Generic CZE method for charge variant analysis of mAbs and complex biotherapeutics

Maximilian Meudt
Analytical Development Biologicals
Charge heterogeneity & analysis techniques

- Charge heterogeneity is an important quality attribute with potential impact on safety and efficacy
- CE-based methods are powerful alternatives to LC
- Increasing number and complexity of molecules requires fast and efficient method development

Generic CE-based methods for charge variant analysis

- CZE - Charge to size ratio
- iCIEF - Isoelectric point
Approaches in CZE

- High pi and complex molecules often suffer from low resolution
- Robustness issues due to BGE components quality

**New BGE comprising**
- Increased pH
- Replacement of eACA

**Substitution by tranexamic acid**
- eACA
- Tranexamic acid

**Good’s buffer-based system**
- Optimized additives
- Adapted ionic strength and capillary length

Good’s buffer-based system

• Favorable properties for CE
  – High buffering capacity, low conductivity when used at respective pH
• Sulphonic acid derivate pH adjusted with basic polyol

![Graph showing IgG pI > 9](image)

*Other settings kept according to He et al.

→ 2 mM TETA, 0.05 % HPMC

→ Buffer system applicable
→ pH increased
→ Resolution decreases at pH>6.3, vanishes at pH 7

Publication pending
Dynamic coating & viscosity enhancing additives

- TETA: Loss of functionality at elevated pH
- Test of alternative polyamines spermine and hexamethonium chloride
- Alternatives hydroxypropyl-cellulose (HPC) or polyethyleneoxide (PEO)
- Different MW and concentrations tested

→ Spermine and mid-sized polymers HPC and PEO found to be superior under new conditions

Publication pending
Final method & comparison

- BGE comprising MOPS/Bis-Tris pH 6.3, spermine, PEO 100; 50 cm BFS capillary

- Newly created BGE

- Increased resolution for most tested mAbs

- Applicable to broad range of pI and formats

Publication pending
Method performance check – challenging molecule

- Charge methods IEC, iCIEF & eACA CZE failed
- Method validation possible?

<table>
<thead>
<tr>
<th>Performance Metric</th>
<th>bs DoppelmAb</th>
<th>Acceptance criterion met</th>
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</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>10% - 200% target load</td>
<td>MP: r=1.00, APG: r=0.99, BPG: r=0.94</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Absolute difference to target % peak area</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Repeatability</td>
<td>%RSD</td>
<td>MP &amp; APG: &lt;4%, BPG: &lt;8%</td>
</tr>
<tr>
<td>Specificity</td>
<td>BGE interference &amp; stability samples</td>
<td>No interference, stability indicating</td>
</tr>
<tr>
<td>Robustness</td>
<td>Hold-time</td>
<td>24 h holdtime</td>
</tr>
</tbody>
</table>

Publication pending
• Increase in BGE pH improved separation, especially of high pI molecules
• Method performance indicates generic applicability to a broad pI range and complex formats
• eACA successfully replaced, further additives optimized

• Ongoing: Robustness testing of BGE components
Acknowledgements

Matthias Knape
Martin Pannek
Fabian Higel
Anete Hornauer
Backup
Capillary zone electrophoresis - Method principle

- Separation by charge to size (resp. hydrodynamic radius) ratio
- In a electrolyte-filled capillary under voltage

→ CZE methods for charge variant analysis are described as fast, generic, high resolution (Moritz 2017, Kahle 2018)
Substitution of eACA by tranexamic acid

- Acid/Base Properties of Tranexamic acid virtually identical to \( \varepsilon \)ACA
- Sterically slightly different
- Might overcome robustness issues

![Chemical structures](image)

A. Tranexamic acid (MW = 157.21 g/mol)
   - \( pK_a = 4.3 \)
B. Epsilon-aminocaproic acid (\( \varepsilon \)ACA) (MW = 131.17 g/mol)
   - \( pK_a = 4.4 \)
Tranexamic acid – Lot-to-Lot variability

• Poor reproducibility

<table>
<thead>
<tr>
<th>Lots A, B, C</th>
<th>APG</th>
<th>Main</th>
<th>BPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>% peak area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>30.5</td>
<td>60.0</td>
<td>9.6</td>
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<tr>
<td>% RSD</td>
<td>2.8</td>
<td>2.4</td>
<td>7.9</td>
</tr>
</tbody>
</table>

→ Not advantageous over eACA
New BGE composition

• Overcome robustness issues by replacing eACA by Good’s buffer
  – Zwitterionic, larger than eACA → less conductivity
  – Buffer capacity in resp. adjustable pH range
  – No absorbance at 214 nm

• Increase pH to improve separation → not possible with eACA

Relative charge difference of charge variants of Trastuzumab
• Acidic variant: N deamidation
• Basic variant: C-terminal K addition
Comparison

Bs ZweimAb pI > 9

IgG pI > 9

IgG pI < 7.5