Table 11: What Attributes Have Not Been Explored Using CE Methodologies?

Session 1:

Facilitator: Joel Welch,*CDER, FDA*

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Session 2:

Facilitator: Clare Ryan, *Regeneron*

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Scope:

This roundtable will discuss what we wish we could do on our CE platforms and why we are unable to do so now, and where those novel methodologies would be useful. Topics include molecules of interest, detection methods, and molecular attributes.

Questions for Discussion:

1. When you have a molecule and need to develop a new analytical method, is CE the first method you turn to? Do you have the time to explore novel approaches using CE?
2. What kinds of molecules do you wish you could analyze, ie, polysorbate, exosomes, ADCs, oligos?
3. What other detection methods besides UV and LIF might be employed, ie, full spectrum UV, FT IR, DLS? Are there molecular attributes that would be nice to have, ie, native size, small molecule/protein interactions, kinetics? Once you develop a cool assay, does that stay in R&D or is the technology easily transferred to other teams and adopted?

Discussion Notes:

Session 1:

CE (charge separations) can be a great tool for monitoring target product profile on attributes. CE could be a good surrogate for this purpose. If the charge doesn't change then likely nothing else will change.

- Unmet needs:
    - Aggregation
- Some work on high molecular weight species be CESI MS has been done at SCIEX
    - Peak ID
- new options from vendors for iCE MS (intabio), cIEF MS (Beckman, Agilent), and CZE ms (908 devices), all charge based.
- CE-SDS has been done with MS detection by the lab of Profs. Neususs and Jooss.
    - Affinity Capillary electrophoresis to replace bioassays
    - Native separations should be developed to look at protein unfolding/folding
    - CE NMR
- fraction collection from CE or running capillary through the magnet. Software would be challenging as spectrometric data is often manipulated. For UV typically there is no treatment of the signals from the system. MS and NMR smooth the data.
- Challenges:
    - CE does a good job on low hanging fruit. Can we use analytical QbD? We may find a technique that was not previously used, may get right first time
    - Acceleration does not come from cutting corners, it should be done from working smarter. Are we doing risk analysis at the right time? Are we providing decision makers with the right information?
    - Are there CE specific detection methods that could be developed rather than taking what is available for LC and using it for CE?

- There are issues with material availability. For vendors, there is a challenge getting meaningful samples. For people working in the gene therapy space, yields are low making it difficult to get material for internal development (in addition concentrations are low).
- Can we correlate what we find with our characterization to clinical data?
- Would like to have more info from vendors on product composition (buffers), can more buffers be made available? AZ uses their own buffer recipe for some molecules in development.
- The organization/company may be hesitant to adopt CE or a new use of CE based on existing methods/technology that is currently being utilized. An example of the CZE method that was developed by Janssen for Virus characterization was discussed. CE was new to their network but the technology was rapidly adopted in the company based on the quality of the information provided by the technique.

Session 2:

1. When you have the need for a new analytical method, do you turn to CE?

   Scientists default to the existing platforms that are available in the lab, including SEC, UPLC, CE-SDS, iCE, LC-MS, CE-MS. The availability of the instrument often defines the method that gets developed. Novel approaches sound great but when resources and time are limiting, you use what you have available, rather than explore novel modalities.

   R&D have more time to explore multiple technologies and develop novel assays which then should move into process development and eventually QC. The QC analyst needs to be educated and technically sound, not just serve as a "room temp robot."

   A novel assay can be successful in R&D but not be adopted elsewhere within the organization. Some of the factors that limit adoption include expense, regulatory concerns, physical space, and expertise. A good example of limited adoption is mass spec, a great tool in R&D but not utilized in QC. Philosophically, every scientist wants more and better data but no one wants to adopt a new method late in the game that yields results that are surprising.

   Technical demand for more sensitive assays can drive adoption of the science, even if the method is difficult. If the method is sufficiently well developed, interns can be trained to execute novel assays.

2. What molecules do you wish you could analyze by CE?

   Exosomes, mAbs, AAVs, ADCs, and oligos can be analyzed by CE now. Polysorbate analysis by CE is desirable. Degradation of polysorbate can result in particulates that induce an immune response. The cause(s) of the degradation may include low level lipases from the culture or peroxide. Current analysis is completed by RP-HPLC with DLS detection or cobalt extraction with UV detection.

   A CE method would likely be nonaqueous with 70% organic and possibly require enrichment of the sample with C18.

   ADCs can be monitored by CE-SDS with LIF. Additional data can be gleaned by CE-MS for peak ID with single dalton differences. CE is more QC friendly with compliance, data integrity, calibration, and a quick turnaround of data that allows for speedy release.

3. What other detection methods would be nice? Once developed, are novel technologies easily transferred?

   Native size and native fluorescence, protein interactions, a changeable fluorescent filter for ADCs to match the wavelength of the drug. FTIR and NMR sound like good ideas but likely do not have the sensitivity for a flowing stream and would require sample collection.

   Tech transfer from a university to a company is like tech transfer between R&D and QC. The innovators may have a new toy that does wonderful things but they need to think in advance about adoption and how the instrument will be used. The assay should be easy to operate, with automatic data interpretation, an understanding of the outcome and pitfalls, and defined invalids. The ideal assay would review data in real time, recognize the atypical data, and re-inject, all according to predefined parameters. The goal is take the decision out of the hands of the operator and remove any subjectivity in interpretation. The manual check of the data after automatic data generation is also necessary.