CESI-MS a technique for the characterization and analysis of both intact and digested biopharmaceuticals

Dr. Stephen Lock
Agenda

- The use of CE in Biopharma
- What is CESI-MS?
- Applications using CESI-MS in BioPharma
  - 100% sequence coverage and PTM detection for monoclonal antibody studies.
  - The use of CESI-MS in intact protein analysis in relation to size and charge heterogeneity profiling.
  - How CESI-MS can be used in glycoprotein analysis in the characterisation of EPO & IFN
- Summary
Traditional CE System Schematic

- Electrode
- Capillary Inlet
- Reservoir
- Electrolyte Buffer
- Detector
- Capillary Outlet
- Reservoir
- HVPS
- Data Acquisition

SciEX
“Capillary electrophoresis uses one or more capillaries as migration channels for electrophoresis and increasingly has become the procedure of choice when an electrophoretic separation method is needed. This is because CE is easier to perform, requires less time, and allows better precision and robustness than PAGE.”

USP Guideline for Submitting Requests for Revision to USP-NF, v3.1

1. CE-SDS = Purity analysis

2. CIEF & CZE = charge heterogeneity analysis

3. Glycan analysis = microheterogeneity determination
Multi-site Studies in Industry Illustrate Portability of CE

- 2006: CE-SDS Gel: Chromatographia, 64, 359-368; A series of collaborations between various pharmaceutical companies and regulatory authorities concerning the analysis of biomolecules using CE

- 2011: CIEF: Chromatographia, 73, 1137-1144; Intercompany study to evaluate the robustness of C-IEF technology for the analysis of monoclonal antibodies:

- 2012: Imaged CIEF: J. Separation Science, 335, 3124-3129; Robustness of Imaged Capillary IEF methodology for the analysis of monoclonal antibodies: An Inter-laboratory Study

- 2013: N-Glycan Mapping Study: CE Pharm 2013: Pending Publication


Synopsis:

“CE Methods Can Be Reliable, Robust, and Transportable Across Sites”
What is CESI – MS?
Combining CE & ESI-Mass Spectrometry

CESI 8000 High Performance Separation-ESI Module

Ultra-low flow rates of < 30nL/min

High resolution, ultra-low flow rate CE separations coupled with high resolution, high sensitivity MS

TripleTOF® 5600+ and 6600 Systems

CESI - “The Integration of Capillary Electrophoresis (CE) with Electrospray Ionization (ESI) Into a Single Dynamic Process Within the Same Device”
Implementation of CESI-MS Through a Commercial Interface

CESI - “The Integration of Capillary Electrophoresis (CE) with Electrospray Ionization (ESI) Into a Single Dynamic Process Within the Same Device”
Ultra-low flow

Conductive liquid reservoir
IS voltage is applied

Eliminate interference from Oxidation/reduction products

Enable IS voltage connection to the liquid in inner lumen

Increase ionization and decrease ion suppression

“The Integration of Capillary Electrophoresis (CE) With Electrospray Ionization (ESI) Into a Single Dynamic Process Within The Same Device”
Influence of Flow Rate on Sensitivity

Evolution of the peak intensity of Angiotensin I (a) and detection sensitivity as a function of the flow rate (b) Experimental conditions: capillary electrophoresis; bare fused silica capillary with a porous tip, total length 88.5 cm × 30 μm i.d. × 150 μm o.d.; Infused sample, Angiotensin I at 2 ng/mL in 10% acetic acid. Mass spectrometry; capillary voltage, -1350 V; detection range, 50-3000 m/z.
Reducing Ion Suppression Bias at Low Flow Rates

Monitoring maltotetraose suppression in the presence of neurotensin

Analyte suppression decreases logarithmically below 50 nL/min
Multilevel characterization of mAbs by CESI-MS
Multilevel characterization of mAbs by CESI-MS – Bottom up analysis

Evaluation of CESI-MS for the comprehensive characterization of mAb forms

IgG1, 2, & 4

Peptide mapping

Reduce, alkylate & digest

Level 1
mAb Characterisation Workflow

- Primary structure characterisation workflow based on bottom-up proteomics strategy

In-solution tryptic digestion → Analysis by t-ITP CESI-MS/MS →
- Amino acid sequence characterisation
- Glycosylations (structure)
- PTMs hot spots characterisation

CESI8000 coupled to 5600 TripleTOF MS

Slide courtesy of Dr Yannis Francois, University of Strasbourg
Biosimilarity Case Study: Cetuximab

- A single analysis of each sample sufficient to conclude on the complete similarity regarding AA sequence
- Complete sequence coverage is obtained through peptides without misclavages or PTMs
- CESI-MS/MS enabled to confirm an error, recently reported in the litterature

Gahoual R. et al., mAbs, 2014 (6), 1464-1473
Amino Acid Sequence Characterisation

**MS/MS spectrum of digested peptides LT04**

- APK
  - (m/z 315.2039 ; 2+)

**MS/MS spectrum of digested peptides HT15**

- CESI-MS detects both very short and long peptides in the same separation

- DYFPEPVTSVWNSGALTSGVHTFPAVLQS
- SGLYSLSSVVTVPSSSLGTQTYICNVNHKP
- SNTK
  - (63 amino acids ; m/z 1119.898 ; 6+)

Slide courtesy of Dr Yannis Francois, University of Strasbourg
## Broad Range of Analysis for MAb Glycan ID

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<tr>
<th>Glycopeptides identified as R.EEQYN(Glycan)STRY.V</th>
<th>mAb glycan abbreviation</th>
<th>Glycan mass (Da)</th>
<th>Monoisotopic mass [M+H]⁺ (Da)</th>
<th>Mass accuracy (ppm)</th>
<th>Average migration time (min)</th>
<th>Average relative abundance (%)</th>
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<td>FA2G1 (G1F)</td>
<td>1606.5867</td>
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1 Error was calculated as standard deviations from triplicate technical CESI-M5 replicate runs.
2 Relative abundances were calculated using peak areas of glycosylated peptides of the same sequence and charge state (-3).
3 [M+H]⁺ mass has a -18.0106 Da loss due to water neutral loss.
Glycoform Characterisation: Fd Region

- Fd glycosylation site characterisation

Glycoforms exhibited by the candidate biosimilar are significantly different from cetuximab

- 30% of glycans contains N-acetyleneuraminic acid

Rejected as biosimilar

Gahoual R. et al., mAbs, 2014 (6), 1464-1473
N-terminal glutamic acid cyclization characterization

- CE mechanism separates of peptide with N-terminal glutamic acid cyclization from the unmodified peptide

Results suggest partial modification of sample

Favorable conditions to estimate sample modification level

Gahoual R. et al., Anal. Chem., 2014 (86), 9074-9081
Separation of Aspartic Acid Isomers: Confirmation in Synthetic Peptides

- Aspartic acid isomers separation by CZE confirmed using a synthetic peptide

Synthetic peptides sequences:

\[ \text{NH}_2\text{-GLEWIGYSY} \text{ } \text{D} \text{ } \text{GTNNYKPSLK-OH} \]

\[ \text{NH}_2\text{-GLEWIGYSY} \text{ } \text{isoD} \text{ } \text{GTNNYKPSLK-OH} \]

Slide courtesy of Dr Yannis Francois, University of Strasbourg
Multilevel characterization of mAbs by CESI-MS – middle down analysis

IgG1, IgG2, & IgG4

Disulfide mapping
Alkylate & digest
~25 ng of digest analyzed

*Disulfide scrambling between mAb heavy and light chains can result in functional differences.
Disulfide linkages identified on IgG4
Multilevel characterization of mAbs by CESI-MS – top down analysis

Immunglobulin G (IgG)

IgG1, 2, & 4

Intact IgG
Charge Heterogeneity & Reduced Analysis

Level 3
Charge heterogeneity analysis of IgG1 by CESI-MS

CESI-TripleTOF® 6600 MS

~ 10 mg/mL sample desalted into 50 mM ammonium acetate, pH 4

BGE – 3% acetic acid (tITP-CZE mode)

~3.5 nL injection → ~ 35 ng injected

*Reverse migration order

pl 8.15

pl range – 0.31

*Main species

Basic species

Acidic species

Acidic species

Basic species

Main species

cIEF-UV
Charge heterogeneity analysis of IgG1 by CESI-MS

Single analysis at the intact level unifies multiple inferred analyses

Acidic, higher MW intact IgG1 specie and glycoform

Main intact IgG1 specie and glycoform

Potential clipped IgG HC-HC or Fab impurity

Potential clipped IgG1 species

Potential clipped IgG1 species
Reduced IgG1 CESI-MS analysis

IgG1 light chains

IgG1 heavy chain
### EPO Glycoform Heatmap by CESI-MS of Intact Protein

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**74 distinct glycoforms detected**

Sensitive Glycoform Profiling of β-Interferon-1α (Avonex) and recombinant human Erythropoietin by CESI-TOF-MS – Haselberg et al.
The CESI 8000 Plus – a new range of detection capabilities . . .

- LIF
- UV/Vis
- PDA

CE Assays & Method Development  MS Analysis
Summary

- CE is a robust and reliable technique – well integrated into Biopharma development and control processes.

- CESI-MS – Effectively integrates capillary electrophoresis with electrospray ionization serving to reduce/eliminate ion suppression and increase ionization efficiency for:
  - Insulin Impurities
  - Peptide quantitation
  - Multi-level mAb characterization
    - 1) Intact mAb/protein analysis
    - 2) Reduced mAb analysis
    - 3) Peptide mapping and PTM characterization
  - Proteoform/glycoform characterization

- We are just starting to see where this technique will take analytical chemistry.

- Take Home Message - “Flow Matters”
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