Scalability of Mechanistic Models for Ion Exchange Chromatography under High Load Conditions

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INTRODUCTION

We present an industrial case study on an intermediate purification of a monoclonal antibody based on high protein load densities ion exchange chromatography. Under the prevailing circumstances of a high protein load density and a low salt concentration in the protein sample, an unusual elution peak shape can be observed. This phenomenon cannot be modeled with the commonly used equations for ion exchange chromatography.
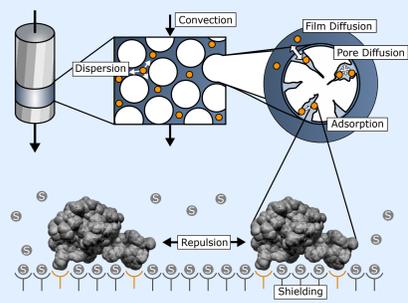
Fig. 1: Effects included in the mechanistic chromatography model.

A second challenge is the transferability of model parameters between different column scales, which is important for process scale-up and the incorporation of small scale data.

MATERIALS AND METHODS

The case study covers a range of experimental systems from commonly used 16 mL lab scale and 1 mL small scale columns, down to 0.6 mL RoboColumn format. The experiments in 1 and 16 mL scale were carried out using an ÄKTApurifier 10, the RoboColumn experiments with a Tecan Freedom Evo liquid handling station.

Poros 50HS adsorber was used for this CEX. The running buffer for all experiments was a 10 mM sodium citrate buffer at pH 5.0 with additional sodium chloride.

The monoclonal antibody is of IgG class and derived from CHO cultivation. The mAb pool was purified by preparative affinity chromatography. It has an antibody concentration of 12.7 g/L, with a monomer content of 98.3% quantified by SEC

Chromatogram simulation and isotherm parameter estimation were carried out using GoSilico's ChromX software. ChromX provides the functionality to create models from raw chromatograms and allows in-silico process scale-up/scale-down.

CV 16mL
Determine column parameters
Estimate isotherm parameters
Prediction 16mL -> 16mL

CV 1mL
Determine column parameters
Estimate isotherm parameters
Prediction 1mL -> 16mL

CV 0.6mL
Determine column parameters
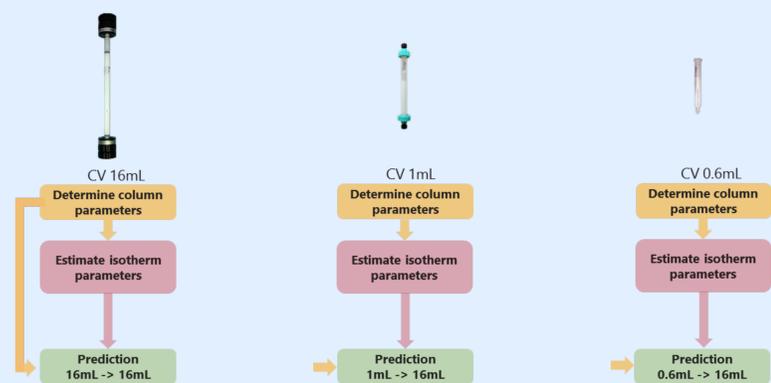Estimate isotherm parameters
Prediction 0.6mL -> 16mL

Fig. 2: Overview of model building: column parameters were determined independently; isotherm parameters were transferred directly.
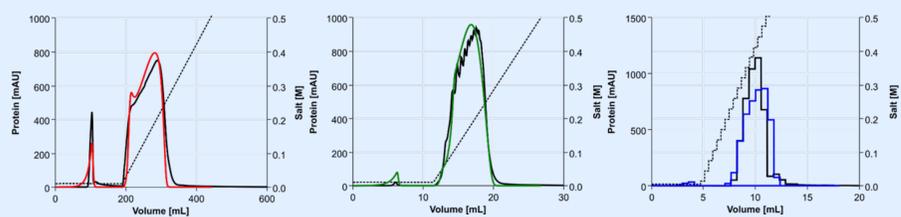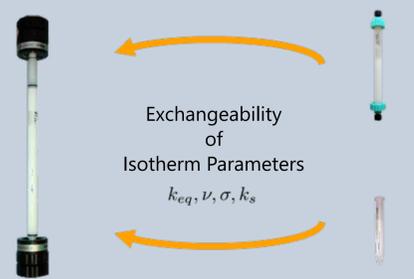
Fig. 3: Example chromatograms at constant residence time, salt concentration, and column loading.

MODEL CALIBRATION

Under low load conditions, a typical Gaussian peak shape can be observed that transforms into a trapezoidal shape with increasing load.

$$k_{kin,i}\frac{\partial q_i}{\partial t} = k_{eq,i}\left(\Lambda - \sum_{j=1}^{k}(\nu_j+\sigma_j)q_j\right)^{\nu_i}\tilde{\gamma}_i\,c_{p,i} - c_{salt}^{\nu_i}q_i$$

$$\tilde{\gamma}_i = \exp\left(K_{p,i}\cdot c_{p,i} + K_{salt,i}\cdot c_{salt}\right)$$

Eq. 1: Mollerup's GIEX isotherm with asymmetric activity coefficient $\tilde{\gamma}$.

Using Mollerup's generalized ion-exchange isotherm (GIEX), the observed elution peak shapes could be recovered. To this end, the GIEX isotherm introduced two additional parameters to approximate the asymmetric activity coefficient.

It could be shown that the parameters can be determined by inverse peak fitting and allow fast and easy model-based process development.
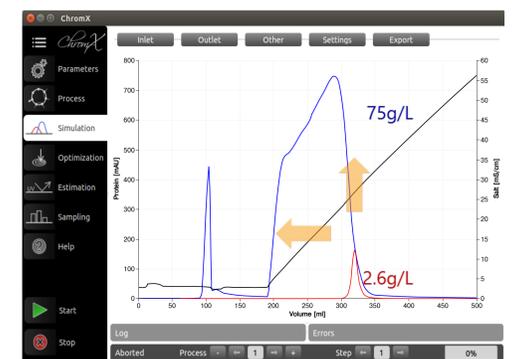
Fig. 3: ChromX GUI with annotated change of peak shape with increasing load.

IN-SILICO SCALE-UP/SCALE-DOWN

We applied the scale invariant model parameters derived from the 1 mL and 0.6 mL scale for the prediction of the elution behavior on the 16 mL scale.

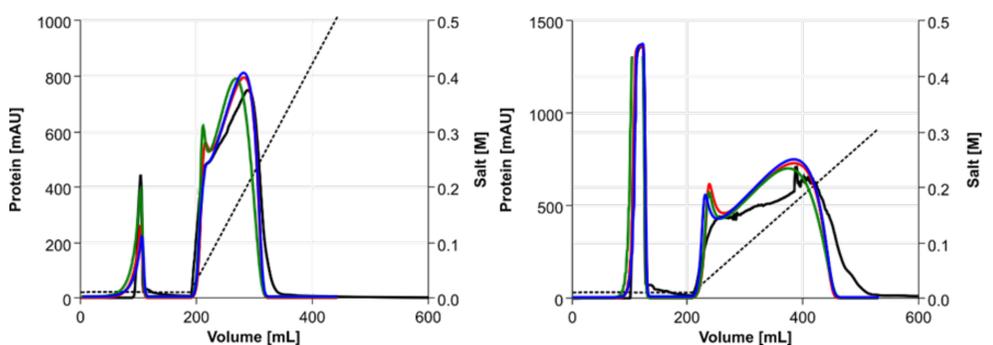Just the column dimensions, porosity and ionic capacity remain scale-dependent.

Fig. 4: 16 mL simulation (red) repeated using model parameters from 1 mL scale (green) and RoboColumn scale (blue) under low load density (top) and high load density (bottom). Original 16 mL scale chromatograms are shown in black.

PARAMETER QUALITY

While the model fits are very good, the median of the difference of the estimated parameters is 12 % in the 0.6 mL and 20 % in the 1 mL scale. Most of the small-scale parameters lie within the 95% confidence intervals of the 16 mL scale. Only k_p is not well identified.

Param.	16mL 95% CI	0.6mL estimate	1mL estimate
k_{eff}	± 7 %	-13 %	-20 %
k_{kin}	± 78 %	-48 %	-41 %
k_{eq}	± 20 %	+12 %	+32 %
ν	± 4 %	-2 %	+3 %
σ	± 0 %	-1 %	+5 %
k_p	± 8 %	-47 %	-293 %
k_s	± 4 %	+0 %	+1 %

Tab. 1: 95 % confidence intervals of 16 mL parameter estimates and deviations of the 0.6 mL and 1 mL parameter estimates.

SUMMARY AND OUTLOOK

This case study supports the fundamental assumption of in-silico scale-up and scale-down of chromatography, that only the fluid dynamics outside the pore system change. Once inside the pores, the same mechanism applies to robotic and production columns.

Even for the observed complex adsorption behavior, the models calibrated from three gradients at 0.6 and 1 mL scale were able to accurately predict the 16 mL scale. A further scale-up to pilot and production scale is expected to work successfully.

Exchangeability of Isotherm Parameters
k_{eq}, ν, σ, k_s

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