Table 3: New Modalities

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Scope:

Pipelines are evolving in the biopharmaceutical industry. Now we are not only working on mAbs but also many emerging new modalities such as viral vectors, nucleic acids, engineered proteins, ADCs, etc. With these challenging new modalities, we have started to find unique advantages of applying CE methods for product characterization, process monitoring, and QC testing thanks to CE's high sensitivity and resolution. But on the other hand, unlike with mAbs, the application of CE is usually not well defined for the analysis of new modalities, either due to technical difficulties or limited product knowledge. In this roundtable we will discuss the industry's current trend and application of CE based technologies on new modalities, with a focus on the success, challenges, and lessons learned during method development. Case studies are welcome so that everyone can share in the learning experiences.

Questions for Discussion:

1. What are the new modalities you are working with using CE based technologies, e.g. AAVs, Plasmid DNA, mRNA, ADCs, engineered mAbs (fragments, bispecific, fusion proteins), or any other?
2. What CE applications have you been working on each modality and what have been successful/unsuccessful so far? Characterization vs. QC?
3. What are the key advantages for using CE based methods on the new modality versus other type of methods?
4. What is the biggest challenge for developing CE methods on these Frankenstein molecules? Sensitivity, resolution, or robustness? Case studies are welcome.
5. How could we optimize CE methods for new modalities? Think about opportunities in the future and in your opinion what the current gap is?
6. How could the industry and vendors work together to solve the problems we are facing?

Discussion Notes:

1. What are new modalities that you are using CE with?

- C: we use CE to analyze adeno-associated viruses (AAVs) for capsid purity analysis
- we also use CE to analyze single stranded DNA (ssDNA) inside capsids
- One main limitation for this study is that there is no commercially available single strand DNA ladder
    - C would like to learn how others make ssDNA happy on a capillary
- Z: we use CE on AAVs and heavily glycosylated fusion proteins
- Cong: we use CE methods on monoclonal (mAb) variants, antibody chemical conjugates (not ADCs), large molecule modalities, fusion proteins, in vivo samples with complex matrices
- K: Fabs (the top portion of mAb) and small proteins
- Trouble lies in forcing methods developed for mAbs onto these novel formats
- D: FC variants (smaller than mAbs)
- Working to find a general approach for these formats
    - -Protein to protein variation is a bigger issue than what is seen with mAbs

2. What CE applications do use for these new modalities?

- -mAbs utilize CE methods for purity and charge variants assessments
- Companies have utilized CE methods to assess AAV capsid purity
- O: Empty vs. full analysis for viral vectors
- A key limitation observed was that technology may not be sensitive enough compared to other non-CE methods
    - S then inquired about how sensitivity could be improved
        - -Issues of note include:
            - Complex profiles -> get several peaks (sometimes additional peaks appear)
- These complex profiles show large batch to batch variability
    - AUC gets more consistent data than CE, but AUC is more labor intensive
    - S saw better results for a CE based empty full assay with Adeno Virus (AV) -> (AV) is a bigger virus than AAV
        - S noted that cIEF works better for AAV empty full analysis than AV empty full analysis
- K: New modalities are forcing people to look into developing CE methods
    - -i.e. Mispairing assays-> HIC methods may not work for all classes of a particular molecule

3. What are key Advantages to using CE?

- More complex molecules drive the need to develop CE methods
- Liquid chromatography methods may show limitations with modalities outside of protein work (i.e. intact protein level)
    - We need a reproducible and easy method to transfer to QC

        - B asked if we use CE primarily as an orthogonal method to give a more complete picture of the molecule
    - -i.e. AUC or electron microscopy as the gold standard for empty vs. full analysis
    - -CE is a good orthogonal method to speed up analysis
- D: noted a similar thinking in charge heterogeneity analysis
        - IEC vs. cIEF -> should we run both if we don't have much info on the analyte
    - -This way you know that you are not missing something in one method
- B then asked if primary knowledge from mAbs can be applied to gene therapies
        - A noted that if you get a team together with different backgrounds (analytical chemists and molecular biologist) you can do a group think to determine what knowledge can be transferred (literature versus current thinking)
        - Z noted that a starting point is always the current knowledge base, but there is always the notion that this knowledge may need to be adapted

4. What is the biggest challenge with CE method development?

- D noted that the challenge is in the characterization part
- We could have a good CE separation, but buffer is not Mass spec friendly
- It can be quite time consuming for characterization
- K brought up the idea that lack of previous knowledge and literature to fall back on makes it difficult to deliver a CE
- You are the expert and need to figure things out
- Z highlighted sensitivity issues -> for AAV you don't have a ton of material to work with
- Lack of sample and low concentration
- C seconded Z thought on sensitivity
- He then highlighted problems he faced working with in vivo samples (low abundance in complex matrix)
    - For C sample enrichment and then detection is key
- K also noted that new modalities may have more complex profiles with not as nice peak separation
    - Profiles could make you nervous on whether regulators will accept the data

5. How did you overcome these challenges?

One idea raised was whether we can we break up the giant molecules into smaller pieces and then look at it

- D gave an example of splitting the fusion protein and performing peptide mapping on each portion to characterize it
- C suggested doing middle down analysis via enzymatic digestion -> get small pieces to possibly boost resolution
- S noted that CE hyphenated techniques are more popular
- She highlighted that more complex profiles increase the need for identification
- If we can characterize peaks better ten it is easier to go to QC with it

- Side topic raised: Sensitivity issues with Gene therapy products-> have you used different detectors to increase sensitivity?
- S's company is developing a native fluorescence detector -> still in early stage
- Is sample concentration a possibility?
- O noted that buffer exchange for other assays like CE-SDS were not an issue for QC transfer as long as explanations are given for why it is needed
- Z asked about trying to use laser induced fluorescence (LIF) instead of UV for gene therapy product
    - S said their company got good signal with LIF, P5O3 dye got good sensitivity for capsid purity
- A noted that UV can detect up to 10E12 concentration (1:100 dilution)
- How can we work together to improve issues and solve the problem?
    - B and S both put out the call for collaborations because instrument vendors lack real materials for development
        - Can only use commercial materials which may not be the same
- Collaborations with companies will help develop better methods
- O noted that a company has been working with two companies to develop AAV methods