The Effect of Glycosylation on Peak Resolution of Antibodies on SCIEX BioPhase 8800 CE-SDS System

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CEED | API Proteins
Discovery, Product Development & Supply | Janssen Research & Development, LLC
Outline

- Janssen Pharmaceuticals: Who we are
- Closing the reliability and robustness gap of CE-SDS
- Case Study
  - Labchip GXII reduced and non-reduced run
  - PA 800 reduced and non-reduced run
  - BioPhase 8800 reduced and non-reduced run
  - Degly sample runs
- Conclusions
- Acknowledgments
Who we are

- **Janssen Pharmaceuticals**
  - Cell Engineering and Early Development group in Drug Product and Development Sciences (DPDS)

- Our goal is to use orthogonal methods to develop cell lines with high titer and high product quality
  - Large molecule drug products
  - Evaluate reduced and non-reduced purity to identify low and high molecular weight product-related impurities and potential HCPs
Characterization of monoclonal material for product quality leads to clone selection for clinical drug development

- Clones cultured in 96 well plates
- ~24 clones received from ambr bioreactors
- Cut down to 8 clones based on MS and CE-SDS data
- Top and back up clone selection using various analytical techniques

4 months
Role of capillary electrophoresis sodium dodecyl sulfate (CE-SDS) in Cell Line Selection

• Characterization of product quality in order to
  • Determine developability for clinical manufacturing
  • Determine product quality for multiple clones
  • Pick the best clone and back up clone for cell banking

• Characterization of Low molecular and high molecular weight species to determine yield and purity for downstream manufacturing

• Evaluation of new drug modalities on product quality

▪ This Data is critical to the timely selection and development of new drug products for Janssen
Closing the reliability and robustness gap of CE-SDS

• BioPhase 8800 system parallel processing of 8 samples
• Inter- and intra-capillary robustness and reproducibility facilitates sample comparison
• Improved timeline for quicker course correction in cell development process
• Improved resolution of normally coeluting heavy chain peaks
Research plan-Bispecific Antibody

- Comparison of Labchip GXII (Perkin Elmer), PA800 (SCIEX) and BioPhase 8800 (SCIEX)
  - Reduced and Non-reduced samples
- Comparison of peak resolution after treatment with PNGase F
## How do the CE-SDS systems compare

<table>
<thead>
<tr>
<th></th>
<th>Labchip GXII</th>
<th>PA800</th>
<th>BioPhase 8800</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Prep Time</strong></td>
<td>6 hours</td>
<td>2-3 hours</td>
<td>2-3 hours</td>
</tr>
<tr>
<td><strong>Sample Run Time</strong></td>
<td>40 sec</td>
<td>50-60 mins</td>
<td>50-60 mins (34-44mins) X 8</td>
</tr>
<tr>
<td><strong>Detection system</strong></td>
<td>FLR</td>
<td>UV or LIF</td>
<td>UV and LIF</td>
</tr>
<tr>
<td><strong>Automation</strong></td>
<td>Compatible</td>
<td>limited</td>
<td>Compatible</td>
</tr>
<tr>
<td><strong>Sample volume</strong></td>
<td>5µl</td>
<td>24 µL/136 µL</td>
<td>45 µL/57 µL</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
</tr>
</tbody>
</table>
Case Study: Effect of CE-SDS systems on Peak resolution

Evaluation of Peak Splitting on Bispecific antibodies
Sample 1 on Labchip GXII

- Expected Peak of Non-reduced antibody at ~140kDa
- Coelution of HC1 and HC2 peaks
Sample 1 on PA800

- Observance of characteristic single peak on non-reduced sample and LC and HC peaks on reduced sample.
Sample 1 on BioPhase 8800

- Observed peak splitting on reduced run not previously seen on GXII and PA800 system
Evaluating impact on resolution due to glycosylation

- Samples treatment with PNGase F
  - PNGase F- cleaves N-linked High mannose oligosaccharides (glycans) off asparagine residues on glycoproteins
  - MW of 35.5kDa
  - Evaluate the effect of Deglycosylation on CE-SDS separation

- Re-evaluated on BioPhase and GXII systems
Sample 1 on GXII with PNGase F treatment

- Observed peak splitting on reduced sample only after treatment with PNGase F
Sample 1 on BioPhase 8800 with PNGase F treatment

- Observed better peak resolution and an expected shift to lower kDa for heavy chains after treatment with PNGase F.
Conclusions

• The BioPhase allows for better peak resolution than other CE-SDS
  • BioPhase 8800 showed an increased ability to resolve the HC1 and HC2 peaks of a bispecific antibody without any additional treatment
  • We observed that BioPhase can separate glycoproteins that are only 1200Da difference (HC1, HC2
  • We observed an increased resolution after treatment with PNGase F induced deglycosylation
  • The increased resolution assists with better identification of known and unknown species

• We are collaborating with SCIEX to better understand the new detection system and the ability to resolve the heavy chain peaks
Pushing the envelope of CE-SDS - traditional vs lightning methods

<table>
<thead>
<tr>
<th>Method</th>
<th>BioPhase 8800</th>
<th>PA 800</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>80 psi 2 min</td>
<td>80 psi 2 min</td>
</tr>
<tr>
<td>Base</td>
<td>20 psi 5 min</td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>20 psi 5 min</td>
<td>20 psi 5 min</td>
</tr>
<tr>
<td>Water</td>
<td>20 psi 3 min</td>
<td>20 psi 3 min</td>
</tr>
<tr>
<td>Gel</td>
<td>80 psi 10 min</td>
<td>80 psi 10 min</td>
</tr>
<tr>
<td>Run</td>
<td>35 min (NR)/25 min (R) at 15 kV</td>
<td>35 min (NR)/25 min (R) at 15 kV</td>
</tr>
</tbody>
</table>