Session 1: Peak Characterization - CGE, Peak Characterization CZE, Peak Characterization - CIEF

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Abstract:

CE has the best separation for almost all modalities, small molecules, proteins, carbohydrates, RNA, DNA, polymer, viruses, bacteria, and yet it is the hardest to characterize each peak. Peak characterization for any mode of CE is almost a "nightmare", but it is not impossible. There are some advancements of methods for CE peak characterization such as in-line MS and off-line collection. MS seems to be the most common one currently, of course, with some challenges in connecting to CE. In this roundtable we will discuss the strategy of CE peak ID and the current most common method(s) to characterize CE peaks in CGE, CZE, cIEF with focus on challenges, industries trends, success stories, and lesson learned. It is the intent of discussion to share each company's experience, strategy, and new suggestions.

Questions for Discussion and Notes:

1. ***What is your strategy in CE peaks characterization in CGE, CZE, and cIEF? Even if the plan does not go well, what are your alternative solutions?***

    - CGE (CE-SDS) & cIEF are both routinely used, but perhaps CE-SDS is more critical as there is no alternative LC method with comparable separation power (like IEX for IEF)
    - When beginning any new peak investigation, it is important to consider the current stage in development for how to begin with your analytical approach
    - Characterizations of CE peaks are typically done using orthogonal LC-MS separations, or a series of fraction collection and subsequent analyses. The best case is to have CE-MS direct coupling, but if that technology is not available, then use orthogonal methods
    - One common approach is to use IEX-MS with fraction collection, then inject each fraction on cIEF for peak characterization
        - However, with any fractionation method it is important to keep in mind that low abundance or unstable variants may not be recovered
    - As a strategy to build methods that are QC-ready, it is important to focus on CE-MS technologies as there is greater consistency when compared with LC-MS methods

2. ***What other methods/techniques could one employ in CE peak characterization beside MS, offline? Or, do any other such methods/tools exist?***

    - Online digestion of fractions from CZE can aid characterization using caproic acid, but there are additional challenges of using other MS-incompatible buffers

3. ***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?***

    - Maurice Flex: analysis by cIEF with fraction pooling in a 96-well plate
        - If fractions are collected and pooled using an MS-friendly (volatile) buffer like ammonium acetate, each fraction can be analyzed by MS
        - The approach is not directly online, but it is straightforward and user-friendly
        - Suitable for intact and subunit analysis to identify bispecific mAb peaks
    - A key characterization challenge is that many commercial CE-MS technologies are expensive
        - Using commercial coupling technologies, you could prepare your own CE-MS system

4. ***How about peak characterization in CZE such as 908 device?***

    - ZipChip (908 Devices): a microchip CE-MS platform
        - Demonstrated for intact, subunit, and charge variants with direct MS-coupling
        - Not yet robust for peptide mapping, but advantageous for rapid analysis

5. ***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?***

    - CE-SDS is not compatible with MS, the SDS needs to be removed first; CE-SDS is critical for size-based purity analysis, so it cannot be replaced by other charge-based separations
    - *One preliminary online approach*: Use a cationic surfactant to form a precipitate with SDS and remove it using a second dimension of separation via a switching valve. Once SDS is removed, the protein is compatible for online MS.
        - This method is not straightforward and requires a highly skilled/dedicated person to use, but it can be effective for peak characterization
    - There are no existing commercial platforms that can quantitatively remove SDS for online MS of size variants → the field should work towards this goal
    - CE fractionation methods have been demonstrated for CE-SDS peak characterization
        - The small loading capacity in each capillary requires >100–200 injections to collect enough material for further MS characterizations after SDS removal
        - This approach can work, but it is challenging and requires a lot of instrument time
        - Use of IEX (HPLC) might be better than CZE or IEF (CE) for fractionation as there is a higher material loading capacity
    - *Future opportunities:* if there are labs that are equipped to perform CGE-MS approaches could train others or collaborate, this would have a major impact on the industry. There needs to be significant investment in technologies that can enable CGE-MS in the future