Roundtable Session 2 – Table 5 - Characterization of Single Peaks from CE Separations

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Abstract:

Capillary Electrophoresis (CE) is an analytical technique widely used in the biopharmaceutical industry for the separation and characterization of complex biomolecules. Despite its broad applications, the characterization of individual CE peaks, such as those obtained from cIEF (Capillary Isoelectric Focusing), CGE (Capillary Gel Electrophoresis), and CZE (Capillary Zone Electrophoresis), presents unique challenges. This roundtable will discuss the traditional methods used for characterizing these peaks and explore the advancements in emerging technologies. Join us to share insights, experiences, and strategies for advancing the capabilities and applications of CE.

Discussion Questions:

1. What are the traditional methods for characterizing cIEF, CGE, and CZE peaks?

2. What are the emerging technologies for online CE-MS? What are the advantages and disadvantages compared to traditional methods?

3. What are the emerging technologies for offline fraction collection? What are the advantages and disadvantages compared to traditional methods?

4. What are the most pressing challenges and unmet needs for the characterization of CE peaks?

Notes:

- In general, all participants have experience with Maurice Flex, Free-flow electrophoresis, CEInfinite, or Intabio.

cIEF peak characterization

- For cIEF, the identification of basic peaks is considered easy and straightforward by using several digestion enzymes. The problems start with the acidic variants. Acidic peaks typically consist of a mixture of various modifications. In this case, several options are mentioned by the participants for peak characterization/identification:
    - Peptide mapping together with forced degradation studies.
    - Combining the information from cIEF-MS and peptide mapping.
    - Fraction collection from IEX. However, this approach offers several limitations due to the different separation mechanisms, cIEF and IEX do not provide the same

expected information (e.g. different number of variants under the selected peak in cIEF in comparison with IEX).
    - Fraction collection with CEInfinite followed by MS analysis or followed by peak characterization with other techniques.
- At this point, a discussion starts about the purpose of collecting peaks, if identifying the peak is sufficient or it is necessary to fully understand the impact of the modification/charge variant. A potency assay from the collected peak is mentioned to be recommended to understand the real impact of the modification. Some participants comment that, from CEInfinite, it is possible to get enough material for a potency assay. The amount of sample required for potency is discussed in the roundtable, but it is also emphasized the importance of having pure fractions. Collecting pure fractions over multiple cycles to get enough material is an important point. Forced stress samples are sometimes used for fraction collection to reduce the number of cycles. Concerning the necessity of reinjecting each collected fraction to verify the identity and purity, it is commented in the roundtable that with CEInfinite this step is not completely necessary. For MauriceFlex to verify the identity after each collection run is recommended.

CE-MS online

- Online CE-MS is mentioned as a valuable tool for peak identification. However, the generally sharp peaks of a CE separation might not match the acquisition time in the MS, leading to low quality spectra. This mostly affects Orbitrap instruments. With the Zipchip instrument from 908 Devices, it is mentioned that the resolution sometimes needs to be reduced in order to maintain the spectra quality.
- Some other participants comment that the alignment of the cIEF and MS profiles in online cIEF-MS is sometimes difficult due to peak diffusion during mobilization.

CGE-SDS peak characterization

- Characterization of some low abundant peaks in the region between LC and HC is a question from one of the participants. Some suggestions for peak identification are:
    - peptide mapping or LC-MS to try to identify the peaks and to match with the CGE-SDS profile.
    - Western to at least understand if the peaks are related with the LC or HC.
    - HILIC, but under certain conditions HILIC can promote additional variants or generate some artifacts.
    - Cutting bands from SDS-PAGE, but only in case of high abundant peaks.
- At this point, the participants of the roundtable discuss why it is important to identify the peaks from CGE-SDS. Authorities require the identification and understanding of peaks in a CGE-SDS profile to a certain extent.