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

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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:

Best practices:

- Sample preparation
    - Centrifuging multiple times to eliminate bubbles
    - Vortexing vs gentling inverting based on samples
    - Is a sonicator necessary to use during sample prep?
    - Desalting samples depending upon the matrix using spin filters. Some notice bad recovery others say good recovery.
- Use of PNGase
    - Try using SHS
- BME or DTT as reducing agent
    - 3-5% should reduce well
- Impact of temperature and time

    - Can we fully reduce and stabilize? 65-70 degrees
    - Robustness. Evaluate lots of areas where time and temperature come up.
    - Creating a library of robustness studies can be helpful for troubleshooting and investigations.

Use of repeater pipet is super helpful. Positive displacement pipet can be better than reverse pipetting. Microbubbles can show up.

- Loading samples into the capillary; electrokinetic vs pressure
- Pressure can help with salt. Ex) 500 mM sodium chloride.
- Good precision from pressure. Electrokinetic is more sensitive but can be difficult with salty samples.

- Changing voltage and injection time to get different TCA outputs
- Electrokinetic 99s is max; you can change function and increase voltage and time.
- Applying pressure to just one side during separation
- Applying pressure can help minimize surfactant inference

- Maximum field strength used: Voltage for a given capillary length. 15 KV should be the max. Increasing voltage can give faster separation and better resolution.
- Joule heating can happen if voltage is too high.
- The cooling system only is applicable for separation window. It's not the entire capillary
- Check the current trace to make sure it's constant. Current can help to determine the lifetime of a capillary. Study the current with different applied voltages and plot.

- Comparison of pre-cut capillaries and manually cut
    - There shouldn't be a difference, but it needs to be cut well.
    - Struggling with manually cutting. Sanding it down. Using ceramic cutter
    - Gentle cutting to create less rough edges

- Reduced vs Non-Reduced CE-SDS
    - High pH can cause reduction of disulfides
    - IAM vs NEM

Sciex pH sample buffers. pH 9 is used but most people make their own for lower pH buffering

- Limitations: Big one is the sensitivity and detection
- Can't lower the SDS concentration too far
- Is labelling worth it? LIF? Can we be sure that we are getting 100% labelling, and is it worth going to QC?
- Try comparing UV and Fl. LMWS may not fully be tagged.
- If you have super low signal, try concentrating it.

Are all of the players in the market achieving what we need? In terms of instruments and consumables.
There is no gel that can resolve everything. Separating lower weight species and high weigh species with different gels