Mathematical Modelling of Chromatography as a tool for process understanding and development acceleration

# Mathematical Modelling of Chromatography as a tool for process understanding and development acceleration


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## Introduction

TO BRING A NEW BIO-PHARMACEUTICAL PRODUCT TO THE MARKET

- Expensive and time-consuming
- Optimal and safe process
- Explore a large number of parameters

**CURRENT APPROACH**

STATISTIC DoE → EXPERIMENTATION AT LAB SCALE → KNOWLEDGE GAINED AT LAB SCALE ASSUMED AS VALID IN FULL SCALE

- LARGE AMOUNT OF SAMPLE NEEDED
- LONG TIMES
- PARTIAL KNOWLEDGE

**GOAL**

BUILD A NEW WAY OF WORKING MODELLING THE CHROMATOGRAPHIC PROCESS WITH IN-HOUSE CODE OR COMMERCIAL SOFTWARES

**NEW APPROACH**

STATISTIC DoE+ MECHANISTIC MODEL → IN-SILICO EXPERIMENTATION → VALIDATION OF THE MODEL AND OPTIMIZATION

- REDUCE THE SAMPLE AND TIME NEEDED
- SPEED UP THE PROCESS OPTIMIZATION
- COMPLETE KNOWLEDGE
- PREDICT THE SYSTEM

## CASE STUDY

Downstream Process:
- Homogenization, centrifugation and filtration
- Capture chromatography
- Intermediate chromatography
- Polishing chromatography (Chromatographic Purification)
- UF/DF
- Final filtration

**Polishing step of a recombinant protein purification**

**H**ydrophobic **I**nteraction **C**hromatography

MASS TRANSFER ✓

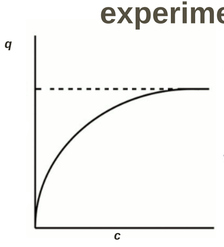
ADSORPTION ?

**MODELLING COMPARISON**

- **PREDICTIVE APPROACH:** Fit experimental isotherms and use parameters to predict elution behaviour
- **ESTIMATION APPROACH:** Use elution tests to find model parameters and use them to fit experimental isotherms

**POC**

Equilibrium adsorption isotherms of commercial proteins are experimentally determined




→ **Static Method**
High-Throughput tests with 96 wells Filter Plates

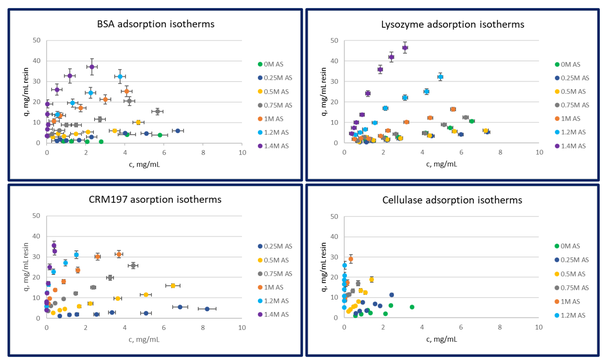
→ **Dynamic Method**
Breakthrough curves with HiTrap columns

BSA, Lysozyme, Cellulase, $CRM_{197}$ → Inexpensive, Well-known

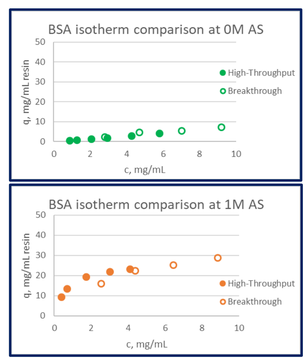
## Results

### EXPERIMENTAL ISOTHERM DETERMINATION

**HIGH-THROUGHPUT** experimentations in 96-wells filter plates, protein concentration increases from 1 to 10 mg/mL for six different salt concentrations in a butylic resin.



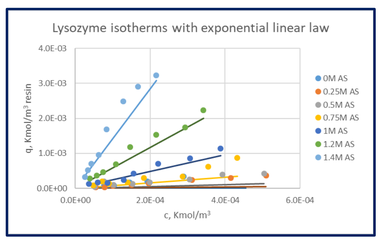

**BREAKTHROUGH** experimentations in 1 mL column with butylic resin at four different protein concentrations and three salt conditions.




**Breakthrough isotherm points compared to high-throughput isotherms seem follow the isotherm shape reaching higher protein concentrations**

### PREDICTIVE APPROACH

**SIMULATION WITH A IN-HOUSE CODE**




PDE System:
- Mass balance in the interstitial liquid
- Mass balance in the pores of the beads
- Equilibrium between solid and liquid

→ Isotherms parameters from experimental data

Test fitting equations that describes adsorption behaviour:

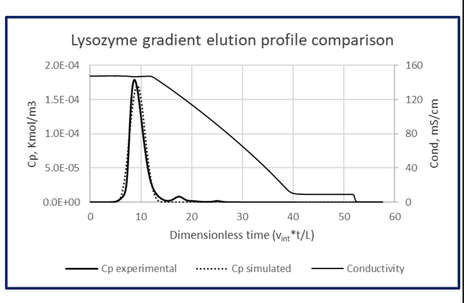
$$q = \frac{\lambda b e^{kC_{salt}} C_p}{1 + b e^{kC_{salt}} C_p}$$

$$q = \frac{\lambda b C salt^{\alpha} C_p}{1 + b C salt^{\alpha} C_p}$$

$$q = a e^{kC_{salt}} C_p$$

- $V_d$, $\varepsilon_p$, $\varepsilon_c$ and $D_{ax}$ are found experimentally with Acetone and Dextran injections.
- $k_f$, $D_m$ and $D_p$ are found with empirical correlations.

**Bind-elute tests with both isochratic and gradient elution are performed and then simulated. Elution profiles are compared to validate the model.**




### ESTIMATION APPROACH

**A commercial software is used to find a suitable model**

CHROM X & DSP X from GoSilico

Optimization algorithm + Curve fitting → Parameter estimation

**Bind-elute experimental tests with isochratic and gradient elution are used to find model parameters**

**Isotherm laws used from the software to model HIC are complex.**

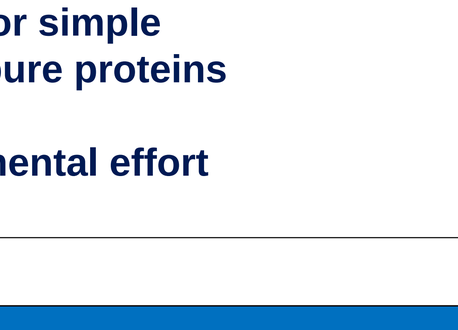
**The laws are (in equilibrium conditions):**

- the law developed by Mollerup et al.

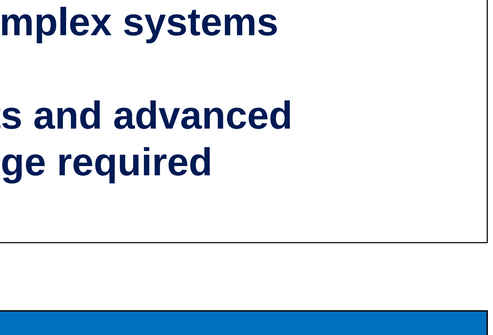
$$q = k_{eq}\left(1 - \frac{q}{q_{max}}\right)^n \exp[(k_s C_{salt} + k_p C_p) C_p$$

- the law developed by Wang et al.

$$q^{1+n\beta} = k_{eq}\left(1 - \frac{q}{q_{max}}\right)^n C_p$$




**Isotherm parameters found from chromatograms fitting are used, as a validation, to fit experimental isotherms**




## Conclusions

### EXPERIMENTAL ISOTHERM DETERMINATION





- High-Throughput method needs small amount of product but requires high experimental effort
- Breakthrough tests are experimentally simple but require a large amount of sample
- Isotherm points obtained with two methods are on the same isotherm

### PREDICTIVE APPROACH



Experiments for parameter determination → Simulations → Experiments to validate the model

- Feasible for simple systems and pure proteins
- High experimental effort

### ESTIMATION APPROACH



Experiments in different operative conditions → Curve fitting → Parameter estimation

- Good for complex systems
- Licence costs and advanced knowledge required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Mollerup J. M., Hansen T. B., Kidal S., Staby A., *Journal of Chromatography A*, 1177 (2008) 200-206.