Roundtable Session I – Table 4 – Technology Advancements in CE

Facilitator: Dr. Mohamed Dawod, *IGM Biosciences*

Scribe: Dr. Jane Luo, *SCIEX*

Abstract:

CE is experiencing a rapid transformation driven by advancements in instrumentation, detection, and automation. Recent innovations, including microchip-based CE, enhanced detector sensitivity, and CE–mass spectrometry (CE–MS) coupling, have expanded its analytical power for a wide range of applications. In the biopharmaceutical field, CE has become a key platform for a wide range of molecules (including biologics, proteins, and glycoproteins), including charge and size heterogeneity, glycoform characterization, and in-depth evaluation of complex biologics such as Fc-fusion proteins. These technological improvements have enabled faster, more robust, and higher-resolution methods, while also facilitating high-throughput and automated workflows for both research and quality control. Despite these advances, challenges remain, including method transferability between sites, the cost of implementation, and harmonization with global regulatory expectations. This roundtable will bring together experts to discuss the latest CE technologies, share practical experiences, and explore future opportunities, including emerging trends in sensitivity enhancement, regulatory acceptance, and integration with complementary techniques such as liquid chromatography.

Discussion Questions:

1. What recent CE innovations have most improved biologics, protein, glycoprotein, plasmid, and RNA analysis?
2. How does CE–MS enhance the characterization of complex biologics compared to traditional CE?
3. What are the biggest challenges in CE method transfer and regulatory approval?
4. How is automation shaping CE workflows in QC and research environments?
5. What future trends could make CE a primary rather than supporting analytical platform?

Notes:

1. What recent CE innovations have most improved biologics, protein, glycoprotein, plasmid, and RNA analysis?
    a. Recent CE innovations include IntaBio and MauriceFlex. IntaBio couples iCIEF directly with Mass Spec, making it great for charge variant identification. MauriceFlex performs icIEF fractionation. It's not dependent on a single mass spec. Each cartridge supports 15 injections, so you can pool the fractions from multiple injections and concentrate them for further analysis, such as mass spec.
    b. Previously, big variations were often observed. Now, there are so many kits from vendors available that help to do it readily, consistently, and for both characterization and QC. In addition, you don't have to coat your own capillaries and pour your own gels. Furthermore, with pre-assembled cartridges, you don't have to cut the capillaries.
    c. Of course, there are pros and cons for both kitted products and customized (homebrew) gels. Using kitted products saves time, but sometimes, lot-to-lot variation or ghost peaks are observed. When making homebrew gels, it is essential to be diligent in screening the raw materials used to produce the gel. Continue to test these raw materials before producing each batch of homebrew gel is also important. It's a lot of work, but we have full control of it. But not everyone has the resources and time to continue screening raw materials. It would be great if vendors could provide information such as the equivalent type of polymer or column, even for the kitted gels. That might be a challenge. For example, the Sample Loading Solution from SCIEX is a highly purified formamide, while the formamide from other vendors is not purified to the same level of quality.
    d. New software: Being able to transfer data to Chromeleon for further analysis is good. Although a lot of places to click, it's good to have options. It would be nice to have customers involved in the software development so that customers' feedback could be incorporated. Having an open system also helps.
    e. The BioPhase SW is good and easy to use with different colored traces, much improved over the 32 Karat. The LabChip GX Touch is faster. However, if you run the BioPhase overnight, the time per sample is approximately the same as that of the GX Touch. The BioPhase has better resolution. For protein analysis, both reduced and non-reduced, the PA 800 Plus and the BioPhase gave equal performance.
    f. Automation of sample prep for protein analysis is getting popular. Many use the Hamilton liquid handler.

2. How does CE–MS enhance the characterization of complex biologics compared to traditional CE?
    a. Compared to traditional CE, where the charge variant identification is done based on prior knowledge, direct MS detection in CE-MS allows confident identification of charge variants. Direct coupling of CE and MS is also more efficient than traditional CE followed by MS, as different peaks separated by CE can be

analyzed by the mass spec immediately, and resolution is maintained during transfer to MS.

b. AES CE Infinite also uses direct coupling of icIEF with MS, and has had promising results so far. It's good to know about some of the unknown peaks and be able to call out specific peaks. CE Infinite with icIEF is working well for characterization.
c. IntaBio offers high resolution in separating charge variants, even the ones with a small pI difference, where traditional CE has challenges. Together with the Biologics Explorer (BE) software, IntaBio enables confident identification of charge variants, including those with small pI differences.
d. Limitations of CE-MS platforms: i) The master mix needs to be MS-friendly for both IntaBio and CE Infinite. ii) CE Infinite is not super QC-friendly yet. It would be great to have a simple method that QC people can run. The transfer from urea to formamide needs some extra effort. iii) IntaBio is only compatible with SCIEX mass spec.
e. Limitation of MauriceFlex: The kit and cartridge used for fractionation are different from the ones for regular icIEF. It is essential to verify that the same results are obtained and the profiles remain unchanged.

3. What are the biggest challenges in CE method transfer and regulatory approval?
    a. The scientists and management have different preferences. Scientists seek a straightforward method that yields optimal results for a single molecule, whereas management prefers platform methods that are effective for multiple molecules. In reality, it is always a balance between the two.
    b. In academic environments, it is easy to develop a method. In the industry, it is more difficult because the method needs to be easy to follow for different analysts. Therefore, ready-to-use kits and coated capillaries are invaluable.
    c. Regulatory folks want to know about all the peaks, while scientists focus on the important ones.

4. How is automation shaping CE workflows in QC and research environments?
    a. Advantages of automation: A: Eco-friendly; B: Saves time. Several people can do the sample preparation together and then split the samples for different assays.
    b. Limitations of automation: i) Hamilton needs a sample volume of 150 to 200 µL. ii) In our CE-LIF sample preparation, we had to use a low volume, such as 10 µL, in order to save dyes. That is an issue with Hamilton, as it does not handle small volumes like this as well as manual methods. iii) The Tecan liquid handler had higher RSD values than the manual method when the formulation buffer was used to dilute the samples. Therefore, we go manual only for this step.
    c. Connecting the liquid handler with the CE instrument: i) Can we connect the liquid handler with the BioPhase? That is the goal for a future project; ii) Maurice: the liquid handler taps Maurice, then the door is open. Iii) The Agilent 5400 fragment analyzer has automation integrated for nucleic acid analysis. It has 96 capillaries in an array. The robot opens the door to load samples.

d. Choosing the optimal master mix through DOE experiments, and then, keep using the same method. This will save time and facilitate automation.
e. It would be great if the entire analysis process could be automated, starting from creating the sample plate, pulling the files or plates by barcodes, making the sample set, integrating the peaks, and generating the Excel report. Some people are already doing this. The size and the tolerance range of the size or migration time need to be defined for confident peak identification for CE-SDS.

5. What future trends could make CE a primary rather than a supporting analytical platform?
   New kits, new detection methods like NFD, and new capillaries can all help. In some companies, CE is already the primary analytical platform. It would be great if we could find out what each peak is. Mass spec would be needed to identify them. Ion exchange is a slower and more expensive process.