Roundtable Session 2 – Table 1 – Strategies and Challenges with Peak Integration in CE

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Abstract:

Peak integration in capillary electrophoresis (CE) is a critical step for accurate quantitation and characterization of biomolecules, yet it presents a range of technical and analytical challenges in both R&D and GMP environments. This roundtable will explore and discuss strategies employed to achieve reliable peak integration, including the selection of optimal integration parameters, management of baseline drift and noise, and approaches for resolving overlapping or poorly defined peaks. By sharing experiences and solutions, participants will gain insights into improving data quality and reproducibility in CE assays.

Discussion Questions:

What are the biggest challenges you've experienced with peak integration? How do you resolve them?

-Software, data acquisition, peak integration protocol

-Test procedure instructions, Training, Method Transfer

-Data quality, noisy baselines, Matrix interference, peak migration variance

-Inconsistent resolution, Impact of peak shape, Tailing peaks, matrix effects

-Low abundance peaks, signal to noise

How do baseline drift and noise affect peak integration, and what strategies can be used to mitigate these issues?

Baseline correction, smoothing algorithms

Manual vs automated integration

Is there an opportunity to provide new innovative solutions such as leveraging AI-driven algorithms or automated integration strategies?

How do molecule type and assay format impact peak integration?

RNA, DNA, fusion proteins, mAb, ADC, ...

CE-SDS, CGE, CZE, iCIEF, CIEF, CZE, CE-MS, ...

What approaches are most effective for integrating closely spaced or overlapping peaks?

How do you ensure consistency in peak integration when transferring methods between labs or instruments? What are the most critical peak integration instructions to document when training and transferring methods?

Notes:

1. Biggest Challenges in Peak Integration & Resolutions

Software, Data Acquisition, Protocols

- **Empower** and **Chromeleon** are the most commonly used software.
- Manual integration is often used for CE-SDS with UV detection ~~and~~ (aka CGE) due to wavy baselines or unclear peak boundaries (where to start and where to end).
- Auto-integration is preferred when possible, but often requires manual adjustment; subjectively in manual adjustments requires a thorough review by highly trained approvers~~.~~
- Some labs prefer~~red~~ to use continued baseline and dropline for integration;
- Some labs use hybrid approaches: combining valley-to-valley and drop-line methods, but emphasize **consistency** within a product or method.

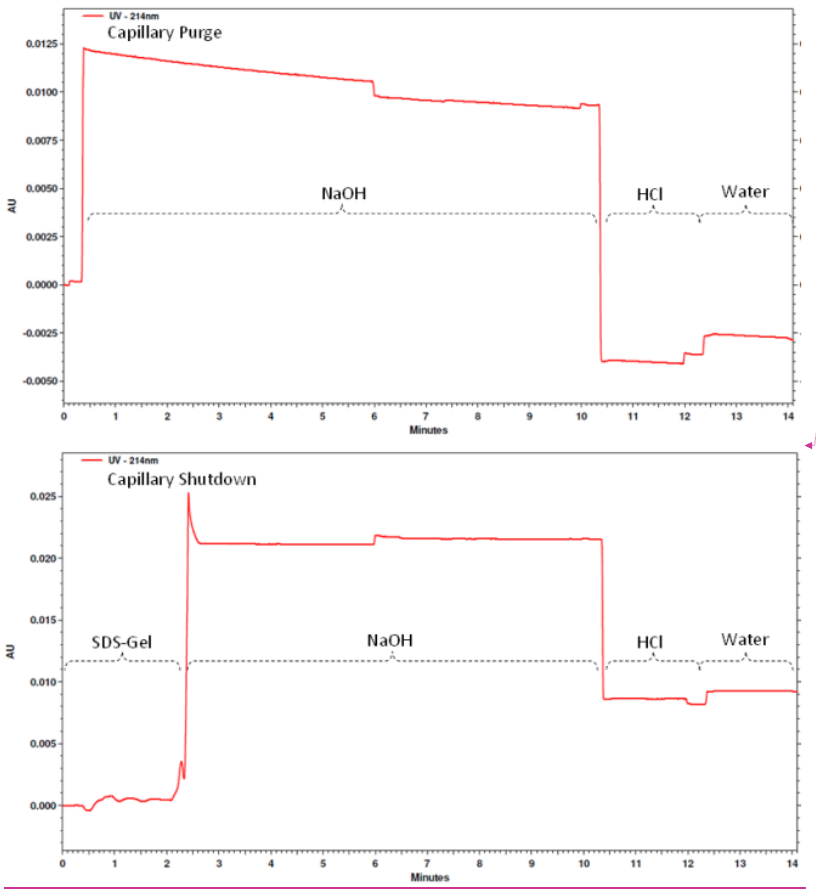
Test Procedure Instructions, Training, Method Transfer

- Variability in human judgment can lead~~s~~ to disagreements; **review and approval** processes are used to align integration decisions.
- SOPs often include integration parameters, but analysts are allowed to use judgment if results are consistent. One way to alleviate person to person variability is to provide explicit examples (often multiple figures) of acceptable integration protocols within the test method
- Method transfer challenges include differences in instruments, software, and analyst interpretation.
- Recommendation: avoid transferring overly complex methods to QC; use ~~premade~~ vendor-supplied reagents (e.g., Sciex sieving ~~gels~~ ,gel), buffers and pre-made cartridges to reduce variability.

Data Quality, Noisy Baselines, Matrix Interference

- Wavy baselines are a common issue, especially with UV detection.

- **Blank injections** (preferably ~~placebo~~made using the product's formulation, not just water) are used to overlay and identify true peaks.
- **LIF and NFD detection** offer improved baselines and are preferred over UV in some labs.
- Integration ~~rely~~relies on data quality. Instrument maintenance (e.g., replacing D-lamps) and gel lot variability can significantly affect baseline.
- CE-SDS: High molecular weight (HMW) peaks are particularly difficult to ~~define~~observe due to their ultra-low abundance and the broadness of the peak(s).
- System diagnosis: perform c~~C~~onditioning runs (~~-~~with data acquisition) at the beginning of sequences help diagnose system issues (e.g., bad gel, leaking coolant, broken detection window, etc).
    - For example, rinse your capillary with NaOH, HCl and water before and after (shutdown) the sample testing sequence. By enabling the detector during these rinses, the observed traces provide the user with critical information about the integrity of the detector and capillary window. Below are example profiles showing the expected responses of reagents passing through the capillary before start of the sequence (top) and at the end of the test session (bottom). Note how each reagent affects the detector response; these responses are reproducible when the system is performing as expected.



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Low Abundance Peaks, Signal-to-Noise

- Peaks below LOD (based on height or S/N=3) are excluded in peak integration.
- S/N determination varies: some use **peak-to-peak**, some use **RMS**; results can differ by a factor of 3.
- Use ~~of~~ a flat ~~30s~~ region within the electropherogram ~~near the main peak is common for noise~~ to estimate the magnitude of noise~~ion~~.

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2. Baseline Drift and Noise Mitigation

- **Smoothing algorithms** are used in some lab (e.g., in Chromeleon for IC~~s~~IEF/CIEF).
- **Manual integration** is often necessary when baseline drift is significant.

- **Blank (-placebo, not water) overlay** is a key strategy to distinguish real peaks from noise.
- **Buffer exchange** is used in LIF detection to avoid formulation interference and blank without formulation is used for analysis using LIF detection
- **Continued baseline and dropline integration** are preferred over valley-to-valley in some labs.

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3. Innovative Solutions: AI and Automation

- AI has been **explored** but not widely adopted due to:
  - Lack of transparency ("black box" issue).
  - Need for manual data upload and adjustment.
  - Limited success in handling complex profiles.
- AI is more promising in **high-throughput screening** scenarios with thousands of samples and different assays.
- Reference profiles and blank injections are essential for AI-based integration.
- For spike peaks identification, can set threshold and use learning algorithms for the AI to learn how to identify the spikes

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4. Molecule Type and Assay Format Impact

- **mAbs and ADCs**: most discussed in this session.
- **RNA/DNA**: not discussed in detail,
- **CE-SDS/ CGE with UV detection**: wavy baselines, especially for HMW peaks. often requires manual integration due to UV detection issues.
- **iCIEF**: smoother baselines with native fluorescence detection.
- **LIF/NFD**: preferred for better baseline and data integrity.

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5. Integration rule of thumb

- Use of **reference profiles** (e.g., control and stressed samples) to guide integration.
- Preferred integration: **continued baseline + drop line**.
- Avoid valley-to-valley unless justified.
- Consistency across batches and analysts is emphasized.

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6. Ensuring Consistency in Method Transfer and Training

- SOPs should clearly define:
    - Integration parameters (thresholds, width, etc).
    - S/N determination method.
    - Criteria for peak inclusion/exclusion.
- Use of **placebo blanks** recommended for better peak identification.
- **Conditioning runs** help ensure system readiness and data quality.
- Avoid transferring methods that rely on homemade reagents or complex manual steps.