CE/MS: Method Development, Application and Implementation in Biopharmaceutical Development

Abstract:

Mass spectrometry (MS) offers high sensitivity and high mass accuracy, which has become a powerful technique to identify, quantify and characterize small and large molecules. Capillary electrophoresis (CE) separates molecules based on their electrophoretic mobility with the use of an applied voltage. It is widely used for biotherapeutics characterization, which is very suitable for top-down proteomics analysis. CE is also a powerful complement to LC, excels with charge or polar analytes. The combination of CE with MS allows highly sensitive and selective identification and detailed characterization of a wide range of molecules, including pharmaceuticals, biopharmaceuticals, metabolites, peptides, and proteins. This roundtable will discuss the utilization and application of CE-MS. Besides, we'd like to discuss challenges, method development and implementation of CE-MS in biopharmaceutical development.

QUESTIONS FOR DISCUSSION:

1. What are applications of CE-MS? Compared with LC-MS, what are the pros and cons of CE-MS? When will you choose to use CE-MS instead of other techniques?

2. What are the main challenges you have when you use CE-MS? How do you solve the problem? What parameters could be optimized when performing CE-MS method development? Do you have any routine methods for CE-MS?

3. Do you have tips or tricks of using CE-MS? How robust is your method?

4. What is the future of CE-MS in biopharmaceutical development? What could be improved? Instrument? Accessories? Software?

September 25, 2023

Scribe: Deanna Digrandi, *Regeneron*

Facilitator: Lin Zeng, *Amgen*

Notes:

CMP EMASS-II interface

- Peptide mapping
- top down and bottom-up proteomics
- glycosylation

- Very hydrophobic ADCs
- Highly glycosylated peptide mapping
- Challenges? Develop robust method, what coating, need more coatings
- CC1 coated capillary
- Difference between CC1 and PS1 - cation coated (CC), PS (Protein separation)
- PS1 – more resistant to high pH hydrolysis than PS2
- Other coatings in development PS3-→PS8
- Minimize buffer exchange and go directly to sample dilution buffer
- capillary coating – very universal
- cIEF-MS – minimum amount of pressure
- CZE-MS – no pressure

Sciex Intabio

- Add reduction agent
- Able to watch peaks
- Shorter the separation length, allows for higher protic solvents (methanol, acetonitrile…)
- Uses formamide to maintain solubility 50-60%
- CESI and Beckman – long capillaries give that extra time to have issues with volatile solvents.

Genovis

- Observed pulling proteins apart makes them more sticky
- Able to use enzymes to localize glycation
- HC/HC is really heard to meld to get resolution
- How do you address?
- Evaluate different mAbs and different formamide concentrations
- But data analysis is time consuming

AAV formulation/LNP formulation

How do you see these instruments supporting these areas?

- No DTT, no BME
- Sciex Intabio
    - 50-60% formamide with reduction agent
    - Need to make sure you get focusing
    - Chip was optimized
    - 14kDa proteins to fusion proteins to noncovalently bound structure
    - Water rinse between samples
    - Carryover is minimal
    - Chip is borosilicate and laser cut

- Glass is treated
- Optimize collision energy and scan range for the 200-300 compounds analyzed
- Collect all your metadata to make sure system is running properly even though requires a lot of storage.
- Run Reference Standards every 10-12 lines in sequence

Future of CE-MS

- Advantages of CE – more amenable to large proteins
- Faster in some cases
- LC is more amenable to MS over CE
- Don't always need to remove the sialic acid unless high levels
- Best way to ID glycation and deamidation is to remove the glycans first and look for the 162 Da difference.

September 26, 2023

FACILITATOR: Lin Zeng, Amgen, Inc.

SCRIBE: Nicole Langlois, Northeastern University

Notes:

1. ***What are applications of CE-MS? Compared with LC-MS, what are the pros and cons of CE-MS? When will you choose to use CE-MS instead of other techniques?***

Advantages of CE-MS:

- Use of one capillary for multiple molecules and modalities
- No carryover between samples or need to change columns
- Short run times (e.g. 5-6 min or less using microchip technologies)
- CE-MS is simpler to work with than nano-LC-MS

Limitations of CE-MS:

- cIEF-MS is limited in linking to in-process data, and difficult to integrate with chip-based technologies due to complexities of field focusing
- CE-SDS is the standard for purity, but it cannot ID new peaks as it is not MS-compatible
- CE-MS requires starting with a relatively high sample concentration (mg/mL) in small volumes

Current applications of CE-MS:

- Clone selection and development, cell culture screening, facilitating clinical to commercial
- Reduced peptide map, oligonucleotide analysis, intact charge variant analysis

When to use CE-MS over LC-MS?

- Depends on the target applications
- CE-MS may be superior to LC-MS for charge-based separations (e.g. cIEF > IEX)

2. ***What are the main challenges you have when you use CE-MS? How do you solve the problem? What parameters could be optimized when performing CE-MS method development? Do you have any routine methods for CE-MS?***

Challenging methods:

- For fast separations (e.g. ZipChip), how do you ensure accuracy and good resolution?
  - If you are able to attain sufficient data to support your application, then getting the highest resolution for a separation is not required
- Consistency of migration times between methods is a challenge:
  - Subtle variations in the coating process between chips cause time variations (< 1 min)
  - Use of a spiked mobility standard improves some of that variability
- High initial sample concentrations work for many applications, but some use cases require higher sensitivity such as with the analysis of samples from mouse models
  - Underscored importance for developing solid-phase pre-concentration on chip CE-MS

Challenging samples:

PEGylated proteins, highly glycosylated proteins, membrane proteins, LNPs, and AAVs

- Large heterogeneity in a sample overwhelms the MS analysis
  - Simplification with enzyme treatment or denaturation has been shown to improve
- Solubilization of membrane proteins is also a primary challenge without micellization
- For complex samples, purification is also a significant challenge
- For AAVs, a challenge in supporting robust applications is the difficulty in attaining standards
- Removal of excipients (phosphate buffers, etc.) is critical for good MS signal

3. ***Do you have tips or tricks of using CE-MS? How robust is your method?***
   - Addition of ITP sample can ensure focusing of analytes with good MS peak shapes
   - In the case of peak tailing, lower sample concentrations or alter sample buffer compositions

4. ***What is the future of CE-MS in biopharmaceutical development? What could be improved? Instrument? Accessories? Software?***

Instrumentation:

Intabio ZT - Integrated chip-based iCIEF with MS (TOF) (Sciex)

- Fast analysis of samples on ~nL scale to effectively resolve sample attributes such as deamidation, glycation, acidic variants, etc.
- Suitable for intact and subunit analysis, peptide mapping, etc.

Rebel system and ZipChip (908 Devices)

- Geared toward bioprocessing with a small footprint; designed for cost/complexity reduction

Future developments desired:

- Compatibility for CE-MS/MS with high sequence coverage
- Expanded access to peptide mapping applications → with open capillaries, it is difficult to maintain consistent migration times between different methods
- Improvements to loading capacity & sample prep limitations: use of 2D-chip separations coupled with integrated solid phase extraction, immobilization, or immunoprecipitation

Improvements envisioned:

- The quality of microchips for separations → how can manufacturers ensure robustness? There is a maximum limit of sample runs per device, but no guaranteed number of runs possible
- Field applications teams need to be able to train new users as the technologies advance
- Demos of new instrumentation are also critically important to show the tools in action
- Software data processing is the bottleneck for rapid, chip-based technologies
    - There is a need to bring CE-MS software up-to-speed to help with method screening and searching multiple PTMs at once