Round Table Session 1 – Table 3 – icIEF Becoming the CE expert at your organization

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Abstract:

Imaged Capillary Isoelectric Focusing (icIEF) is a popular tool for characterizing protein charge heterogeneity based on its isoelectric point (pI). This roundtable focuses on the crucial role of icIEF experts during the development and quality control of therapeutic proteins. Join us to discuss best practices, common analytical challenges, and strategies for keeping pace with emerging icIEF trends.

1. What are some common challenges that an icIEF expert helps address?

2. What are the most essential skills for an icIEF expert to have?

3. What are the most effective strategies for acquiring these skills?

4. How can icIEF experts best adapt to emerging icIEF-MS and icIEF fractionation technologies?

5. How can icIEF experts best adapt to emerging modalities such as antibody-drug conjugates (ADCs) and lipid nanoparticles (LNPs)?

Discussion Questions and Notes:

1. What are some common challenges that an icIEF expert helps address?
    - Countries require resolution criteria for the methods, could propose a challenge with unfamiliarity of the instrument
    - Questions surrounding the characterization of the peaks. Addressed by increasing the amount of characterization work of the peaks in earlier stages
    - Increase issues with integrity of Cytiva Pharmalytes (making product specifications fail due to the differences in the lots)
    - Lack of knowledge on what the pharmalytes are compose of
    - Increased noisy baseline between different types of pharmalytes
    - Conducting bridging studies when method changes (ie. iCE3 to Maurice or UV280 to native fluorescence)
    - Conduct method robustness in earlier stages of the platform methods before method development in order to guide you in the method development for the new products as needed
    - Challenges with the HPLC/UPLC column packing and lot to lot differences causing shifts of charge variant methods from IEX to icIEF

2. What are the most essential skills for an icIEF expert to have?
    - Proper training and knowledge sharing
    - Knowing all the critical reagents and the purpose of each: carrier ampholytes, solubilizer, stabilizers, additives, etc.
    - Familiarity with the sample preparation, method and instrumentation
    - Needing to understand each method and the technology in full (IEX vs cIEF vs CZE)
        1. Understanding stationary phase on IEX (could be nonspecific interactions with molecule) would help explain that cIEF would be better fit
    - Do more characterization work in the earlier stages to understand the acidic and basic peaks and which you would leverage to other similar modalities
    - Close connections and contact with vendors
        1. Emphasis on having to order specific lots of pharmalytes
        2. Staying up to date on instrument changes and best practices
        3. Vendors publish technical notes and whitepapers to help troubleshoot
    - Having to learn from experience meaning lots of trial and error in order to become an SME
3. What are some of the most effective ways of acquiring these skills?
    - Leveraging platform methods to continue method development
        1. Only use platform to establish a baseline then optimize in the later stages
    - Need to know the goal/purpose (weigh in early vs late phase) before starting development
        1. JMP useful to use for DOE for method development and give you the most optimize conditions
        2. AE fusion software (comparable with JMP) to give you DOE. Use DOE softwares to save time on optimizing different conditions one by one
        3. Knowing what is fit for the purpose of the specific study, product, and/or what project stage
4. How can icIEF experts adapt to emerging icIEF-MS and icIEF fractionation technologies?
    - Bridging the need to replace urea with formamide in addition to changing methyl cellulose with other coating agents as both are incompatible with MS
        1. This leads to changing in the method parameters in order to fractionate
        2. Best practice would be to run the fractions back with your icIEF method
    - Historically fractionation work was using liquid chromatography method to fractionate but more companies are moving towards CE-base methods to collect fractions as this may be more representative and save you time on developing a IEX method
    - Consider the use of the following instruments: Sciex Intabio ZT vs Maurice Flex vs Advanced Electrophoresis Solutions CEinfinite
        1. CEinfinite gives you a focused electropherogram at each of the fraction collection, but the flex does not give you that
        2. AE capillaries are already coated, which helps you not have to use MC
        3. Then run the fractions back on your iCE/Maurice method
        4. Maurice Flex has some method development to get the enough volume for each fraction
    - Consider use the fragment analyzers to get fractions and then evaluate/characterize on your iCIEF method
5. How can icIEF experts adapt to emerging modalities such as antibody-drug conjugates (ADCs) and lipid nanoparticles (LNPs)?

- AVV or fusion proteins uses native fluorescence detection using Maurice for method development
    1. Higher amounts of solubilizers (ie. urea, BME, glycerol) may be required for new modalities than with other mAbs
    2. Keep in mind temperature and stability of the capsid during method development
    3. Challenges with co-formulation of similar pI molecules

Other Notes:

- Maurice vs. iCE3: very similar profiles, overall noticeable improved resolutions (exception of 2 molecules, where the solution was to recommend that method has to be optimized before bridging to Maurice)
- Recommending not using arginine for most methods (only for 10.1) when using the Maurice. Suggestions that arginine may not be required for the Maurice instruments vs. iCE3
- Maurice experiencing more right shifting in profile with similar focus times from the iCE3, which could be attributed to the difference in detections
    - Maurice detection showing signs that could be better than iCE3
- iCE3 pI markers don't all have native fluorescence on the Maurice
    - Some pI markers also have residual peaks
    - Recommend changing the pI markers to use the Maurice specific pI markers (which all have fluorescence)
- Issues with increased in basic/acidic peaks compared to main peak (maybe due to quenching?) with moving to fluorescence detection
- Perform instrument to instrument comparability then reference ICH to follow their validation and qualification guidelines
    - Historical experience involving the switch valve causing problems with different charge variants between iCE280 and iCE3 that affected with product specifications for commercial product
        - Follow up with characterization and more comparability studies
        - Integration was inconsistent (back to training)
        - Had to change specifications due to the instrument changes
        - Might have to re-validate the method
- New modality molecule could be highly glycosylated and sialylated which would require cathodic stabilizer and more method development
- High pI markers: 9.77 or higher falling off the window (9.5 was more stable)
    - Wide range between the low and high markers could push it off (this affects method with long FTs)
    - Happening more with Maurice flex (since you require longer FTs for Maurice Flex methods)
- Experience with asking Protein Simple Vendor to adjust your instrument camera
- AEslyte are comparable to Cytiva (switched from GE) pharmalyte
    - Problem with the lot differences could be when Cytiva switched from GE which showed pI may shift more than 0.1 pI units
- Recommend look at the slope of the COA of the pharmalyte lots will help with reproducibiltiy, then request to order these specific lots from manufacture
    - Since pharmalyte lot batches are different

- Recommend qualification of pharmalytes to ensure the reliable of the pH gradients
- Suggestion to switch to native fluorescence from UV absorbance detection with the icIEF method
    - To help with carrier ampholyte interference and baseline problems