- Establishing a chromatographic process is labor- and time-intensive
- Calibrating a **mechanistic model** can be done with fewer experiments
- Such a model can then be used to quantify the impact of **Process Parameters** on **Quality Attributes** and **Process Performances**
- This provides the basis to determine the **Proven Acceptable Range** of process parameters

MATERIALS AND METHODS

Experimental details

This case study extends previous work done for RPC using a Kromasil 100A 10 µm C8 medium packed in a 25 x 0.46 cm column (see *Guetaz et al.* 2013 Journal of Chromatography A).

Mechanistic model

The model uses the mixing cell approach with the Linear Driving Force approximation together with a specialized isotherm for reversed-phase binding.

$$\bar{\bar{C}}_i = \underbrace{\frac{H_{1,i}\,C_i}{1+\frac{H_{1,i}}{\bar{\bar{N}}_{1,i}}C_i}}_{\text{Langmuir type interaction}} + \underbrace{\frac{H_{2,i}\,C_i\left(1+I_{ii}\frac{H_{2,i}}{2\bar{\bar{N}}_{2,i}}C_i\right)}{1+\frac{H_{2,i}}{\bar{\bar{N}}_{2,i}}C_i+I_{ii}\frac{H_{2,i}}{\left(2\bar{\bar{N}}_{2,i}\right)^2}C_i^2}}_{\text{Moreau type interaction}}$$

where $\bar{\bar{C}}_i$ is the concentration in the solid phase, C_i is the concentration in the liquid phase, $H_{1,i}$ and $H_{2,i}$ are the Henry coefficients for Langmuir and Moreau interactions, $\bar{\bar{N}}_{1,i}$ and $\bar{\bar{N}}_{2,i}$ are the capacities for the Langmuir and Moreau interactions, and I_{ii} is an adsorbate–adsorbate interaction parameter

RESULTS

Excellent agreement between experiments and simulation for the product

- Experimental and simulated data show:
  - Langmuir type, tailing elution peak for low loading amounts (≤ 0.2 g/L column)
  - Moreau type, fronting elution peak for higher loading amounts

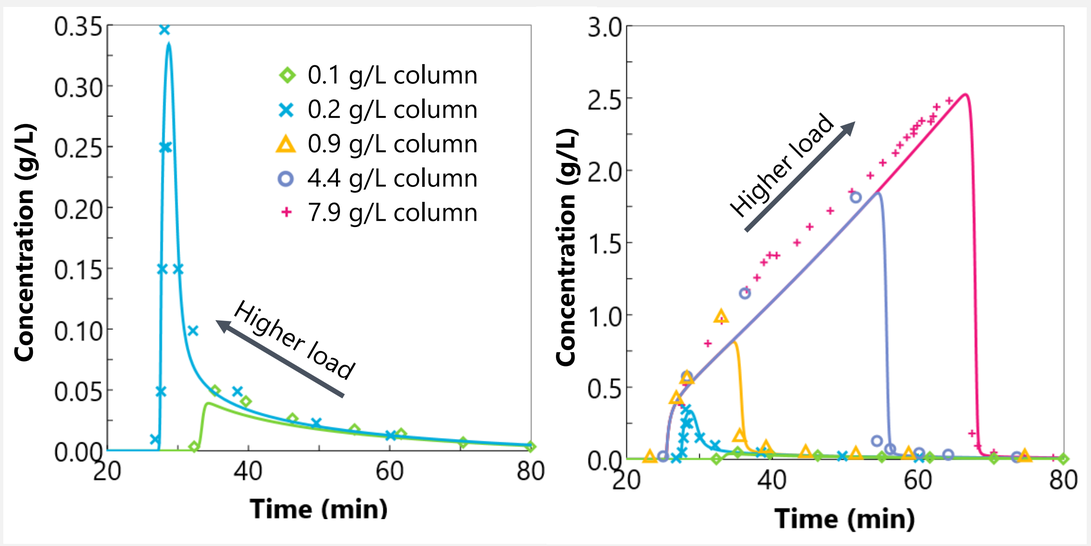

Fig. 1: Prediction of isocratic (25.58 (v/v)% acetonitrile in water) peptide elution over a range of loading amounts. Scatter points show experimental data, lines show simulation predictions.

Excellent agreement between experiments and simulation for competitive binding with impurities

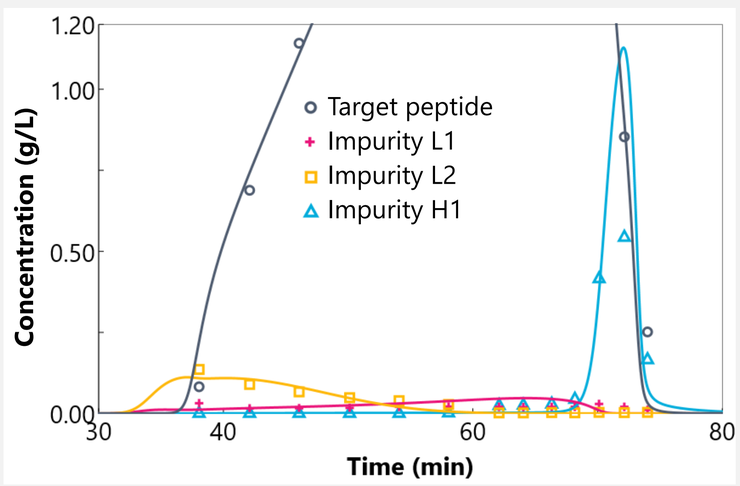

Fig. 2: Prediction of isocratic (25.58 (v/v)% acetonitrile in water) peptide elution for a 7.5 g/L load including impurities. Scatter points show experimental data, lines show simulation predictions.

RESULTS

The following process is considered for preparative purposes:

- 2 Bed Volumes equilibration with 22 v/v% acetonitrile in water
- Variable loading over a range of 1.6 to 6.5 g/L column
- Elution gradient from 22 v/v% up to 36 v/v% over a range of 40-160 minutes

Quantification of the impact of process parameters

- **Simulations** were performed to explore the **Design Space** by varying load (in g/L column) and gradient duration (in minutes)
- The collection strategy was defined
  - To: Maximize yield
  - While: Maintaining purity > 97%

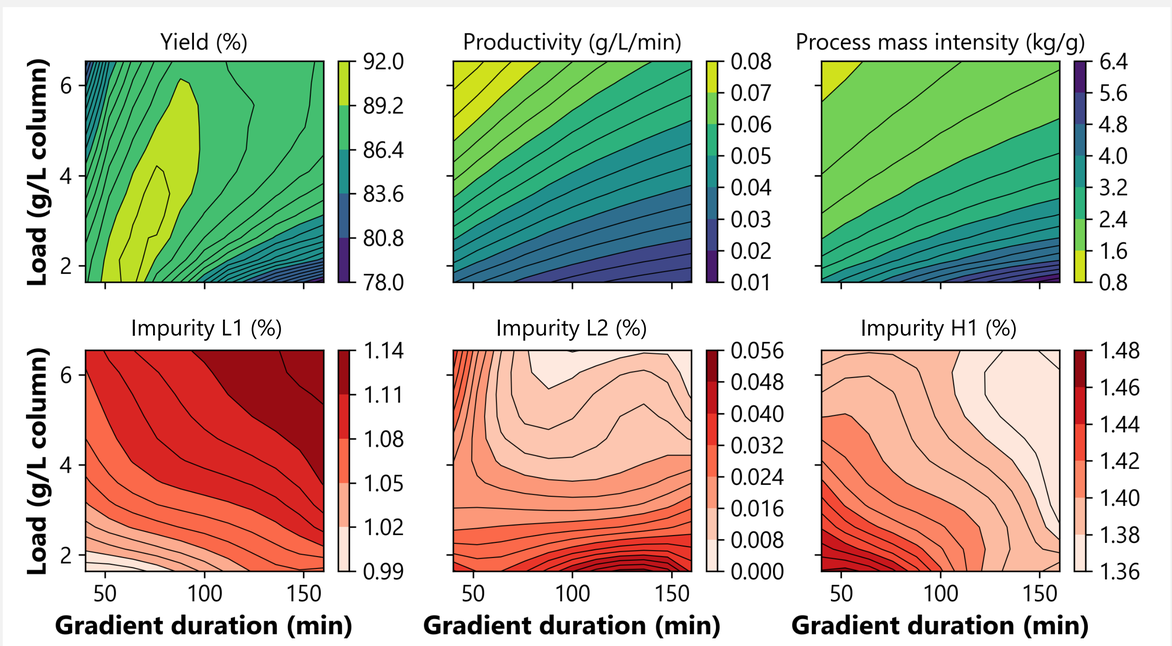

Fig. 3: Simulated impact of process parameters.

Recommendation of the Proven Acceptable Range

- **Simulations** were performed to determine
  - the **Edge of Failure** (quality constraints: L1<1.1%, L2<0.032%, H1<1.4 %)
  - **economically viable conditions** (performance constraints: Yield>86%, Productivity>0.03 g/L/min)
- The following **Proven Acceptable Range** was recommended
  - Load: **2.3 – 5.8** g/L column
  - Gradient duration: **51 – 114** min

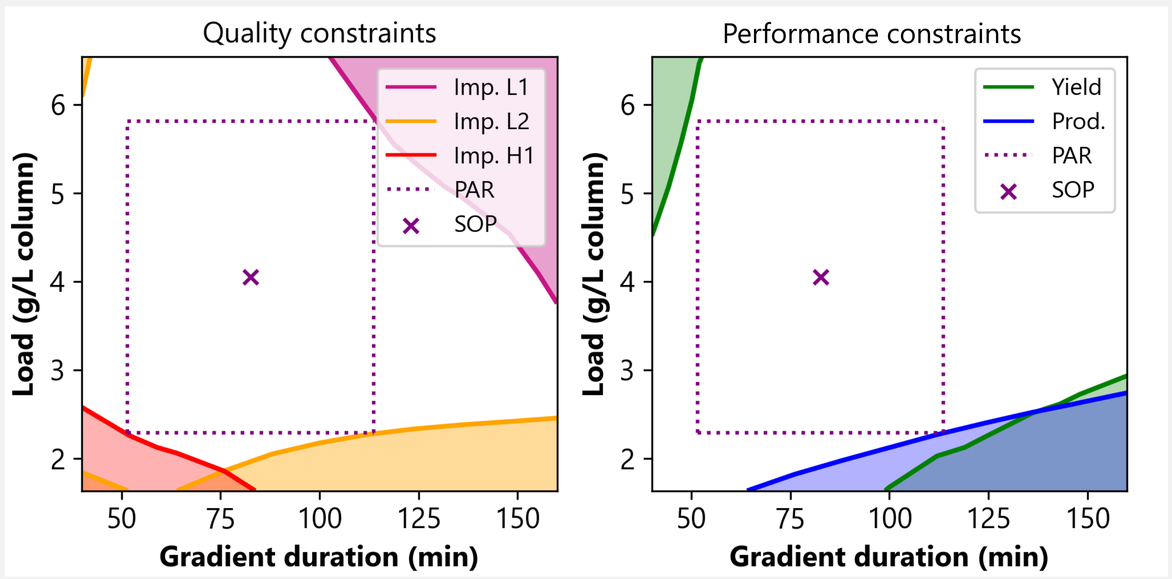

Fig. 4: Determination of the Edge of Failure (left) and economically viable conditions (right). Recommendation of the standard operating point (SOP) and Proven Acceptable Range (PAR).

CONCLUSION

- A mechanistic model for reversed-phase peptide chromatography was developed. The model was proven capable of representing **complex peptide interactions** with the solid phase, leading to peculiar behaviors.
- The model was then used to identify the range of **process parameters** allowing to meet both **quality** and **performance constraints**.
- This provides a solid basis to determine the **Edge of Failure** and provide recommendations on the **Proven Acceptable Range**.

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