Application of DOE for the Purification of Proteins Using Novel Polymeric Cation Exchange Chromatography

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ABSTRACT

Efficiency in the separation and purification of therapeutically important peptides and proteins becomes increasingly significant as biotech companies look for ways to find manufacturing capacities for new and existing drugs. Various methods are routinely being explored to increase throughout in the purification. This poster demonstrates how Design Of Experiment (DOE) can be used to maximize binding capacities of proteins by optimizing mobile phase conditions. The chromatographic media employed here is a unique, polymeric based strong cation exchanger (PolyCSx) with mixed mode functionality. It is designed for downstream process applications with high throughput requirements. Experimental results show this media to be suitable for the separation and purification of hydrophobic peptides and proteins.

Objectives

Apply and demonstrate utility of Design Of Experiments (DOE) methodology to optimize hydrophobic protein or peptide binding capacity.

Steps:

- Selecting a suitable chromatographic media
- Selecting the proper buffer concentration
- Selecting the appropriate pH range
- Selecting a suitable solvent to maximize the solubility of the protein
- Conducting the DOE and determining the optimum condition for maximum capacity

Properties of Target Protein

- Molecular weight: 5,700 Da
- Isoelectric point: 5.7 with moderate hydrophobicity
- Total number of amino acids: 51
- Solubility of selected protein depends on pH and percentage of organic solvent

Criteria for Selection of Chromatographic Media

- Strong cation exchanger for binding below pH 5.0
- Ability to withstand organic solvents needed due to hydrophobicity of protein or peptide stability
- No irreversible binding
- Ability to support operation at high pressure and high flow rate

Properties of Selected Chromatographic Media

- PolyCSx: Strong cation exchanger with mixed mode functionality
- Particle size of 35µm and 550 Å pore size
- Solubility of selected protein depends on pH and percentage of organic solvent
- High chemical stability ensuring extended lifetime
- Minimized irreversible adsorption
- Enhanced mechanical strength, enabling easy column packing and operation at high velocity and pressure

Design of Experiments

Factors:

- Buffer concentration
- Percentage of organic solvent
- pH
- Center point: Number of replicates: 2
- Total number of experiments: 10

Minitab Calculation

Partial P: Break versus Buffer Content, Prop 1

Estimated Effects and Coefficients for Break (coded units)

<table>
<thead>
<tr>
<th>Source</th>
<th>Effect</th>
<th>Coef</th>
<th>SE Coef</th>
<th>T-Value</th>
<th>P-Value</th>
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<tr>
<td>Buffer Content</td>
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<td>0.690</td>
<td>0.200</td>
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<tr>
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Analysis of Variance for Break (coded units)

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<tr>
<th>Source</th>
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<th>DF</th>
<th>MS</th>
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<tr>
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<td>0.000</td>
<td>0.000</td>
<td>1.0000</td>
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<td>Residual Error</td>
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<td>Total</td>
<td>5.715</td>
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<td>0.572</td>
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Desity of Experiments

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<tr>
<th>Buffer Content (mM)</th>
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<th>Saturation Capacity (mg/ml)</th>
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<tr>
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<td>6</td>
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<td>19.91</td>
<td>23.56</td>
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Surface Ligand Structure of PolyCSx

Conditions for DOE

- Solubility of the protein:
  - Screened from pH 2.5 to 4.5
  - pH 3 was chosen for dissolving the protein
- Buffer composition
- Buffer concentration
- Percentage of organic solvent

Effect of Buffers on Protein Binding

- Peptide adsorption profile in (Blue) glycine buffer
- (Red) malonic acid; (Pink) lactic acid; (Orange) glycine buffer
- Effects of buffer concentration and organic solvent content on the protein binding capacity

Surface Plot

- The Interaction Plot of Buffer Content with Propanol Concentration
- The Main Effects Plot

Results of DOE Experiments

<table>
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<tr>
<th>Experiment</th>
<th>Buffer Content (mM)</th>
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STEPS:

Application of DOE for the Purification of Proteins

- Minimized irreversible adsorption
- PolyCSx: Strong cation exchanger with mixed mode functionality
- Strong cation exchanger for binding below pH 5.0
- Various methods are routinely being explored to increase throughput in downstream process applications with high throughput requirements.
- Efficiency in the separation and purification of therapeutic peptides and proteins becomes increasingly significant as biotech companies look for ways to find manufacturing capacities for new and existing drugs.
- DOE was successfully applied to optimize hydrophobic protein binding onto PolyCSx media.
- The concentration of buffer has minimal effect on protein binding capacity.
- There is no irreversible binding of protein on the PolyCSx. Total protein recovery is close to 100%.
- Hydrophobic peptides and proteins purification can be efficiently purified using PolyCSx.