Table 10: Troubleshooting Complex Separations

Session 1:
Facilitator: Mohamed Dawod, *Merck & Co., Inc.*
Scribe: Sarah Wright, *University of Notre Dame*

Session 2:
Facilitator: Hermann Wätzig, *University of Braunschweig*
Scribe: Colleen Reed, *Pfizer, Inc.*

Scope:
One of the most notorious limitations in electrophoretic analyses is the adsorption of analytes/matrices onto the walls of the separation channel. This adsorption is reported to contaminate the surface and cause a progressive non-uniform ζ potential across the separation compartment which leads to unavoidable band broadening and/or peak asymmetry.

In this roundtable discussion we'll shed light into causes as well as potential solutions to address, reverse and eliminate this issue.

Questions for Discussion:
1. What is the root cause and adverse effects of analyte/matrix adsorption?
2. Have you developed any successful strategies to prevent/reverse adsorption?
3. What different types of capillary coatings have you used?
4. Have you experienced limitations when using capillaries with different types of coatings?
5. What is/are your source(s) of capillaries and how do you evaluate the performance of their coatings?

Discussion Notes:
Session 1:
Question 1: What is the root cause and adverse effects of analyte/matrix adsorption?

Protein charge in biological pH is typically the source of adsorption which negatively impacts both the quantitative results acquired, as well as the lifetime of the capillary.

Question 2: Have you developed any successful strategies to prevent/reverse adsorption?

Be mindful of pH if using bare silica capillaries. Alternatively, the use of particular dynamic coatings such as CSDS may help prevent adsorption.

Question 3: What different types of capillary coatings have you used?

Dynamic coatings need to be re-applied frequently, but are often easier to work with and tend to be more affordable. Permanent (static) coatings are irreversible and include LPA, PVA, and MPA among others. Coatings start to hydrolyze outside of the recommended pH range.

-ScieX uses dynamic coating for a sheathless CE-MS interface and provides all necessary reagents.

-Beckman mutual capillary is fairly reproducible and uses amed coating. For these, pH range between 3-9 is ideal, 10 is okay, but it's not recommended to go under 3.

-LPA is compatible with a pH range of 2-10, but it is destroyed by the sodium ion. TRIS buffer is compatible

-SMIL coated capillaries tend to be more reproducible and have less adsorption due to degradation. It can go for 450 runs before being swapped out

Question 4: Have you experienced limitations when using capillaries with different types of coatings?

In general, coated capillaries experience issues with reproducibility, below are some strategies to counteract this:

Selection of Coating:

-Be mindful to select the right coating that is compatible with your analyte to produce ideal results

-Note that some dynamic coatings may not be compatible with different detection modes such as CE-MS

Coating & Cleaning:

-Recoating your dynamic capillary frequently improves reproducibility

-Cleaning frequently (between each run/set of runs) will improve results

-Use 0.05M HCl flush to get rid of proteins adsorbed onto surface of capillary

-If you have bubbles in the line, consider increasing your hold pressure from 20 psi to 50 psi. If you don't apply pressure to both sides, the current with crash frequently.

Lifetime of capillary:

-Ramp down voltage slowly (over 5 min). The static voltage could damage a capillary or sprayer over time, but a slow ramp down will release this voltage from the system. This is an ideal solution for complex protein separations but would not be as beneficial for high throughput operations.

-While dynamic coated capillaries might have issues with detection, permanent coatings might be stripped or degraded over time.

-Check recovery and peak shape periodically to ensure optimal performance

-Stickiness will cause tailing on the peak shape, see coating & cleaning suggestions

-Perform a routine reference standard check, particularly if you're unsure if the capillary coating is in top shape

-If you have a near zero EOF, you can use an EOF marker compatible with your coated capillary. Note that this might not be possible with MS

Question 5: What is/are your source(s) of capillaries and how do you evaluate the performance of their coatings?

-Beckman is producing AMED and has a DNA kit

-Agilent has PVA

-SMIL Human Metabolite, cation capillary works well with metabolites and small molecules

-ScieX capillaries tend to have issues with reproducibility, some you order are fine, others are unusable

-908 Chip has a separation channel that is coated. Each chip has 125 injections, but sometimes the chips go bad in the 40's. They're pretty expensive, so when this happens, you can contact the company and they will replace it. It's $8/injection if you get the full 125. Also, the chip stops after 125 since the Maurice cartridge counts sequences and injections, even if the channel is still good. The sequence count doesn't make any sense, but injection count at least represents the lifetime of the capillary.

-Beckman PA100+, 400 runs with capillary and still good!

-LPA coating by Dovichi lab: "Preparation of linear polyacrylamide coating and strong cationic exchange…", Zhenbin Zhang, et. al.

<u>Session 2:</u>

- Categorize trouble shooting - instrumentation, software, and materials
    - Materials
- Improve on gel from kit

- Problems – current issues, system peaks, and baseline wave
- Universities working on improvements
- More complex buffer system seems to cause more system peaks and baseline issues
- Reagent containers – leaching (plastic)
- Capillary
- Inspect for straight cut - DO NOT use Sciex grinder to straighten
- Extra dip steps might be needed to need to remove material on outside of capillary
    - Examples: gel or sucrose containing sample
    - Depends on surface tension and viscosity
- Issues arise using Kits for non-intended applications
- Software
- 32 Karat
- Requires more training than other controller software
    - Training complications can result in problems like instrument plate collisions

Complicated to create overlay images

- For presentations or notebook entries
- Must clear all electropherograms and re-add

No s/n calculations

- Problems with creating method system suitability, assay acceptance, and peak inclusion criteria
- Problems with setting LOD's and LOQ's

Skipped injections

- Sometimes cause recorded in activity log and others no record of cause
- Large problem for GMP labs (investigations)
- Method modifications not saved

- Empower as a controller software is not affordable to universities and industry
- Instrumentation
- Be cautious of power use in lab, may destabilize instrument voltage
- Watch for additional moisture that can contribute conductivity, which can destabilize currents
- Vertical jumps in baseline
- Gel can get stuck on the inside of the pressure valve usually on the Outlet side

Diligent cleaning and maintenance

- Consistent straining on cleaning and maintenance needed for all instrument users
- Do not fill waster vials higher than ¾ height of vial
- Other

Training

General lab training is commonly a root cause of CE analysis problems

- Pipetting with the wrong pipette, particularly with small volumes
- Pipetting viscous solutions/reverse pipetting
- Use of balances in preparations
- Importance of instrument cleaning and maintenance has become a diluted message as more scientists are trained

- Protein adsorption
- Generally smaller issue with CE vs LC
- Should be considered with any sample contact surface
- Some methods require very tight temperature control