Table 1: Troubleshooting Capillary Electrophoresis Instrumentation and Separations

Facilitator: Cassandra Crihfield, *West Virginia University, Morgantown, WV, United States*

Scribe: Xiaoping He, *Pfizer, Inc., Chesterfield, MO, United States*

Scope:

As the use of capillary electrophoresis has become more widespread, so has the awareness of quirks or challenges associated with the technique. Whether it is an instrument error rarely seen or a new peak appearing and disappearing in electropherograms, everyone has been faced with an issue that stopped critical progress on method validation or sample analysis. This roundtable hopes to facilitate discussion between capillary electrophoresis users who face challenges to help spread knowledge on how to prevent or quickly address issues. Discussion will center on both problems associated with instrumentation and those related more to method development. Topics that have been discussed in the past may also be focused on, such as the "ghost" peaks encountered in CE-SDS analysis of monoclonal antibodies.

Questions for Discussion:

1. What are the most common challenges you face when using capillary electrophoresis?
2. Including peak shifting, seemingly random peak appearance and disappearance, unusual error codes, etc.
3. What have you found to be the most effective way to address these issues?
4. Are there any specific software or instrumentation issues, such as error codes, freezing during analyses, etc., that you have had problems with? How did you address this?

Discussion Notes:

1. CESI-MS issues:
   SCIEX system – We spent most time on troubleshooting. Wonder if other people had issues with the system?
   a. Possibly causes for the issue: new capillary, spray issue, ion source, conductivity issues, positive mode mostly, etc.
   b. The new MS Bruker's triple TOF 6600+ helped.
   c. Some other thoughts: quality of capillary matters, base fuse silica for intact protein analysis, neutral surface ones recently, migration time reproducibility, etc
   d. Spray stability is one of the big issues.
2. CIEF solubility upon focusing: what helps to prevent from aggregation as molecules approaching a net charge of zero?

    a. Urea and other additives, DMSO, Simplsol, NDSB (1M final conc, mixture of 11 forms, 2 worked better for us), reduced protein concentration for CIEP preps, Tween 20 helps (?%)
3. Baseline noise for PA800+: uv detector, hilly baseline of CE-SDS, quite random, come and go
    a. Aperture cleanness, robust clean of the entire system to rid of gel accumulating on the interface, deep cleaning after every assay before & after
    b. Pressure pump related, in this case, it will need service engineer from the vendor
    c. Tubing cleaning
4. LIF detector module issue with PA800+:
    a. Random errors around system control board not detected, have to abort the run. Service engineer came in to fix it, to date I still have the issue from time to time.
    b. We had the same issue. And the vendor extended our warranty, because it's an internal issue.
    c. A 3rd person added: we saw the issue during a switch between detectors. Errors occurred only when high voltage supplied. SCIEX engineers took preventive approaches mainly, e.g. insolating tapes, etc.
5. Dip before the peak: labChip (touch) system, not salt related, or Buffer related, software parameters for baseline may help.
6. Empower control for PA800 or iCE3:
7. PA800+ is under Empower controlled for some customers for a couple of years now. Driver 1.1.5 still involves 32 Karat. With later versions such as driver 1.3.Eempower controls all.
8. Some labs are evaluating a 3rd part demo driver for Empower for the PA800+
9. iCE3 is still in the process to develop the Empower direct control, though Maurice the newer generation of iCE system has been operating under Empower data acquisition and integration.
10. Security guarded local drive to collect iCE3 data for QC purpose is the current industrial practices using ProteinSimple's iCE3 instruments.
11. Gel buffer caused migration time shifting for nrCGE
    a. Likely attributed to lots differences of the Gel buffer. Seems to be a common thing. Many people observed similar happening. Buffer bottle opening time matters. New one appears goo Could it be viscosity related?

Other resources:

https://www.proteinsimple.com/documents/2019_CEPharm_SimpleSol_Poster.pdf

https://www.proteinsimple.com/consumables_ice.html

https://doi.org/10.1002/elps.201900449 (DMSO in CIEF)