**Roundtable Session 1 – Table 3 – CE-MS: Current Trends and Applications in Biopharmaceutical Development**

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**Abstract:**

Capillary electrophoresis has been coupled to mass spectrometry for decades now and numerous publications exist showing the applicability and benefit of CE-MS for the characterization of (bio)pharmaceuticals. However, in the past CE-MS was considered a rather unrobust technology that is difficult to use in routine practice. This seems to be changing in recent years and more and more commercial systems are becoming available: These facilitate the coupling of CE to MS for multiple CE assay formats and make the technology more applicable to routine use. This roundtable focuses on the current use of CE-MS assays in biopharmaceutical development.

**Discussion Questions:**

1. What types of CE-MS applications do you use, or did you use in the past?
2. At what stages of the development do you apply CE-MS assays, and why?
3. Do you use CE-MS data in INDs/CTAs or BLAs/MAAs?
4. What is your experience regarding assay performance, stability, and ease of use?
5. What improvements would you wish for?

**Notes:**

**Roundtable participants**

- Most participants are familiar with MauriceFlex from Bio-Techne.
- For direct MS, Intabio ZT from Sciex, CEInfinite from Advanced Electrophoresis Solutions, and ZipChip from 908 Devices are also other technologies applied.
- Several participants are new in the field of CE-MS and want to gather new information and insights.

**Purity, fraction pooling, and stability of cIEF collected fractions**

- Purity and stability of the fractions are considered critical factors during cIEF fractionation.
- Good resolution is essential for ensuring fraction quality. Resolution can be particularly challenging for acidic variants.
- Pooling fractions across vials should be approached with care. Confirmation runs for Maurice Flex users are recommended prior to pooling to verify fraction quality. Some

participants report challenges during confirmation runs, such as migration time shifts and material loss for the run. Others note that CEInfinite runs performed on the same day tend to be more reproducible.
- Re-buffering (buffer exchange) or adding formulation buffer or other additives can help to improve stability, but these steps may introduce sample dilution.
- Sample stability in the collected fraction is particularly important when working with viral proteins (AAV).

**Amount and concentration in cIEF collected fractions**

- The decision to collect multiple cycles or to work with diluted samples depends on the MS requirements or the final intended use of the fractions.
- For peptide mapping, one strategy mentioned is collecting several similar species to have enough material. It was also mentioned that large amounts of material are not always needed, concentration is often the critical parameter.

**Direct MS analysis**

- Some participants highlight that using direct cIEF-MS coupling with Intabio ZT as an initial step can offer early insights, helping to guide next steps.
- Experience with the Intabio system suggests that method development is often necessary and highly molecule dependent.
- The relation between Intabio and BioPhase 8800 data is raised as an open question. Participants express interest in having tools or workflows that support better correlation between these platforms.
- The ZipChip system offers customizable background electrolyte (BGE) solutions to fine-tune separation performance.

**CE-SDS peak identification**

- The correlation between CE-SDS separation and molecular weight is not strictly linear, which can limit its accuracy for mass determination.
- Cutting bands from SDS-PAGE gels is mentioned as a practical approach for further mass spectrometry characterization.
- Employing orthogonal analytical techniques is suggested as an strategy to complement CE-SDS data.

**Other applications for CE-MS**

- Some participants report using CE-MS for peptide mapping or for analyzing released glycans as part of their characterization strategy.
- EMASS-II interface from CMP Scientific is mentioned as being used for these applications.
- CE can offer higher separation efficiency for released glycans compared to LC-MS, but with CE-MS being more labor-intensive. CE-MS becomes more valuable when in-depth characterization is required.