Hydrophobic Interaction Process Scale Separation of Protein, MAb and Antibody Drug Conjugate

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INTRODUCTION

Hydrophobic interaction chromatographic (HIC) separation applies to all stages of biological sample purification process including capturing, polishing protein purifications. Aggregates, fragments and different forms of proteins can be isolated due to the hydrophobic interaction between the sample and the resin solid support. The hydrophobic interaction can be controlled by a number of factors such as protein structure, salt concentration, pH, temperature and organic solvent additives. HIC separation is a great addition to the native protein purification process including ion exchange, size exclusion, and affinity chromatography.

In this paper, three different HIC resins are introduced for protein, mAb and antibody drug conjugate process scale purification. Sepax Generik & Polar MC-HIC chromatographic resins are designed for hydrophobic interaction chromatography. The polymeric resins are composed of a methacrylate polymer (also referred to as PMA) bead with high mechanical and chemical stability. The resins have mean particle size of 30 and 60 µm, and pore size of 800 Å. On the resin surface, alkyd groups or aryl groups are attached via a proprietary chemistry, so that the resin can selectively interact with different bio-molecules through different hydrophobicity.

The characteristics for the 30 µm particle size are discussed for Generik & Polar MC-HIC. Protein separation examples are presented for the resin applications. Sepax Generik MC30-HIC Butyl, Polar MC30-HIC Butyl and Polar MC30-HIC Ether resins provide excellent high efficiency and recovery separation of bio-molecules such as mAb (monoclonal antibody), ADC (antibody drug conjugate) and related protein fragments, DNA and oligonucleotides. The HIC media also tolerates high pressure operation up to 100 bars. All three phases are applicable at different stages from laboratory discovery, pilot-scale purification to industrial process chromatography.

EXPERIMENTAL

<table>
<thead>
<tr>
<th>Media Type</th>
<th>Generik MC30-HIC Butyl</th>
<th>Polar MC30-HIC Butyl</th>
<th>Polar MC30-HIC Ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packing</td>
<td>70% v/v slurry in 20% ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>Hydrophilic polymethacrylate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle Size</td>
<td>20 ± 45 µm (mean 30 µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pore Size</td>
<td>800 Å</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dynamic Binding Capacity*</td>
<td>Generik MC30-HIC Butyl</td>
<td>Polar MC30-HIC Butyl</td>
<td>Polar MC30-HIC Ether</td>
</tr>
<tr>
<td></td>
<td>45 ± 5 mg lys. / mL</td>
<td>35 ± 5 mg lys. / mL</td>
<td>15 ± 5 mg lys. / mL</td>
</tr>
<tr>
<td>pH Stability</td>
<td>2-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operating Temperature</td>
<td>Up to 40 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operating Pressure</td>
<td>Up to 100 bar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobile Phase Compatibility</td>
<td>Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, or methanol.</td>
<td>Typical buffers: phosphate, Tris, &amp; acetate.</td>
<td></td>
</tr>
<tr>
<td>Linear Flow Rate</td>
<td>Up to 1800 cm/hour</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ORDER INFORMATION

<table>
<thead>
<tr>
<th>Product</th>
<th>PN#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar MC30-HIC Ether (30 µm, 800 Å, 4.6 x 50 mm)</td>
<td>258130-4605</td>
</tr>
<tr>
<td>Polar MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)</td>
<td>258030-4605</td>
</tr>
<tr>
<td>Generik MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)</td>
<td>248030-4605</td>
</tr>
</tbody>
</table>

RIBONUCLEASE A AND LYSOZYME ANALYSIS ON GENERIK & POLAR MC30-HIC

Column: Polar MC30-HIC Ether (ID 10 mm, volume 5 mL)
- Polar MC30-HIC Butyl (ID 10 mm, volume 5 mL)
- Polar MC30-HIC Ether (ID 10 mm, volume 5 mL)

Flow rate: 4 mL/min; Detection: UV 214 nm;
Mobile Phase A: 25 mM Phosphate + 2M (NH₄)₂SO₄, pH 7.0;
Mobile Phase B: 25 mM Phosphate pH 7.0;
Injection Volume: 100 µL; Gradient: 0-30 min 0-100%; Sample: ribonuclease A, lysozyme (5 mg/mL); Instrument: FPLC

POLAR MC30-HIC BUTYL PRESSURE LIMIT TEST

Column: Polar MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)
Instrument: HPLC
Mobile Phase: 25mM Phosphate + 2M (NH₄)₂SO₄, pH 7.0;
Flow Rate: 1-24 mL/min

MAB321 ANALYSIS ON GENERIK MC30-HIC BUTYL

Column: Generik MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)
Flow rate: 2 mL/min; Gradient: 0-50 min, 50-100%;
Mobile Phase A: 25 mM Phosphate + 2M (NH₄)₂SO₄ pH 7.0;
Mobile Phase B: 25 mM Phosphate pH 7.0;
Injection Volume: 50 µL; Gradient: 0-50 min, 50-100%; Sample: MAB321 (2.5 mg/mL); Instrument: FPLC

POLAR MC30-HIC BUTYL CIP TEST 10 CYCLES

Column: Polar MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)
CIP: 1 M NaOH, Flow rate: 0.2 mL/min, 15 min;
Wash: 25mM Phosphate + 2M (NH₄)₂SO₄, pH 7.0;
Flow rate: 1.5 mL/min, 10min;
QC: Mobile Phase A: + 2M (NH₄)₂SO₄ pH 7.0;
Mobile Phase B: 25mM Phosphate pH 7.0;
Injection Volume: 20 µL;
Sample: ribonuclease A, lysozyme, chymotrypsinogen (2 mg/mL);

POLAR MC30-HIC BUTYL LIFETIME TEST

Column: Polar MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)
Flow rate: 0.5 mL/min;
Detection: UV 214 nm;
Mobile Phase A: 25mM Phosphate + 2M (NH₄)₂SO₄, pH 7.0;
Mobile Phase B: 25mM Phosphate pH 7.0;
Injection Volume: 10 mL;
Sample: FPLC

POLAR MC30-HIC CIP TEST 10 CYCLES

Column: Polar MC30-HIC Ether (ID 10 mm, volume 5 mL)
CIP: 1 M NaOH, Flow rate: 0.2 mL/min, 15 min;
Wash: 25mM Phosphate + 2M (NH₄)₂SO₄, pH 7.0;
Flow rate: 1.5 mL/min, 10min;
QC: Mobile Phase A: + 2M (NH₄)₂SO₄ pH 7.0;
Mobile Phase B: 25mM Phosphate pH 7.0;
Injection Volume: 20 µL;
Sample: ribonuclease A, lysozyme, chymotrypsinogen (2 mg/mL);

CONCLUSION

- Sepax Generik & Polar MC-HIC resins offer stable and consistent performance.
- Sepax Generik & Polar MC-HIC resins can tolerate high pressure, which allows faster flow rate and shorter running time.
- Sepax Generik & Polar MC-HIC resins provide excellent high recovery and resolution in separating proteins.
- Sepax Generik & Polar MC-HIC resins offer a potential solution for ADC separation.