Roundtable Session 2 – Table 5 - mRNA Vaccine CE Applications

Facilitator: Cristina Montealegre, *Solvias AG*

Scribe: Tyler Davis, *SCIEX*

Abstract:

As of 2025, mRNA therapeutics are increasingly being explored and developed, expanding well beyond their initial success in infectious disease prevention. Capillary electrophoresis is expected to play a central role in analytical development and process optimization in mRNA vaccines. CE enables high resolution separation of RNA fragments, allowing for the detection of incomplete transcripts and degradation products that may arise during synthesis or storage. Recent studies have also highlighted the role of CE in characterizing lipid nanoparticle (LNP) formulations.

This roundtable will explore the current applications of CE in the mRNA landscape, with a focus on technical strategies, instrumentation, and analytical challenges.

Discussion Questions:

1. In what ways CE is being applied for mRNA analysis (e.g. purity, integrity/size, stability, plasmids, poly(A) tail)?
2. What are the current limitations or challenges in applying CE to mRNA vaccine analysis?
3. Technical deep dive:
    - Use of commercial kits vs. custom buffer/gel systems
    - CE instrumentation (e.g. PA800 Plus, Fragment Analyzer, LabChip GXII): advantages and disadvantages.
4. CE for mRNA drug product: Do you apply CE for intact mRNA-LNP analysis (e.g. encapsulation efficiency, surface charge)?

Notes:

Overall summary:

The session was not well attended on day 2. Table included 2 industry and 3 vendors

Recent policy changes are significantly impacting mRNA vaccine development, leading to funding cuts and strategic shifts across the biotech industry. Companies are adapting by pivoting from infectious disease vaccines to cancer-related applications, which are seen as less politically sensitive.

Technical discussions focused on mRNA sample preparation, encapsulation efficiency, and stability testing. Analytical methods like CE and LC were compared for RNA characterization, with emphasis on software tools and data integration challenges. Lipid

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nanoparticle (LNP) analysis and encapsulation metrics were also highlighted as critical areas for ongoing research.

Policy & Industry Impact

- Policy changes are significantly affecting mRNA vaccine development.
- Fear and stigma around “vaccines” are influencing funding and public perception.
- Concern that Cell & Gene Therapy (CGT) and AAV are also impacted.
- European companies likely affected, though not confirmed.

Market Trends & Shifts

- Despite setbacks, reagent sales remain stable—no growth, but ongoing work continues.
- Some researchers are shifting focus from infectious diseases to cancer (less controversial).
- Companies are still working due to long development timelines.

Sample Preparation & Analytical Techniques

- **Lipid Nanoparticles (LNPs) & mRNA Encapsulation :**
    - Sample prep methods: IPA precipitation vs. detergent with mild heat.
    - Detergent not suitable for LC.
    - SCIEX observed no difference between IPA and detergent methods.
    - High RNA yield is not always achieved.
    - Encapsulation efficiency requires careful sample prep to avoid interference.
    - Reliable assays needed to measure encapsulated vs. released RNA.
    - Comparison methods are used to assess release efficiency
    - LC used to characterize lipids.
- **Stability Testing:**
    - Stability is condition dependent.
    - Fragment characterization and ladder estimation used.

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Analytical Tools & Software

- **Capillary Electrophoresis (CE) vs. Liquid Chromatography (LC):**
    - Regeneron paper compared poly-A tail characterization using both methods.
        - Journal of Pharmaceutical and Biomedical Analysis 236 (2023) 115692
    - CE: Length distribution by CGE.
    - Compared to LC-MS, poly-A analysis by CGE requires markers to determine the length of the poly-A (e.g., customized from IDT ssDNA at 120nt).
- **Software & Data Integration:**
    - BioPhase, 32 Karat, Empower, and Chromeleon discussed.
    - 32 Karat allows easier baseline integration adjustments compared with BioPhase software.
    - Pa800+ on Chromeleon is user-friendly with common stats and reporting templates.
    - Empower is the preferred CDS (even Agilent customers are requesting it).
    - CDS (Chromatography Data Systems) are critical for standardizing large datasets.

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