



Protein A Resin Lifetime Study

Evaluation of Protein A Resin Performance with a Model based approach in continuous capture

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Introduction

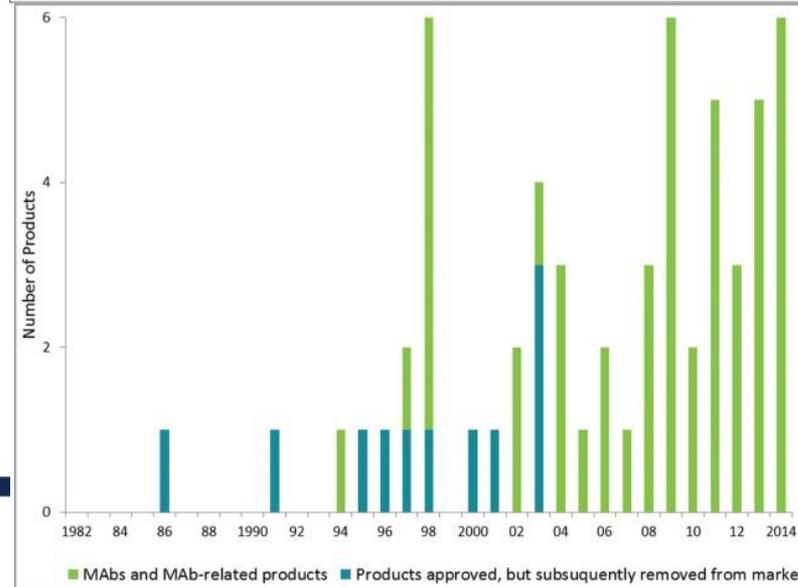
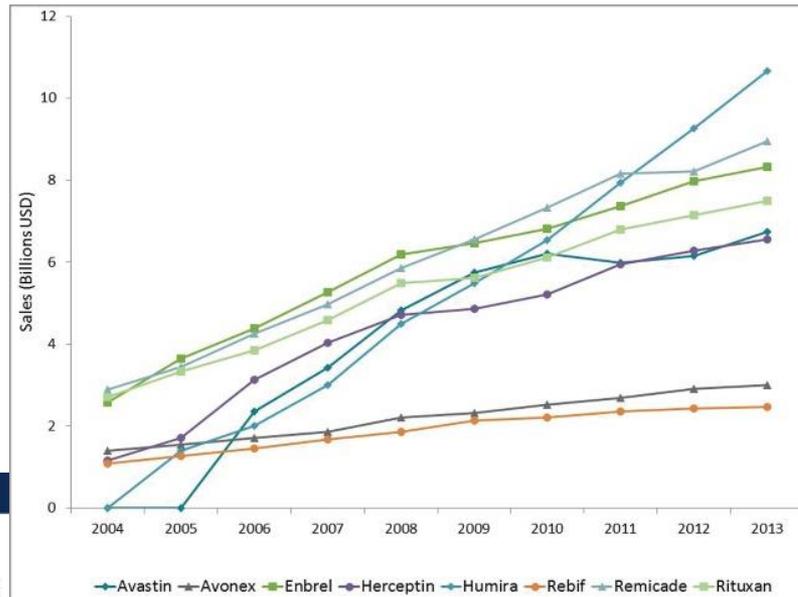
Current Problems & Approach

Model Development: Modified Shrinking Core Model
(MSCM)

Results

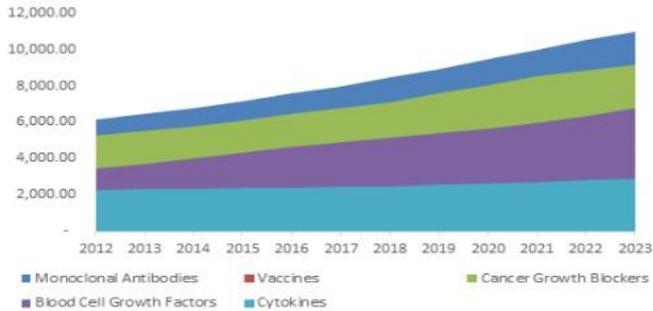
Conclusion

Biopharmaceuticals



Source: Ecker et al, mAbs, 2015

U.S. Cancer Biological Therapy Market size, by product, 2012 - 2023 (USD Million)

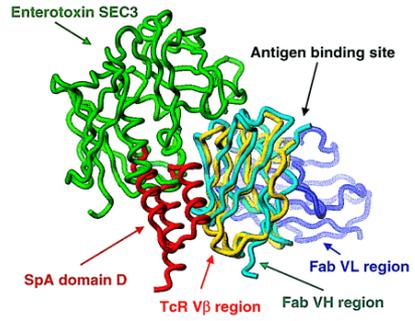


<https://www.gminsights.com/request-sample/detail/676>

Protein A resin

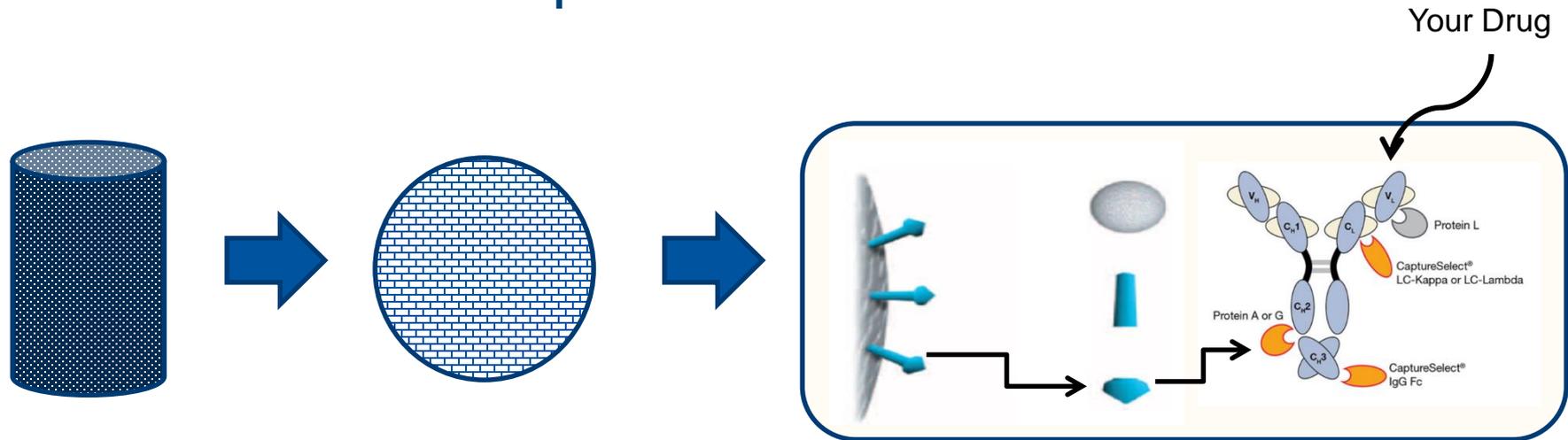
- ❖ Protein A – 42KDa
- ❖ Found in cell membrane of *S.aureus*
- ❖ Functionally made up of 5 domains

- ❖ Affinity binding resin – Capture step
- ❖ Various recombinant forms available
- ❖ Provides > 95% product purity



Disadvantage: Expensive & Protein A degradation

Protein A resin in a packed column

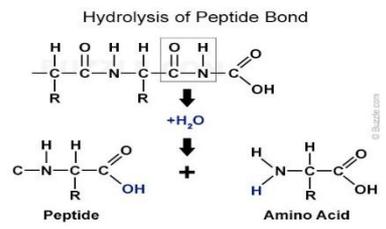
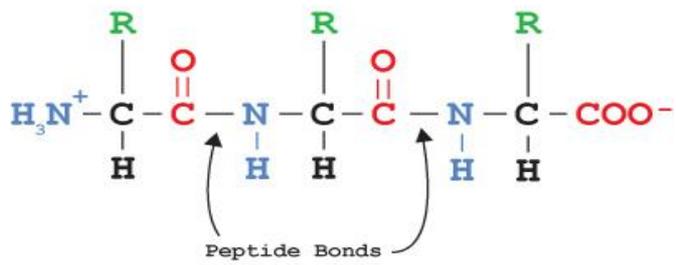


Source: Applied biosystems & GE healthcare

- The column is packed with resin particle
- Each resin particle has numerous pores
- Within each pore are the Protein A ligands
- The ligands are bound to the resin at multiple locations via linker
- *Antibody in the feed specifically binds to the Protein A ligand attached to the column*

Impact of caustic on Protein A

- NaOH – popular in biopharmaceuticals
- Breaks down proteins and saponifies fats
- Inactivates bacteria, yeasts, endotoxins, etc.
- Cost-effective



Cause of Protein A degradation

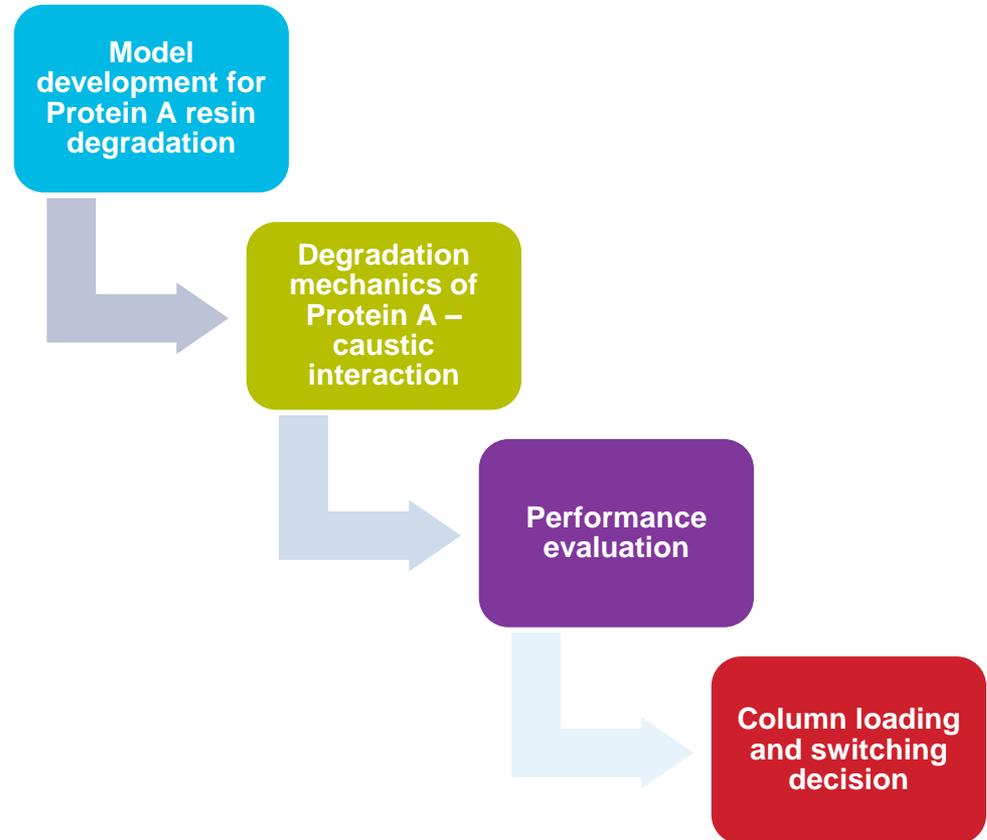
Protein A resin lifetime - Problems

- How do the Protein A ligands degrade?
- What is the effect of NaOH on the Protein A ligands?
- How to compensate for the ligand loss in column switching decision?

Poor understanding of the column after multiple uses

Overview: Protein A resin lifetime study

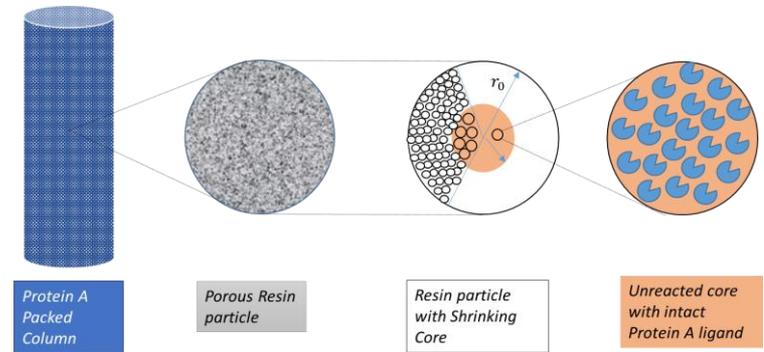
- ✓ **Problem statement:**
 - Protein A degradation
- ✓ **Solution:**
 - A mechanistic model to explain the resin degradation over time
- ✓ **Application:**
 - Provide predictability for the user



Degradation reaction

- leaching of Protein A ligands
- unfolded or denatured Protein A ligands still bound to the resin particle and
- unfolded or denatured Protein A ligands removed during sanitization and elution phase of a typical column run.

Modified Shrinking Core Model (MSCM)



Behere et al, Manuscript under review, Biotech Progress

- Packed column comprises of porous resin particles.
- Each particle has tortuous pore channels
- Protein A ligands bound to the stationary phase matrix.

ISPE March 2017 / Protein A lifecycle study/ Ketki Behere

Model Equations

Fraction of degraded Protein A is given by

Reaction rate at interface

Number of degradable Protein A particles consumed

Reaction rate

Number of degradable Protein A particles available in the resin is given by

$$R = 1 - \frac{r^3}{r_0^3}$$

$$D \frac{dC}{dr} = \alpha k C^n$$

$$\frac{dN}{dt} = -4 \pi r^2 C^n k$$

$$r_e = -k C^n$$

$$\frac{dN}{dt} = 4 \pi r^2 \frac{dr}{dt}$$

R = Fraction of degraded Protein A

r = radius of unreacted core region (cm)

r₀ = initial radius of resin particle (cm)

C = NaOH concentration at a given time (M)

D = diffusion constant (cm²/s)

k = Rate constant

K = k/D

α = stoichiometry factor

N = number of degradable Protein A particles reacted in time t

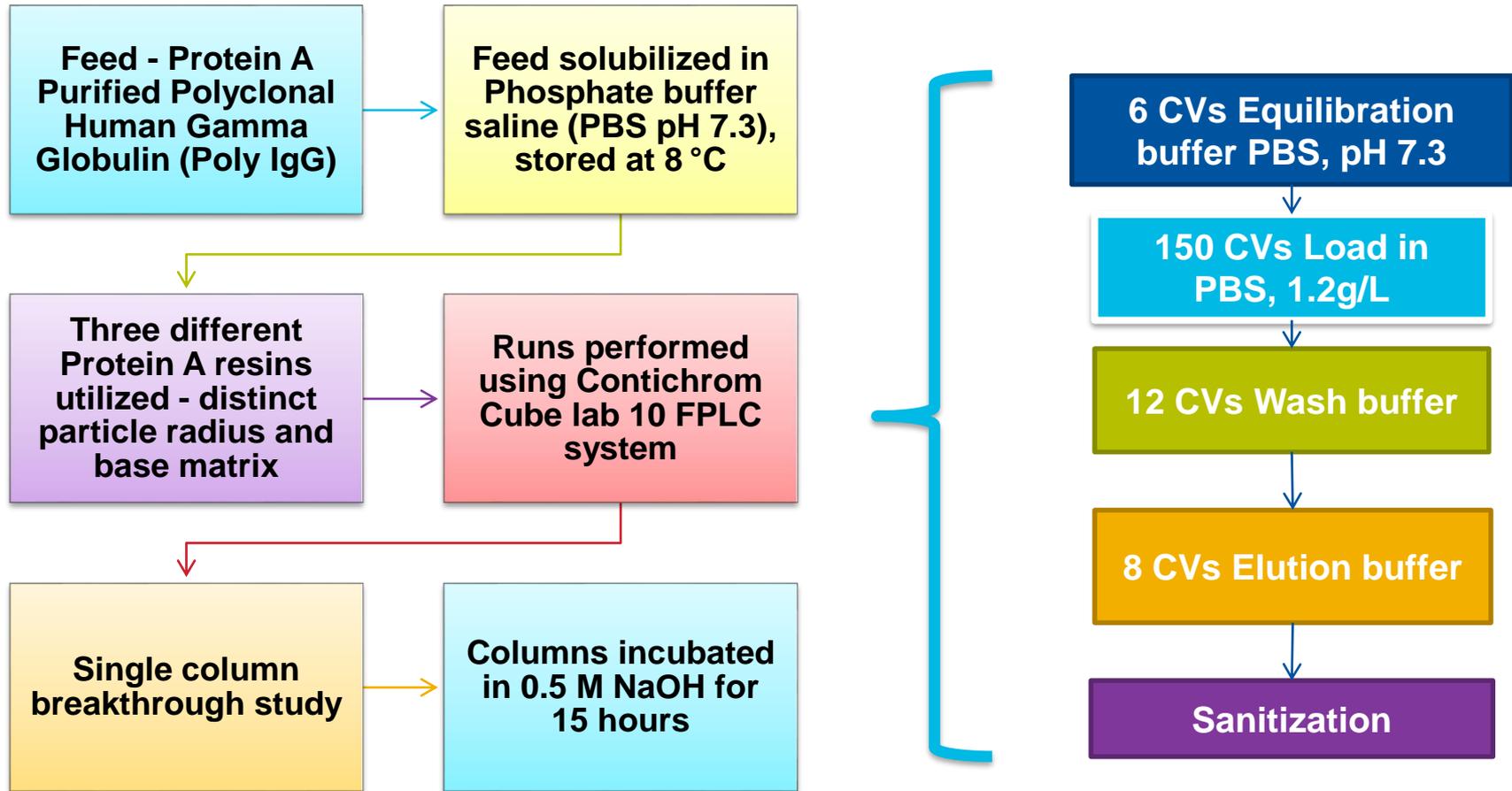
n = reaction order

The model equations were numerically solved using the ode23 solver in MATLAB.

K, n and D parameters estimated using MATLAB

Source for Shrinking Core model: Advanced Separation textbook , Hsu et al 1975

Lifecycle study – experimental protocol



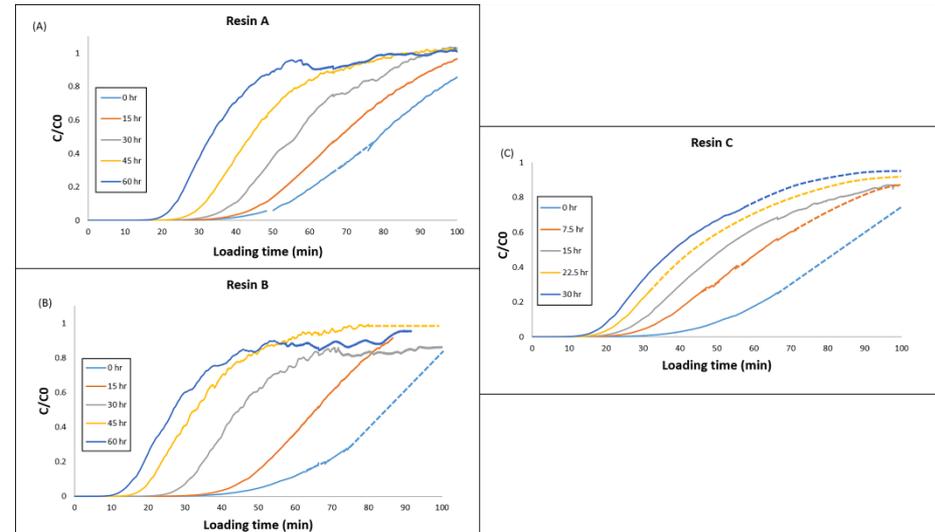
Breakthrough Run Analysis

Various curves represent incubation time of resin in caustic: 0hr (black), 15hr (blue), 30hr (red), 45hr (yellow) and 60hr (green)

The breakthrough curve at higher loading provides a clear distinction of the loading pattern

Variables:

- UV measurement -- Antibody concentration
- Conductivity
- pH
- Pressure



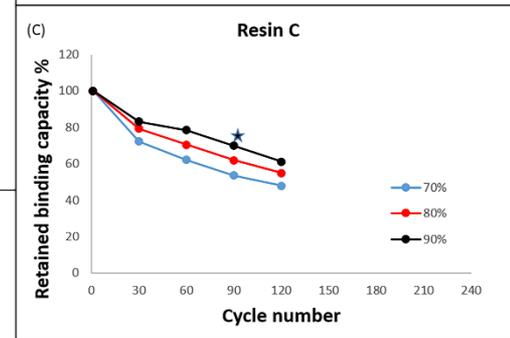
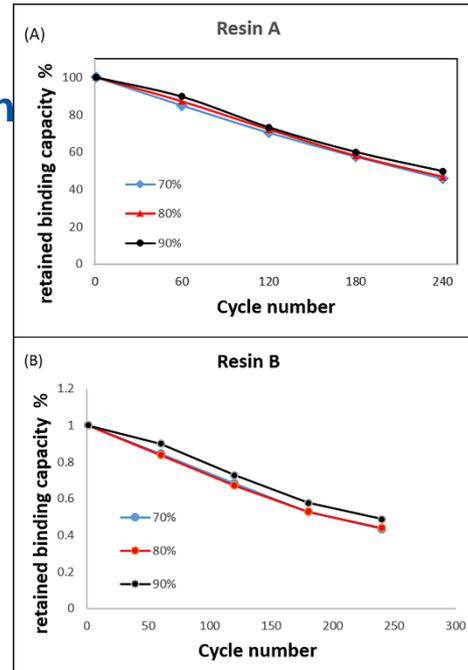
Behere et al, Manuscript under review, Biotech Progress

Retained Binding Capacity

All the experiments were performed in duplicates for reproducibility.

The column performance was assessed at 70%, 80% and 90% of binding capacity.

Binding capacity steadily decreased from 100% to 45%



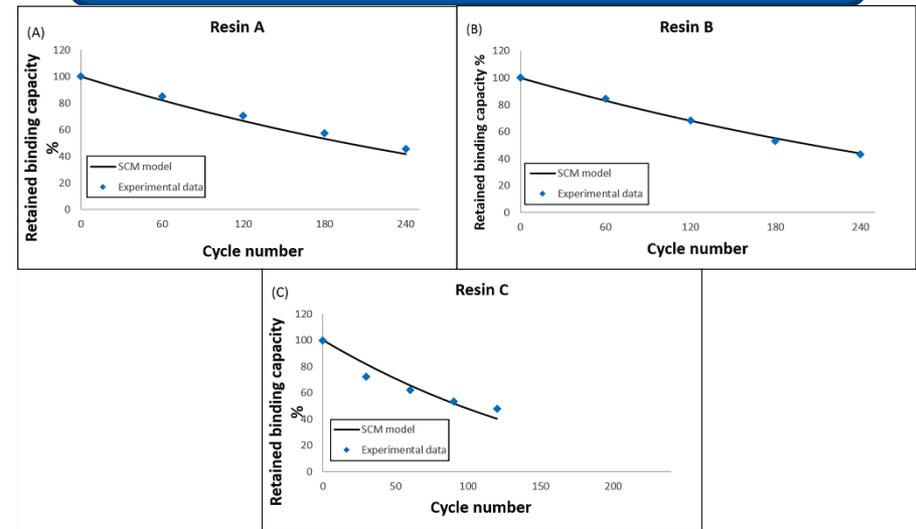
Behere et al, Manuscript under review, Biotech Progress

Model Validation

Resin	Reaction order	Rate constant	Diffusion constant (cm ² /s)
Resin A	3	4E-08	5E-08
Resin B	4	4E-08	5E-08
Resin C	1.65	4E-08	5E-08

The reaction order depends on the complex reaction mechanism occurring within the resin pores

The experimental data at 70% of retained binding capacity was used to perform the model validation and reaction order estimation



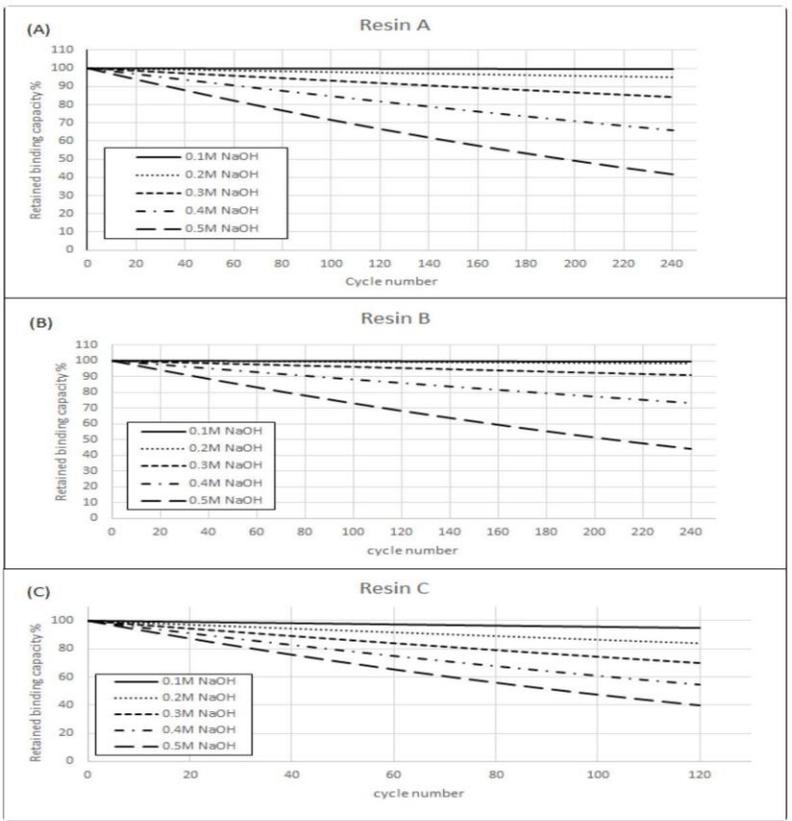
Behere et al, Manuscript under review, Biotech Progress

Model Simulations

Predict the behavior of the binding capacity

Different concentration of caustic (0.1N, 0.2N, 0.3N, 0.4N and 0.5N) used

Cycle number	0.1M NaOH	0.5M NaOH
120	R	R
Resin A	0.01	0.33
Resin B	0.01	0.32
Resin C	0.03	0.62



Behere et al, Manuscript under review, Biotech Prog

Conclusion

Reaction order

- key indicator to qualify the resin performance
- Assess proposed cleaning strategies

Cause of resin degradation

- linker-ligand breakdown
- ligand conformation changes

Significance of higher order reactions

- increased OH^- ions are required to perform hydrolysis
- Reduced degradation rate

MSCM model

Parameter estimation

Experimental validation

Adsorption isotherm determination

Protein A-NaOH predictive model

Industry Applications

Process Development

- Evaluate resin stability to caustic
- Make column switching decision
- Assess excipient type and concentration to improve the Protein A ligand stability

Soft sensor for online column performance monitoring

Regression isotherm toolbox

- Batch and continuous operations
- Ability to make real-time decision for column performance

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Questions?

Please use the microphone indicated so our recording includes audio of your question

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