Roundtable Session 1 – Table 5 – Characterization of Single Peaks from CE Separations

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Abstract:

Capillary Electrophoresis (CE) is a powerful separation method generally applicable for many modalities such as small molecules, proteins, carbohydrates, DNA, mRNA, polymers, viruses, and bacteria. Characterization of each peak is a challenging task for any mode of CE separation, but it is still possible. Recently, some innovations are made for CE peak characterization using off-line fraction collection, and in-line MS analysis. This round table will be focused on discussing how CE peaks in CGE, CZE and cIEF are characterized with focus on challenges, industry trends, success stories and lessons learned.

Discussion Questions:

1. Have you developed method(s) for characterizing CE peaks? What CE methods?
2. What methods do you use for CE peak characterization in CGE, CZE and cIEF?
3. What other methods/techniques could one employ in CE peak characterization beside MS,
4. offline? Or, do any other such methods/tools exist?
5. What are people experiencing with the current CE-MS technology for cIEF such as IntaBio/SCIEX icIEF-UV/MS, or CEInfinite cIEF-MS, or off-line fractionation from Maurice Flex, or even custom coupling from regular CE to MS? Any feedback with advantages and disadvantages?
6. How about peak characterization in CZE such as 908 devices?
7. Lastly, CGE peak characterization? Any attempt beside old school, collect from different separation methods (SEC, RP HPLC) then inject back to CE? Where are we with regard to CGE-MS technology? How about cutting capillary after CGE separation?
8. What are the challenges associated with developing CE peak characterization?

Notes:

The discussion confirmed that, peak characterization of single peaks for CE assays is a relevant topic for all roundtable participants. Two CE-based methods are in focus, namely SDS-CE and ICIEF. With CZE and CIEF being less common in release and stability methods panels, these

assays seemed less relevant, but similarly challenging regarding peak characterization, if required. With protein-based biopharmaceuticals currently being the majority of products in late development stages or being marketed, primarily related assays were discussed. It was acknowledged that, challenges with peak characterization for CE-based assays similarly apply to the increasing number of non-protein-based biotherapeutics such as e.g. AAVs.

Indirect characterization is still common practice to identify peaks in CE-based assays. If feasible, fractions from comparable LC-based assays (e.g. ion exchange chromatography in case of charge heterogeneity) are collected, characterized, and analyzed in respective CE-based assays to deduce peak identity. Samples of (various) forced degradation studies analyzed with multiple assays allowing to determine identity and potency, and likewise analyzed by CE-based assays further support peak identification. In particular cases suspected variants can be produced e.g. by enzymatic treatment (and subsequent purification), and spiked into the respective CE assay to further support peak characterization. Additionally, off-gel fractionation is still used for peak characterization purposes.

Recent developments with respect to ICIEF fractionation and ICIEF-MS coupling considerably facilitate peak characterization for ICIEF (and CIEF) assays. Most roundtable participants use at least one of the available techniques for ICIEF fractionation, or ICIEF-MS coupling. Although ICIEF-MS is reported easy in handling and delivering valuable results, intact mass data is not regarded sufficient for peak characterization. The lack of functional data (e.g. potency / target binding) or at least a precise localization of modifications still requires fractionation. Fractionation and fraction analysis is reported well doable on both, CEInfinity and MaruiceFlex systems, but with each of the systems seemingly comprising individual strengths. LC-MS peptide mapping-based analysis is on top of the assay panel applied to collected fractions, followed by potency bioassays or binding assays. The very limited sample amount of iCIEF fractions remains a major challenge. Another challenge is the fact that, collected charge heterogeneity fractions usually comprise more than only one single variant. CZE-MS (e.g. using the ZipChip) was mentioned by some participants to be useful for charge heterogeneity characterization in general, but of only limited benefit for ICIEF peak characterization due to the different separation principle leading to considerably different electropherograms.

Direct characterization of SDS-CE peaks is still not feasible and regarded the remaining main challenge for CE assay peak characterization. Recent reports in the literature (and at CASSS CE Pharm) to either develop SDS-CE-like assays compatible with MS, to “quench” SDS, or to “remove” SDS by e.g. 2D-CE-MS are recognized but are still deemed inapplicable outside from academic research laboratories. SEC-MS and HILIC-MS are applied by some participants and reported to be useful as supportive tool.

Finally, the discussion arose about how much characterization is actually needed. So far regulatory agencies seem to commonly accept that peak information for CE assays is limited. Roundtable participants involved in biosimilar development noted that, this observation may hold true for originator products but reported on specific peak characterization data being requested for CE assays by authorities in case of biosimilar development projects.