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Evaluation of a continuous chromatography process through process modeling and resin characterization

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Evaluation of a Continuous Chromatography process through Process Modeling and Resin Characterization

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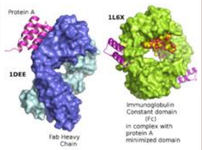
ABSTRACT

Increased product yield at reduced cost and time has been the driving force of any manufacturing process. However, inevitable increase in future demand for biopharmaceutical drugs, along with intensified competition and stringent regulatory laws have exhibited an imperative need for established platform processes. In this regard, the implementation of continuous processing, in whole or in components, has demonstrated to increase the manufacturing productivity with key impact on speed, cost and facility implications. While, upstream operations have confirmed increased productivity by implementation of continuous processing components, such as perfusion culture, similar adaptation for downstream processes has been limited. We have evaluated a continuous process for separation of a mAb in a closed environment by employing a combined approach of process modelling for continuous chromatography column and characterization of the Protein A resin. The process model utilized several semi-empirical parameters namely Peclet number and effective diffusivity from previous batch processes for continuous separation mAbs, which were appraised for process efficiency. The resin characteristics were assessed for the impact of pH, temperature, pressure and shelf life using the Scanning Electron Microscope (SEM). The results and associated outcome confirmed that this model can be employed with significant impact on the process time. The approach elucidates the conditions required to perform the continuous unit operation for mAb capture step, with indications of alleviating the bottleneck posed by conventional batch processes. Overall, the outcomes of the evaluated approach supports the shift away from conventional fed batch processes to continuous processing for improved biomanufacturing.

OBJECTIVE

- ✓ The resin usage per manufacturer's suggestion is 100 caustic cycles.
- ✓ But in most cases the resin is utilized 10-20% of its suggested use
- ✓ **Problem statement:** Poor understanding of the column after multiple uses
- ✓ **Solution:** A model to explain the resin usage
- ✓ Provide predictability for the runs without compromising the column efficiency and qualification

Why is it important?

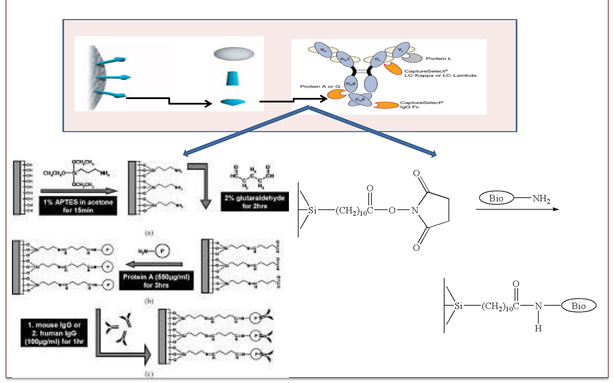


- High specificity of Protein A
- High Protein A resin costs
- Efficient use of resin is required
- Loss of productivity is observed mostly during the regeneration/sanitization step of NaOH wash

Proposed mechanism of NaOH-Protein A interaction: Protein A leaching

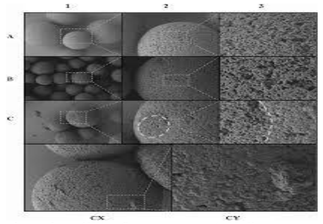
Where does it occur?

- Resin (agarose/silica/organic polymer) is attached to the Protein A via linker
- Caustic (NaOH) is a strong alkali and attacks the linker-Protein A (COO-NH2) bond
- Hydrolysis results in Protein A leaching



MODEL DEVELOPMENT

Shrinking Core Model (SCM)



Model Assumptions:

- Resin Particle is spherical
- Reagent concentration at the interface characterized by "r is not measurably different than the bulk concentration C_0
- Diffusion of ions to and from the interface along an average reaction surface is so rapid that the leaching is characterized almost entirely by the ''surface reaction"
- Leachable Protein A molecules are assumed to be uniformly distributed throughout the resin particles.

Model Equations

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

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

Reaction rate at interface

$$\frac{dn}{dt} = -4\pi r^2 Ck$$

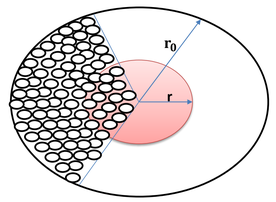
$$\frac{dn}{dt} = -4\pi r^2 k C_0 e^{\alpha k(r-r_0)}$$

Number of leachable Protein A particles consumed

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

After substitution & integration,

$$1-(1-R)^{1/3} = \frac{1}{\alpha k r_0}\ln(\alpha k K C_0 t + 1)$$



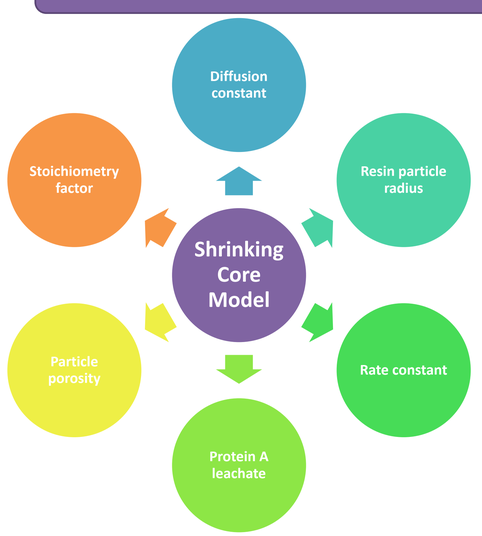
Shrinking Core Model (SCM) for Protein A- NaOH interaction

R = recovery – leachate of Protein A
r = radius of unleached Protein A core region
r_0 = initial radius of resin particle
k = Rate constant
D = diffusion constant
α = stoichiometry factor
n = number of leachable Protein A particles reacted in time t

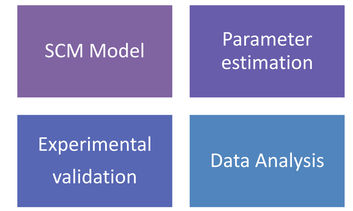
Materials & Methods

Resin Life-cycle study	• 2-3 different resins • Vary caustic (NaOH) concentration
Continuous Chromatography run	• 100-120 cycles with NaOH wash with and without protein • Similar run conditions for different resins for comparability
SEM Analysis	• Impact of pH, temperature and pressure on the resin structure • Resin topography change with column use and NaOH wash
HPLC analytical analysis	• Detection of Protein A leachate • Protein productivity change with cycle size
Data Analysis with SIMCA and/or MS-Excel	• MS-Excel for model validation • SIMCA to build PCA/PLS model

Parameter Estimation



MODEL VALIDATION



Protein A – NaOH predictive model

Protein A-_Linker + NaOH → Leached Protein A

POTENTIAL APPLICATIONS

- Optimum concentration of NaOH in Continuous chromatography run can be determined to ensure:
  - consistent product quality
  - Efficient resin usage
  - Optimize resin costs
- Predictability to the user with data analysis
  - SIMCA Principal Component Analysis (PCA)
  - SIMCA Partial least Square (PLS) model
- Protein A leachate minimization
  - Design space analysis with MODDE
  - Determine sweet spot for minimum leaching

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