

Scalability of mechanistic models for ion exchange chromatography under high load conditions

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Case study

We present an industrial case study on an intermediate purification of a monoclonal antibody (mAb) based on high-protein-load-density ion exchange chromatography (IEX). Under the prevailing circumstances of a high protein load density and a low salt concentration in the protein sample, an unusual elution peak shape occurs. This phenomenon cannot be modeled with the commonly used equations for IEX (Fig 1). We also consider the transferability of small-scale model parameters to process scale up.

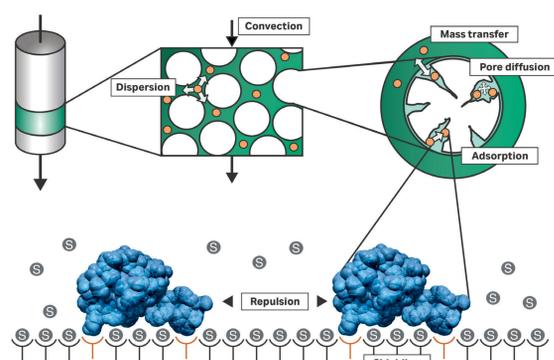


Fig 1. Effects occurring inside a chromatographic column.

Model design and experiments

Experiments

The case study covers a range of commonly used 16 mL lab-scale and 1 mL small-scale columns, down to the 0.6 mL robotic column format. We used an ÄKTA™ chromatography system for the experiments at 1 mL and 16 mL scales and a Tecan Freedom EVO™ liquid handling station for the robotic column experiments.

A strong cation exchange resin was used for this cation exchange step. The running buffer for all experiments was a 10 mM sodium citrate buffer at pH 5.0 with additional sodium chloride. The mAb is of IgG class and derived from Chinese hamster ovary (CHO) cell cultivation. The mAb pool was purified by preparative affinity chromatography. The antibody concentration was 12.7 g/L, with a monomer content of 98.3%, as quantified by size exclusion chromatography.

Simulation

We carried out chromatography simulation and isotherm parameter estimation using GoSilico™ Chromatography Modeling Software (formerly ChromX™ software) (1), which can create models from raw chromatograms and allows *in silico* process scale up or scale down.

A transport-dispersive column model and lumped rate pore model were combined with the general rate isotherm. We used a genetic algorithm for parameter estimation and refined the estimation with the deterministic algorithm CERES.

References

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Model implementation

Isotherm definition

Under low load conditions, we observe a typical Gaussian peak shape that transforms into a trapezoidal shape with increasing load (Fig 2). Using Mollerup's generalized ion exchange (GIE) isotherm (Eq 1)(2), the observed elution peak shapes could be recovered. Compared to the commonly used steric mass action isotherm (3), the GIE isotherm introduced two additional parameters to approximate the asymmetric activity coefficient.

$$k_{kin} \frac{\partial q_i}{\partial t} = k_{eq} (\lambda - \sum_{j=1}^n (v_j + \sigma_j) q_j)^v \tilde{\gamma} c_p - c_{salt}^v q_i$$

$$\tilde{\gamma} = e^{k_p c + k_{salt} c_{salt}}$$

Eq 1. Mollerup's generalized ion exchange model.

Parameter estimation

We showed that the parameters can be determined by inverse peak fitting (4), which suggests that further process development can be done quickly and easily *in silico* (see 2–4 for derivation of the isotherm models and detailed parameter interpretation).

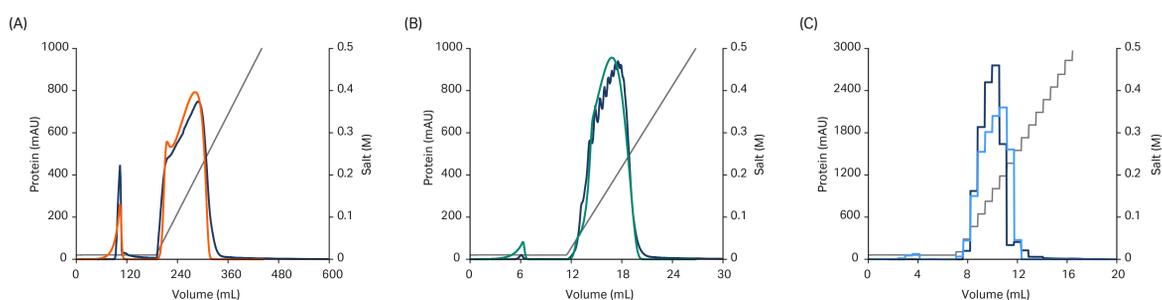


Fig 2. Example simulated and measured chromatograms at equal residence times, salt concentrations, and column loading in (A) 16 mL scale, (B) 1 mL scale and (C) 0.6 mL scale. Simulated curves are plotted in orange (A), green (B), or light blue (C). Measurements are in dark blue. Partially adapted from (4).

Results and discussion

Scale up

To demonstrate scalability, we applied the estimated model parameters from the 1 mL and 0.6 mL scale to predict the elution behavior on the 16 mL scale (Fig 3). The column dimensions, porosity, and ionic capacity remain scale dependent and were determined separately (4). Figure 3 shows that the differences in model parameters lead to only minor changes in the simulated chromatograms. Hence, the differences in peak shape across scales (Fig 2) can be accurately predicted under the assumption that the thermodynamic properties of the system stay constant.

Evaluation

While the model fits (Fig 2) and predictions (Fig 3) are very good, Table 1 shows that the median difference of the estimated parameters from 16 mL to 0.6 mL scale is 12%, and 20% from 16 mL to 1 mL scale. However, most parameters lie within the 95% confidence intervals of the 16 mL scale, so the parameter estimates are transferable. Only K_p is not well identified.

Table 1. 95% confidence intervals (CI) of 16 mL parameter estimates and deviations of the estimated parameter values from 0.6 mL and 1 mL experiments

Parameter	95% CI of 16 mL scale estimates	Parameter deviation from 0.6 mL to 16 mL scale	Parameter deviation from 1 mL to 16 mL scale
k_{eff}	± 7%	- 13%	- 20%
k_{kin}	± 78%	- 48%	- 41%
k_{rel}	± 20%	+ 12%	+ 32%
v	± 4%	- 2%	+ 3%
σ	± 0%	- 1%	+ 5%
K_p	± 8%	- 47%	- 293%
k_{salt}	± 4%	+ 0%	+ 1%

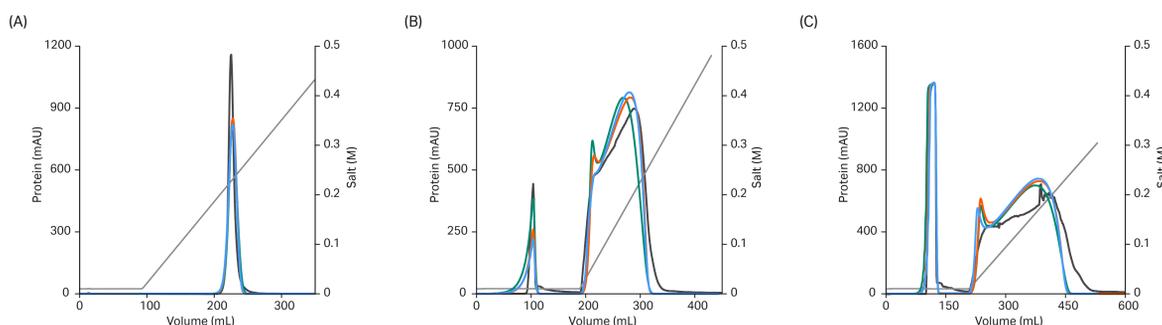


Fig 3. The 16 mL simulation (orange) repeated using model parameters from 1 mL scale (green) and 0.6 mL scale (blue) under (A) low load density and (B, C) high load density. UV measurements are shown in dark gray. Partially adapted from (4).

Summary and outlook

This case study supports the fundamental assumption of *in silico* scale up and scale down.

- Only the fluid dynamics outside the pore system change (Table 2).
- Inside the pores, the same mechanism applies to robotic and laboratory-scale columns.
- The models calibrated from three gradients at 0.6 and 1.0 mL scale were able to accurately predict the 16 mL scale.
- Based on this, we expect scale up to pilot and production scale to be successful.

Table 2. Factors to consider for applying model-based scale up and scale down

Scale down	Scale up
• Packing quality	• Packing quality
• Intermittent flow	• Lot-to-lot variability
• Dispersion	• Dead volume
	• Dispersion