Table 3: CGE - Becoming the CE Expert in Your Organization – Best Practices Exchange

Facilitator: Mohamed Dawod, *IGM Biosciences, Doylestown, PA, USA*

Scribe: Maximilian Meudt, *Boehringer Ingelheim Pharma GmbH & Co. KG, Baden-Württemberg, Germany*

Scope:

CGE, sometimes termed as CE-SDS is a workhorse electrophoretic technique used for size-based separation of proteins and biomolecules. CE-SDS can be thought of as a combination of regular CE and SDS-PAGE. In essence, the BGE is replaced with a sieving gel matrix and the sample is treated with SDS and heated to denature the proteins providing a consistent coating of about 1.4g SDS per gram of protein.

In this roundtable discussion we will shed light into best practice, sharing experience and brainstorming on improvement of the performance of the technique.

Topics for Discussion:

1. Best practices for sample preparation and setting separation parameters.
2. Sample loading strategies
3. Reduced Vs non-reduced CGE
4. Limitations brought by inferior detection sensitivity and possible strategies for enhancement
5. Commercial availability of sieving agents and strategies for enhancing the separation efficiency

Discussion Notes:

1. Best practices for sample preparation and setting separation parameters.
    - Problems with process samples and impurities difficult to overcome
    - Many interferences observed
    - Buffer exchange is an option but a questionable sample preparation step with regards to time and lot sample concentrations
    - Labeling and fluorescence detection might be an option since strong dilution becomes applicable, but comes with many challenges

2. Sample loading strategies
    - Electrokinetic injection mostly used

- Transferability/feasibility of platform methods with electrokinetic injection to hydrodynamic injection possible?
- Especially samples with high salt content could benefit from hydrodynamic injection instead of electrokinetic injection

3. Reduced Vs non-reduced CGE
    - Excursus on method training/knowledge sharing in the organizations:
        - Different mindsets between liquid chromatography and CE users, LC mindset seems to dominate in organizations which makes training difficult
        - Increased training effort required (mindset) and complexity of sample preparation (keyword fluorescence labeling and detection)
        - Advantages/disadvantages of technology vs. project driven organization, discussion on a CE core unit within organizations
        - Since many organizations collaborate with CROs, complex methods might be difficult to outsource

4. Limitations brought by inferior detection sensitivity and possible strategies for enhancement
    - UV detection is commonly used, Fluorescence detection might be advantageous
    - Labeling and fluorescence detection requires several validation steps that might include: Spiking experiments, MS, determination of LOD LOQ and further parameters
    - Validation of CE measurement and labeling steps required
    - FL detection challenging but offers many benefits. 3-5x higher sensitivity, signal to noise, rather flat baseline, easier automation in integration
    - Native/intrinsic fluorescence detection might be the future (for large biomolecules like proteins), since no labeling is required, and sensitivity is high. At the moment difficult since third party detectors would be required (issues with data integrity, implementation.)

5. Commercial availability of sieving agents and strategies for enhancing the separation efficiency
    - Gels for big molecules may be required in the future? (Viruses, Cells)
    - Gel science seems to be ongoing; manufacturers might provide better gels.