Roundtable Session 1 - Table 2 - CGE - Becoming the CGE Expert in Your Organization – Best Practices Exchange

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Abstract:

This panel discussion aims to bring together leading experts and practitioners in the field of Capillary Gel Electrophoresis (CGE) to share insights, strategies, and best practices for mastering CGE techniques. The session will cover a range of topics including training analysts, troubleshooting common CGE issues, and optimizing performance. The Roundtable discussion will highlight examples, providing practical tips and solutions for overcoming challenges and achieving excellence in CGE assays. This roundtable discussion will provide valuable insights and actionable takeaways to elevate your proficiency in Capillary Gel Electrophoresis. The roundtable discussion will look into future advancements and automation in sample preparation and characterization for CGE assays.

Questions for Discussion

1. What are the **key factors** to consider when setting up a CGE experiment to ensure accurate and reproducible results?
2. What are the **most common challenges** encountered in CGE, and how can they be effectively addressed?
3. Could you provide a case study where CGE significantly improved analytical capabilities to monitor product quality of your organization?
4. What best practices would you recommend for someone looking to become a CGE expert and what best practices are for an organization to train their analysts in CGE?
5. How has CGE technology evolved over the past decade, and what future advancements do you foresee, such as automation for sample preparation and characterization of unknown peaks in CGE?

Discussion Questions:

Question 1

- **Manual Prep (for Low Throughput):** Manual preparation should follow a strict SOP to ensure consistency, especially when processing a smaller number of samples.

- **Mastermix Preparation:** Prepare a mastermix rather than preparing individual samples, where possible, to reduce variability.
- **SOP Adherence:** Strictly follow the SOP for consistency in sample handling and instrument setup.
- **System Suitability Testing (SST):** Use well-characterised control samples to verify the method's suitability. E.g **Stressed Samples for Monitoring LMWS:** Add stressed samples to detect low molecular weight species (LMWS)
- **Gel Droplet Handling:** Ensure no gel droplets are around the vial sides to avoid crystal formation, which can affect separation performance.
- **Use of Mineral Oil (MO):** To minimise migration time (MT) shifts in runs longer than 20 samples, include MO. Issues such as odd separation profiles may occur if mineral oil gets introduced into the capillary, often due to low replenish gel volume.
  Solution: Increase replenish gel vial (usually BI:B1 position) volume to 1400 µL and carefully add 25-30 µL (max) mineral oil on top of the gel meniscus. Keep the separation gel inlet and outlet unchanged according to the platform method.

Question 2

- **New Modalities in CGE:** Heavily glycosylated or heterogeneous glycans require careful removal as they can affect separation and peak shapes. Consider removal of sialic acids as part of the sample prep.
- **Peak Tailing & Resolution:** Limited resolution, especially with newer modalities, can cause broad or peak tailing due to secondary interactions. Consider glycan/sialic acids removal as part of the sample prep optimisation process
- **Lack of Internal Markers for DNA:** Without internal markers, aligning data can be difficult. Use third party markers to aid in consistent data integration. Potential example - Agilent DNF marker can be purchased separately. Note: verify buffer compatibility as DNF reagent may cause some shifting due to buffer salt. Start with 8-10 uL per 200 uL sample

- **Contamination in past CE-SDS Gels:** Lot changes in gels can cause issues, such as contamination (e.g., 10 kDa or plastic precursor contamination around COVID-19 pandemic and supply chain). However this seems to be addressed by vendor.

- **Baseline Issues Due to Instrument/Capillary Age:** Older instruments or capillaries can lead to baseline shifts. Visual inspection of UV aperture of the cartridge and Replace the 200 µm aperture if it's burned out from UV exposure, and ensure fibre optic cables are tightened.

- **Excessive Capillary Conditioning:** Some users condition new capillaries up to 10 times, but 4-6 times is typically sufficient in most experience. UV lamp on time may be something to consider to achieve stable baseline as earlier runs can have more disturbance.

Question 3

- **CGE for Nucleic Acid Characterization:** CGE is used for monitoring the purity and stability of mRNA.
- **Highly Heterogeneous Fusion Proteins:** Heavy glycosylation and charged glycans can cause broad peaks due to secondary interactions delaying migration.
    - **Solution:** Use Rapid PNGase F (NEB) to remove glycans for cleaner separation profiles.

Question 4

- **Understanding Protein Chemistry:** Check program molecule amino acid sequence Tailor the sampl buffer pH to the light chain type:
    - Kappa light chain: Tend to be able to tolerate a pH 9 sample buffer for non-reducing (NR) runs. DoE to determine and when to align buffer choice for both NR and R
    - Lambda light chain: Use a lower pH buffer for NR. pH 6-7, decision through DoE data.
- **Sample Stacking:** Improve sample injection using slow electrophoretic mobility and low ionic strength buffers. User PeakMaster https://web.natur.cuni.cz/gas/peakmaster.html
- **Buffer Exchange:** Should buffer exchange is necessary, ensure to pre-rinse filters with Milli-Q water before buffer exchange to avoid huge sample loss.
- **Pressure Injection Method:** For high-salt formulations, (e.g. in process) use pressure injection to standardize sample loading. This avoids the need for buffer exchange and ensures more consistent results.
- **Refer to SCIEX's HR method Pressure Injection Method:** See Page 37 of the IgG kit guide: https://sciex.com/content/dam/SCIEX/pdf/customer-docs/application-guide/igg-kit-appguide-pa800plus-en.pdf
- **Optimising Separation Method:** When using SCIEX CE-SDS pressure injection method, ensure that other necessary changes are made to the separation method according to the guide (water plug)

Question 5

- **Automation for Sample Preparation:**
    - Liquid handling systems, such as Tecan or Hamilton, can increase throughput and consistency.
    - **Considerations with Automation:** Automation has limitations with some reagents, such as the use of BME, which may require tape sealing and ventilation strategies. DTT is not compatible with the SCIEX CGE-SDS kit.
    - Some Sciex kits sample prep with automation https://sciex.com/tech-notes/biopharma/complete-automation-of-the-charge-heterogeneity-assay-using-the-

    - https://sciex.com/tech-notes/biopharma/streamlined-protein-characterization-workflows-for-capillary-ele
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- **Avoiding Bubbles in Automation:** Adjust liquid handler dispensing speed and plate centrifuge samples to avoid bubbles
- **Data Processing Bottleneck:** High-throughput data processing remains a challenge.
    - The **Biophase 8800** system includes HT data analysis capabilities to help handle high-throughput datasets. Manual user verification still necessary to ensure consistent peak integration.
- **Redundancy in Samples:** Incorporating redundant samples helps with data consistency, especially in high-throughput scenarios.

Notes:

Start typing notes here. Be sure to leave out the names and organizations of attendees at the table in order to promote a more free flowing discussion.