Roundtable Session I – Table 5 – mRNA Vaccine CE Applications

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Abstract:

Capillary electrophoresis (CE) has emerged as a powerful analytical tool for characterizing mRNA vaccines, already well-known for high-resolution separations and quantification of nucleic acid components including plasmid DNA and mRNA molecules. This roundtable will explore the various challenges and applications of CE, including mRNA integrity, purity, degradation, and implementation in a quality control environment. Buffer optimization, detection strategies, and compatibility with lipid nanoparticle formulations are just a few critical parameters in CE method development. The discussion will also address challenges such as sample preparation, matrix effects, and throughput limitations. .

Discussion Questions:

What quality attributes have you used CE to study?

What mRNA attributes have caused the most challenges with analysis by CE?

What sample-related difficulties have you encountered in applying CE to mRNA vaccine analysis—such as sample preparation, matrix effects, or throughput—and how have you addressed them?

What are some instrumental limitations that would enable your organization to use CE more routinely? What instrumentation do you tend to use as an alternative and why?

Notes:

1. Quality Attributes Studied Using CE

Capillary Gel Electrophoresis (CGE) is a mode of capillary electrophoresis that utilizes a gel-filled capillary to separate biomolecules, typically nucleic acids or proteins—based on , size. In this discussion, several critical quality attributes (CQAs) of mRNA were highlighted, which can be characterized using CGE-based kits:

- mRNA Integrity and Purity: CE is widely used to assess mRNA purity, distinguishing intact mRNA from degraded or tailless species. It offers excellent resolution, enabling separation of tailless mRNA from full-length mRNA (as demonstrated in a webinar presented by Vernal).

- PolyA Tail Analysis: The ssDNA 100 R kit is used to evaluate polyA tail length distribution and quantification, with single-base resolution.
- Plasmid DNA Analysis: CE is applied to upstream components in mRNA production, including:
    - Plasmid topological forms
    - Linearization efficiency
    - Plasmid identification via enzymatic digestion profiles
- mRNA-LNP Encapsulation Efficiency: Traditionally assessed using the RiboGreen assay; CE (via the RNA 9000 kit) is being explored as an alternative analytical tool.

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2. Challenging mRNA Attributes in CE Analysis

- mRNA-Lipid Adducts: When using the RNA 9000 kit for mRNA purity analysis, additional peaks migrating after the main mRNA peak may represent mRNA-lipid adducts. Further investigation is needed to confirm their identity.
- Secondary Structures: mRNA folding can affect migration behavior and peak shape. Sample preparation strategies such as formamide treatment and heat denaturation are used to minimize secondary structures. However, heat denaturation may lead to mRNA degradation. A study by SINTEF in collaboration with SCIEX observed degradation during sample preparation and developed an optimized method to release mRNA from LNPs while minimizing degradation.
- Low Concentration and High-Salt Samples: For samples with high salt content (e.g., in-process or “dirty” samples), pressure injection is recommended over electrokinetic (EK) injection. Pressure injection reduces matrix competition during sample loading, resulting in better consistency and reduced bias.

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3. Sample-Related Difficulties and Solutions

- Injection Techniques:
    - EK injection may introduce bias and result in reduced sample loading in high-salt formulations.
    - Pressure injection provides more uniform sample loading and is preferred for complex matrices.
- Throughput Limitations:
    - CE has lower throughput compared to other analytical techniques.
    - Automation and batch processing strategies are being explored to enhance efficiency.

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4. Instrumental Limitations and Alternatives

- Detection Sensitivity:
    - UV detection has limited sensitivity for low-abundance samples; LIF detection is commonly used for improved signal clarity.
    - However, LIF detection can introduce bias when analyzing sgRNA and Cas9 RNA together in a single injection. The intercalating dye binds less efficiently to short nucleic acids (e.g., sgRNA) than to longer ones (e.g., mRNA), skewing the ratio.
    - For accurate quantification of sgRNA and mRNA in a single run, UV detection at 254 nm is recommended.

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