Unaddressed Challenges & Technical Innovations

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Abstract

Capillary electrophoresis (CE) is the gold standard for analysis of charge and size of proteins, nucleotides, and small molecules in the pharmaceutical industry. As this field continues to develop and improve there are still numerous unmet challenges and areas for growth. In this roundtable we will discuss, share experience, and brainstorm on unaddressed challenges and potential solutions to those challenges as well as technical innovations.

Discussion Questions and Notes:

1. What CE system do you predominately use? What is the biggest challenge you encounter with that CE system? What modalities are you analyzing with that CE system?

2. High throughput is desired and new multiplex systems are emerging on the market. What are the pros and cons of multiplex systems?

3. With combination products and vaccines emerging, how do you approach co-migration of species?

4. Sample loading can be performed with an electrokinetic or hydrodynamic injections. What are pros and cons of each?

5. Manual integration is time very consuming and there can be analyst to analyst variability. Auto integration can save on time; however, CE is notorious for baseline shifts and irregularities. Do you use or have experience with auto integration? Does auto integration have the capability to address shifts and irregularities in the baseline? Robust shoulder and valley detection?

Discussion Point 1 – Types of CE instrumentation

Topic not discussed.

Discussion Point 2 – Multiplex CE systems

Instruments of discussion were the BioPhase 8800 and the Fragment Analyzer.

Users reported good repeatability with the instruments and think that these systems are going to beneficial to use moving forward. Overall, the view is that development multiplex systems is good for the field.

Having a single broken capillary was not problematic to the Fragment Analyzer but was for the BioPhase 8800 currently.

Discussion Point 3 – How to handle co-migrating species?

Problem statement created – nucleic acid constructs (eg: mRNA) of similar size but different sequence having incomplete separation and co-migration.

Difficulty the species have similar size and electrophoretic mobility in commonly used gels based separation kits. The kits available from vendors do not easily allow for changes to the kits to improve this separation.

Proposed solution – selectively labeling constructs with a dye prior to being mixed together.

Conclusion – Streamline approaches for improving nucleic acid separations are still needed.

Discussion Point 4 – Electrokinetic injection vs hydrodynamic injection

Electrokinetic injection was mentioned as the only injection method used for gels-based methods at the table.

Discussion Point 5 – Data Processing

Problem statement created – regulatory agencies are starting to request automatic integration that does not involve any user interaction with the integration once the autointegration has occurred.

Difficulty is that no one has been able to implement a purely auto integrating process method due to shifting and unstable baselines as well as shoulder peaks. Most common approach mentioned is to have an initial processing method then manually adjusting. Most common integration problems are occurring in non-reduced CE-SDS

Proposed solutions

Using migration markers – might help but does not address unstable baselines or shoulders.

Using a Caesar algorithm as part of processing method – currently not in data processing software.

Having integration software developed with AI (most popular idea) – Currently no vendors at the table were working with AI for data processing.

Conclusion – autointegration software currently available may not meet data integrity requirements in the future and improvements in processing software is still needed.