Table 5: CE/MS: Successes and Challenges in Biopharmaceutical Development

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Scope:

Capillary electrophoresis (CE) is widely used in biopharmaceutical development for the characterization of therapeutic proteins. A remaining challenge is the direct identification of variants as fractionation is limited by low sample amounts / concentration and coupling to MS has also its challenges, e.g. regarding buffer compatibility. In recent years several systems have been commercialized which are allowing the hyphenation of CE separation (CZE, cIEF) and mass spectrometry. This roundtable aims to discuss the technical strategies, challenges, and solutions offered by currently available CE/MS solutions as well as identify new opportunities for development in this space.

Questions for Discussion:

1. What is your application of CE-MS (intact (native/denatured), subunit, peptide mapping; separation/fast detection, CE peak ID)? Which CE-MS methods are you using (e.g. CGE, CZE and cIEF)?
2. What CE-MS systems are you using and what are their pros and cons? What are the pros and cons of CE-MS compared to LC-MS? What are the pros and cons of cIEF-MS compared to fractionation? Are you routinely using CE-MS? Are you using platform methods or is extensive optimization needed? Are you using CE-MS at the intact or subunit level?
3. Which information are you obtaining of separated peaks using CE-MS? Is MS information sufficient or is Top/middle-down sequencing necessary and how can this be obtained? Could this information be obtained differently/easier by e.g. using existing peptide mapping / subunit / intact data?
4. What further developments are needed to establish CE-MS in biopharmaceutical development? What are the actual major pain points?

Discussion Notes:

1. What is your application of CE-MS (intact (native/denatured), subunit, peptide mapping; separation / fast detection, CE peak ID) Which CE-MS methods are you using (e.g. CGE, CZE and cIEF)?

- Common applications:
    - Intact level for charge heterogenicity
    - Subunit

- Peptide mapping
- Peak ID
- Both native and denatured
- Glycosylation profile
- Amino acid analysis with ZipChip
- Common CE-MS method:
  - CZE/CESI-MS
  - CIEF-MS and iCIEF-MS
- ▪ Intabio/SCIEX
- ▪ CE Infinite
- ▪ CMP Scientific (Jun Dai, Anal Chem, 2018)
- ▪ ZipChip

2.	What CE-MS systems are you using and what are their pros and cons? What are the pros and cons of CE-MS compared to LC-MS? What are the pros and cons of cIEF-MS compared to fractionation? Are you routinely using CE-MS? Are you using platform methods or is extensive optimization needed? Are you using CE-MS at the intact or
subunit level?

- LC-MS vs CE-MS
  - LC-MS and CE-MS are two orthogonal methods.
  - CE-MS has advantages for certain modifications. They might not separate well in LC-MS, but CE-MS
- can easily resolve them.
- ▪
- Glycosylations (mannose)
- ▪ Lysine clipping
  - LC-MS is well-established and has methods readily available for many applications.
  - CE-MS needs minimal sample volume.
  - CE-MS has minimal run-to-run carry-over. Blank injections between each sample usually are not
- needed. Less column equilibrium time is needed too.
  - CE-MS spray emitter can get clogged. Tips from the discussion:
- ▪ Use a large diameter emitter (20 µm) when possible.
- ▪ Sample preparation is the key. Emitter lasts longer with clean samples.
  - Certain ion suppression presents in the LC-MS method is eliminated in CE-MS, especially in
- complex samples.
- Platform method
  - Amgen has published a platform CE-MS method for multiple next-generation biologics. (Mei Han,
- Anal Chem, 2021).
  - Existing cIEF platform methods are directly transferable to the Blaze system.

- Some advantages of ZipChip
    - Easy to use compared to LC-MS
    - Compatible with multi-vendor MS
    - Buffer kits are available for different applications

3.   Which information are you obtaining of separated peaks using CE-MS? Is MS information sufficient or is Top/middle-down sequencing necessary and how can this be obtained? Could this information be obtained differently/easier by e.g. using existing peptide mapping / subunit / intact data?

- The mass shift within 1-2 Da is acceptable.
- To identify deamidations, it is not sufficient to solely rely on mass spec data. But cIEF/CZE
- can separate deamidation.
- Top-down analysis for an antibody size molecule might be limited to the terminal sequences.
- It's hard to get to the middle region. Middle-down is an easier way to do it with better coverage.

4.   What further developments are needed to establish CE-MS in biopharmaceutical development? What are the actual major pain points?

- CE-MS is not a new technology, but it's taking off in the past five years. Many new systems
- and applications have shown up.

- Now many major vendors in the field have platform methods, such as CMP, 908, and
- Blaze, etc. They are more applicable in the biopharmaceutical world.
- The users must work with the vendors to help them understand our expectations and ask them to
- improve.
- People are more tolerant of existing technologies like LC-MS. If an LC-MS method has a
- resolution issue, or the LC-MS system has a communication issue or leakage, people think they are
- common. While for CE-MS, some people say it's not robust. We need to keep generating more good data
- and educating people. Also, we need to push the
- vendors to make more robust and high throughput instruments.