Design and Construction of a Chromatographic Separation Process

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Objective

Bioengineers purify drug compounds to meet high FDA purity standards, often employing chromatographic separation. The objective is to provide future Bioengineering students with hands-on experience separating proteins with a chromatography process they will use in industry.

Background

Proteins can be separated by size exclusion chromatography (SEC), which separates proteins based on their hydrodynamic radius. These systems contain a hollow column filled with porous resin (Figure 1). Resin pores determine which proteins are excluded and which diffuse into the resin. Proteins larger than the resin exclusion limit elute quickly, while smaller proteins spend more time in the pores and elute later.

Porous Microsphere

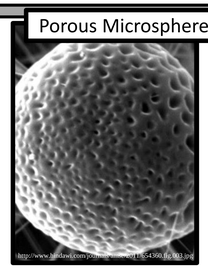

Figure 1. Porous microsphere.

Model

Packed Bed:

Packed Bed

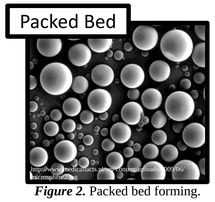

Figure 2. Packed bed forming.

Ergun's Equation

$$\frac{\Delta P}{L} = \frac{150\mu(1-\varepsilon)^2 v_o}{\varepsilon^3 {d_p}^2} + \frac{1.75(1-\varepsilon)\rho {v_o}^2}{\varepsilon^3 d_p}$$

The pressure drop across the packed bed is related to the flow rate of the mobile phase and the length of the packed bed by Ergun's Equation.

Elution Profile and Scale-up:

The goal of scale-up in a separation process is to maintain purity while increasing yield. Scale-up of column throughput can be accomplished by maintaining the sample dispersion ('σ') to preserve the shape of the peak (purity) and increasing the column volume to accommodate a larger sample.

Elution Time Relationship

$$t_o \propto \frac{l}{v}$$

Dispersion Relationship

$$\sigma^2 \propto \frac{v {d_p}^2}{l}$$

Colored Contaminants

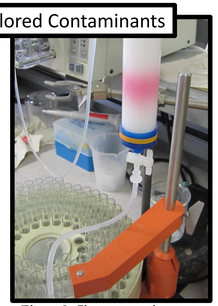

Figure 3: Chromatography process with colored sample components

Elution Profile

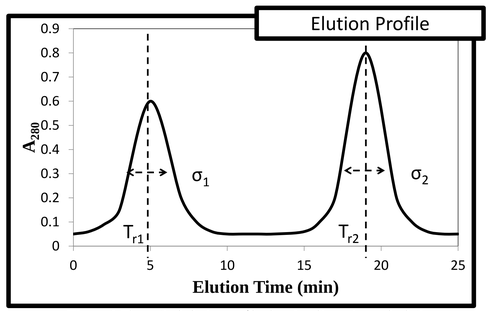

Figure 4: Theoretical elution profile showing dispersion and relative elution times.

Methods

The process will be run by future BioE 415 (Unit Operations Lab) students with the following limitations:

- Student teams will have two 3 hour lab sessions (short run time)
- TAs prepare and regenerate the column (minimal maintenance)
- Process used for multiple lab sections each day (quick regeneration)
- Process run many times per year (low cost of materials)
- Equipment frequently used and stored in offseason (robust, durable)

Size Exclusion Chromatography System:

Process Flow Diagram

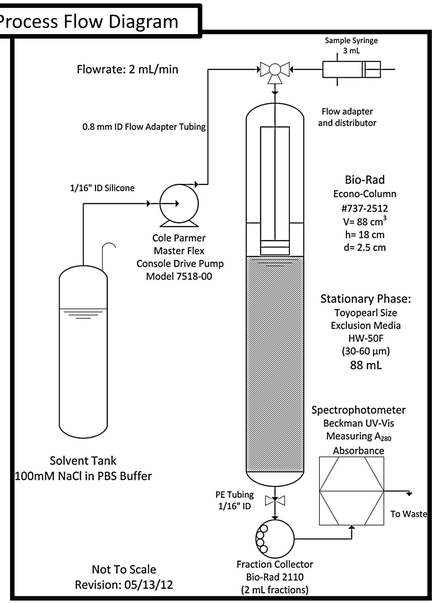

Figure 5: Process flow diagram of size exclusion chromatography system. Mobile phase is pumped into the column after the sample is loaded. Mobile phase then elutes the sample through the stationary phase.

Simulated Contaminants:

Proteins will simulate contaminants in an industrial process.

- Two proteins selected to simulate the sample (1 Dalton = 1 amu)
  - Vitamin B_{12} ~1 kDa
  - γ-Globulin ~150 kDa
- Hydrodynamic radius (R_h) is proportional to the molecular weight

$$R_h \propto \sqrt[3]{MW}$$

- Sample of 2.5% of the column volume
  - containing 0.5 mg/mL B_{12} and 3.5 mg/mL γ-Globulin
  - 2 mL of sample used for bench-scale
  - 8 mL of sample used for lab-scale

Results

The proteins γ-Globulin and Vitamin B_{12} were separated in a bench-scale system.

- Results are shown in Figure 6.

Bench-Scale Data

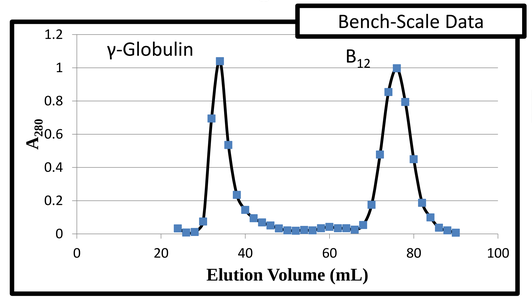

Figure 6. Elution Profile of Bench-Scale System in a 17 cm Bed Length SEC Column

- The column was scaled-up to separate a larger sample with the same purity and yield as the bench-scale system. Results are shown in Figure 8.

Lab-Scale Data

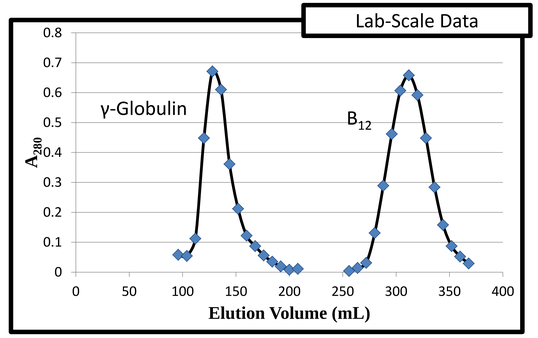

Figure 8. Elution Profile of Lab-Scale System in a 17 cm Bed Length SEC Column

- Some tailing is visible in Figures 6 and 8 (slow decline from peak), because the proteins are interacting with the resin molecules. Tailing can be reduced by increasing the ionic strength of the buffer.
- The absorbance values shown can be converted to protein concentrations using standard curves.

3D Column Model

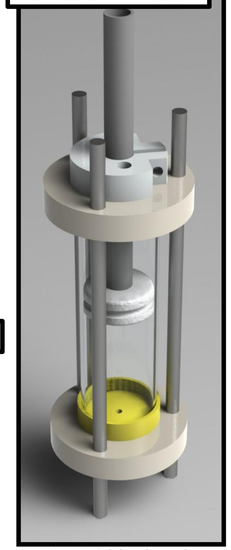

Figure 7. SolidWorks mockup of lab-scale separation column with 5 cm ID.

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