Beyond the mAb – Fusion Proteins, Biospecifics, and Peptides Applications

Abstract:

Over the past 20 years, protein characterization by CE has become widely adopted as an alternative to conventional separation techniques like slab gel based SDS-PAGE and IEF for analysis of mAb size and charge variants. More recently, development of protein variants including ADCs, bispecific mAbs, mAb variants, fusion proteins, and even peptides has increased, creating the need for similar robust characterization strategies. This round table is focused on discussing how CE has been and will be used to profile and test non-mAb protein products. What are the opportunities, limitations and challenges of developing and applying CE methods for these analyses? These include the testing of purity, charge, stability, conformation, etc.

Questions for Discussion:

What non-mAb protein variants are currently or will be in development?

What methods are currently being used for purity/size and heterogeneity analysis?

Can conventional CE assays be applied for characterization of these proteins/peptides?

What challenges exist in the characterization of these proteins/peptides?

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What non-mAb protein variants are currently or will be in development?

<u>What non-mAb protein variants are currently or will be in development?</u>

a. Monoclonal antibodies (mAbs) ADCs, Fusion Proteins, Bispecifics
b. Biosimilars
c. Peptides
d. AAVs, nucleic acids
e. BiTEs
f. Half-antibodies

2) <u>What methods are currently being used for purity/size and heterogeneity analysis?</u>
    a. SDS-PAGE – for legacy molecules
    b. LC methods
        i. Titer
        ii. SEC
            1. SEC-MALS
        iii. Charged based or other orthogonal methods
            1. IEX or AEX

iv. Glycan-LC method
v. LC-MALS

c. CE, CZE, cIEF methods
d. MS or MAM – not all companies have capability
e. Bioassay
    i. ID methods – ELISA
    ii. Potency assays
f. Mass Photometry
g. AUC

3) <u>Can conventional CE assays be applied for characterization of these proteins/peptides?</u>
    a. LC methods first
    b. CZE – difficult separation
    c. Affinity CE
    d. Limitations on sample count – 24 vs 96
4) <u>What challenges exist in the characterization of these proteins/peptides?</u>
    a. Sample types and issues
        i. "Dirty" samples – can/cannot be injected directly into the instrument?
        ii. Peptides small sizes and precipitation
        iii. Fatty acids in albumin samples binds to capillary
        iv. Fusion or PEG-type molecules require non-traditional methods
            1. Method development needed
        v. BiTEs – have O-glycans
            1. Not a lot of companies manufacture enzymes needed for purity and characterization
        vi. Heavy glycosylated fusion proteins sample profiles
        vii. ADCs – multiple components
            1. Different linkers, chiral centers, conformations
        viii. Low concentration samples
            1. Need to concentrate up and perform buffer exchange
        ix. Viscosity and matrix interference
        x. Stability – new peaks growing or observed
        xi. Fragmentation of half-antibodies
    b. Sample preps and consumables
        i. ADC samples require long capillary for separation
        ii. Different solutions for baseline stability
            1. SHS – need to vortex a lot
        iii. Evaporation issues
            1. Use of mineral oils for long runs/sequences
            2. Use of film for cover
        iv. Deglycosylation enzymes needed for glycoproteins
        v. Desalting/buffer exchange protocols - tedious
    c. Methods issues
        i. Migration time shifts
            1. Maybe due to sample evaporation

2. Molecule dependent
3. Need to optimize using linearity for concentration issues

ii. CZE
1. Difficult separation
2. Resolution decreases on some antibodies
3. Capillary stability
4. Buffers – optimization needed to get the right buffers for different molecules
5. pH selection

iii. LIF vs UV detector
1. Stacking issues
2. Use of native fluorescence - Maurice

iv. Methods must be stability indicating

v. Methods need to differentiate sterioisomers

vi. New peaks identification
1. Can be solved with MS for peak ID/verification

vii. Limitation on number of samples that can be analyzed per run

viii. Bridging studies needed to implement new methods
1. SDS-PAGE to CE methods
a. Need to re-file for legacy molecules

d. Attributes
i. Glycosylation
1. N-glycans
2. O-glycans
3. Mannose

ii. Deamidation
1. Kinetics

e. Need for high-throughput assays/capabilities and instrumentation/technology for screening
i. BioPhase 8800 System from Sciex
ii. Maurice from Bio-techne
iii. Use of 96 well-plates instead of vials
1. Availability of small/low volume vials

iv. Use of the shorter end of the capillary or reverse polarity for faster runs
1. Sacrifice resolution
2. Only for “quick and dirty” screening
3. Yes or No trending
a. Minor peaks may or may not be in consideration

f. LC-MS methods not readily available in QC

g. PD workflow
i. Movement from PD to QC need gap assessments, validation and transfers

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- A lot of newer modalities have very similar processing to mAbs
    - No major issues with method development--> only need to make changes for better separation
    - Most difficulties come from species with similar migration times
- Fusion proteins but no CE development yet
- Vaccines, biologics, small molecules
- Seeing a lot of ADCs
    - Most people run LC and utilize enzymes
- Bi-specific, Tri specifics molecules
- AAV, Lentivirus, nucleic acids
- Don't see many peptides
- Multiple mAbs in one mixture like 2-3 in one sample

What methods are currently being used for purity/size and heterogeneity analysis?

- SEC and CEX still used on LC systems
- CE and LC methods hold their own weight
- Use CE/cIEF when you don't need to run LC

Can conventional CE assays be applied for characterization of these proteins/peptides?

- We are seeing a shift from reduced antibodies and now we are seeing a mix of doing something new vs still applying older techniques
- In some cases, you can use existing methods without optimization and in other cases optimization is needed
- For the case of peptides, we need to rethink the assay since we are asking different question

What challenges exist in the characterization of these proteins/peptides?

- There are gaps in current workflows, such as not being able to line up the peaks from SDS-PAGE to CE-SDS so something like CE-SDS MS technology would go hand in hand with upcoming methods
- Being able to visualize what is happening during CE-SDS like high power imaging and going beyond the mAb to understand what you are seeing with new modalities
- No commercially available charge-based standards, something vendors are working on