Emerging and Enabling Technologies in Chromatography of Integrated Biomanufacturing

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Continuous and Semi-Continuous Chromatographic Systems

Periodic Counter-Current Chromatography (PCC): Continuous bind-elute/capture; relatively simple process.

Simulated Moving Bed (SMB) and related approaches: Continuous capture or polishing, can be complex.

Multicolumn Countercurrent Solvent Gradient Purification (MCSGP): Enables gradient operation.

Continuous Countercurrent Tangential Chromatography (CCTG): Enables truly continuous operation, uses resins slurries and membranes.
Figure 5. Three-stage elution step in continuous countercurrent tangential chromatography (CCTC).
Emerging and Enabling Technologies in Chromatography

- Availability of affinity capture agents for new classes of biological products (enables platform process).
- Availability of new high capacity short residence time adsorptive materials for rapid cycling of these operations (reduced footprint).
- Predictive methods for rapidly identifying both materials and modes of operation for use in optimal, robust integrated processes (more rapid process development and more robust integrated processes).
- Proper understanding and utilization of orthogonal modes of selectivity for the removal of process and product related impurities (enables minimal downstream steps and more robust integrated processes).
- Optimal integration of continuous, semi-continuous and batch chromatographic processes into a seamless, integrated downstream process.
Affinity Capture Agents
Work Flow for Identification of Affinity Peptides

Drugs Target

Phage Display

PhysicoChemical Properties

Resin Screening

Drug Target

Drug Design & Discovery

Affinity & Selectivity Maturation

Validation & Refinement

Parameter Optimization

Process Design & Deployment

Eptope Mapping

Point Mutagenesis

Library Design & Discovery

Parameter Optimization

Process Design & Deployment

Affinity Capture Process

O(10^12) Global Minima

O(10^3) Local Minima

2/3° Mutations

Fluorescence Polarization

Fluorescence Polarization

Molecular Modeling

Affinity & Selectivity Maturation

Validation & Refinement

Parameter Optimization

Process Design & Deployment
Resin Screening Procedures

- Load hGH in Cell Culture Fluid (CCF)
- Wash
- Elute
- Strip

**hGH Binding "Isotherms" in Pichia CCF**

- Solution hGH Concentration (mg/mL)
- Q (mg hGH/mL resin)

**hGH Elution (%) From Peptide SH14**

- NaCl Concentration (mM)
- pH

**Batch Identification of High-Binding Peptide Resins**

**Scouting for Column Operating Conditions**

**High Throughput RPLC Analysis for Product Quantification**
Advances in Adsorptive Materials

• Ongoing advances in Resins
• Monoliths
• Membranes
• Nanofibers
• 3-D Resin Materials
High Capacity/Short Residence Time Materials

Natrix HD Membrane – 2 Components

1. Reinforcing mesh “skeleton”
   ✓ Provides mechanical strength & durability to composite membrane
   ✓ Polypropylene – good chemical stability

2. Porous polymer gel
   ✓ 3D macroporous structure provides:
     • a large surface area that contains a high density of protein binding groups
     • Interconnected pores that provide convective flow channels
   ✓ Polymer chemistry can be quickly tailored to fit application

Courtesy of Natrix
Enables very rapid cycling with smaller footprint

4.5 kg mAb sent to Protein A over 10 days

120 kg mAb purified annually in approx. 20 ft² of GMP suite

Productivity of Protein A membrane capture is 200 g/h•L-media

- 15X better than Pro A resin productivity: 13.5 g/h•L-media
Nanofiber Supports (Bracewell lab)
3-D Printed Adsorptive Materials (Fee Lab)

Fig. 3. CAD designs versus printed cutaway columns (a) SC CAD model (b) SC printed model (c) 20× magnification of SC beads (d) PC CAD model (e) PC printed model (f) 20× magnification of parallel channels (g) HC CAD model (h) HC printed model (i) 20× magnification of herringbone channels.

C. Fee et al. / J. Chromatogr. A 1333 (2014) 18-24
Advances in Understanding and Predicting Selectivity

Protein Surface Properties

Ligand Chemistry

Surfaces
MM Chromatography of hGH variant

- Purification of hGH variant on Capto adhere using ArgHCl (A) or CaCl$_2$ (B)

- Significant clearance of product related aggregates and HCP.

- HCP reduction from 50,000 ppm in the load solution to less than 250 ppm in the pool when using ArgHCl. Therefore, this eluent selected for further model development.
Assembling a Mixed-mode Cation-Exchange Ligand

Method: Molecular descriptors and QSPR modeling

3D structure of protein
Local grid points

Various potentials calculated at each local grid point
- Electrostatic
- Hydrophobic
- Aromatic
- Aliphatic

Clustering of potentials to identify strong regions of interactions

QSPR models employed for correlating descriptors to protein retention

Pairs of potentials important for interactions are calculated, and used as descriptors
Predicts 95% of the data in both the training model and test set to an accuracy of ±200 mM NaCl (experimental variation ±100 mM NaCl)
  – Hollow points indicate proteins that were fully retained on linear gradient (did not elute)
• Stable to cross-validation ($R^2 = 0.82$) and y-scrambling ($r^2 < 0.3$) analysis
Methods to Facilitate the Removal of Product Related Impurities (identification of resins and conditions that will focus the selectivity on a specific protein surface patch).
Results: Fab A hydrophobic variants

- Different selectivity trends in CEX, HIC and multimodal systems
- CDR loops is a preferred multimodal ligand binding site
Results: Mixture of hydrophobic Fab A variants on Capto MMC

Karkov et al., Biotech. And Bioeng. 112 (11), 2305-2315 (2015)
Modes of operation for integrated downstream bioprocesses

• Initial product capture with bind elute
• Followed by appropriate combinations of bind elute, flow through, weak partitioning and/or gradient operations (as required).

• Notes: these steps must be fully integrated as discussed on day 1, but not necessarily truly “continuous”.
• These steps must be orthogonal wrt separation of process and/or product related impurities.
Integrated Biomanufacturing
(using affinity capture)
(C. Love, MIT)
Facilitated Downstream Process Design with Orthogonal Selectivity Trains (new approach in development, details to come later…)

- Rapidly employ **detailed** understanding of the chromatographic behavior of the biological product in concert with the behavior of process and product related impurities in various resin systems under a range of conditions to design truly orthogonally selective downstream bioprocessing trains.

- Note: does not necessarily require affinity capture
Example Purification Process:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Product Conc. (ug/ml)</th>
<th>Product Recovery (%)</th>
<th>HCP Content (ppm)</th>
<th>Log HCP Step Clearance</th>
<th>DNA Content (ppm)</th>
<th>Aggregate Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Culture Fluid</td>
<td>23</td>
<td>---</td>
<td>1,976,522 ± 6,715,217</td>
<td>---</td>
<td>278,261</td>
<td>---</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Step Capture Eluate</td>
<td>612</td>
<td>100*</td>
<td>6989 ± 3,023</td>
<td>2.45</td>
<td>28</td>
<td>1.10%&lt;sup&gt;2&lt;/sup&gt;nd s</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Step FT Pool</td>
<td>217</td>
<td>88</td>
<td>66 ± 17.9</td>
<td>2.03</td>
<td>&lt; 23**</td>
<td>1.11%</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; Step Final Polish Eluate</td>
<td>232</td>
<td>91</td>
<td>8 ± 0.8</td>
<td>0.92</td>
<td>&lt; 23**</td>
<td>0.60%</td>
</tr>
</tbody>
</table>

* Value was found to be greater than 100%
** Value is below the limit of detection of the assay

Overall Product Recovery = 80%
Total HCP Clearance (logs) = 5.4
Total DNA Clearance (logs) = >4.1
Final Aggregate Content = 0.60%
Status?

- Availability of affinity capture agents for new classes of biological products (in process, now have commercial suppliers).
- Availability of new high capacity adsorptive materials for rapid cycling of these operations (in process, now have commercial suppliers).
- Predictive methods for rapidly identifying both materials and modes of operation for use in optimal, robust integrated processes (advances in both academia and industry).
- Proper understanding and utilization of orthogonal modes of selectivity for the removal of process and product related impurities (in process).
- Optimal integration of continuous, semi-continuous and batch chromatographic processes into a seamless, integrated downstream process (in process, several industrial examples have been presented in this meeting).
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