

Multi-Attribute Analysis of LNPs with icIEF on the Maurice System

Highlights

- A single icIEF workflow simultaneously measures LNP identity, encapsulation (mRNA), and stability, eliminating the need for multiple orthogonal assays
- Dual detection distinguishes empty LNPs from mRNA-loaded LNPs, enabling empty/full assessment in a single run
- Fluorescence-based detection provides quantitative insight into encapsulated mRNA, with signal proportional to packaged mRNA content
- Charge profiles resolve differences in LNP composition, including variations in lipid content and encapsulated mRNA
- Stress-induced changes in charge and signal profiles reveal LNP degradation and mRNA leakage, providing stability information within the same analysis

Why This Workflow Matters

Lipid nanoparticle (LNP) therapeutics require careful characterization of multiple critical quality attributes (CQAs), including particle identity, encapsulation efficiency, and stability. These attributes are typically assessed using a combination of orthogonal techniques, each requiring separate workflows and sample preparation, making it difficult to correlate results across assay platforms. By integrating imaged capillary isoelectric focusing (icIEF) with dual absorbance and fluorescence detection, the **Maurice™ platform** enables simultaneous measurement of LNP composition, encapsulated mRNA, and particle integrity within a single icIEF workflow.

This consolidated approach not only simplifies LNP characterization but also provides additional insight into how changes in lipid composition, surface charge, and mRNA encapsulation collectively impact particle stability all of which would ultimately impact performance.

Case Study: Multi-Attribute Analysis of LNP Composition, Encapsulation, and Stability Using icIEF

LNP samples containing mRNA payloads were analyzed using the Maurice system to assess multiple critical quality attributes, including surface charge (pI), encapsulated mRNA, and particle integrity. Samples representing different LNP compositions, mRNA payloads, and environmental stress conditions were evaluated using a workflow that combines charge-based separation with dual absorbance and fluorescence detection.

Absorbance detection was used to monitor LNP particles through UV light scattering and mRNA through UV absorption, while fluorescence detection using SYTO 41 enabled selective detection of encapsulated mRNA. This approach allows differentiation of empty and mRNA-loaded LNPs within a single analysis.

All analyses were performed using a standardized icIEF workflow. Details on materials and methods are provided in the Appendix.

Results

Surface Charge Profiling Identifies Different Lipids and mRNA Payloads in Various LNP Formulations

icIEF analysis enables separation of LNPs based on surface charge, providing distinct charge (pI) profiles for particles formulated with different mRNA payloads¹.

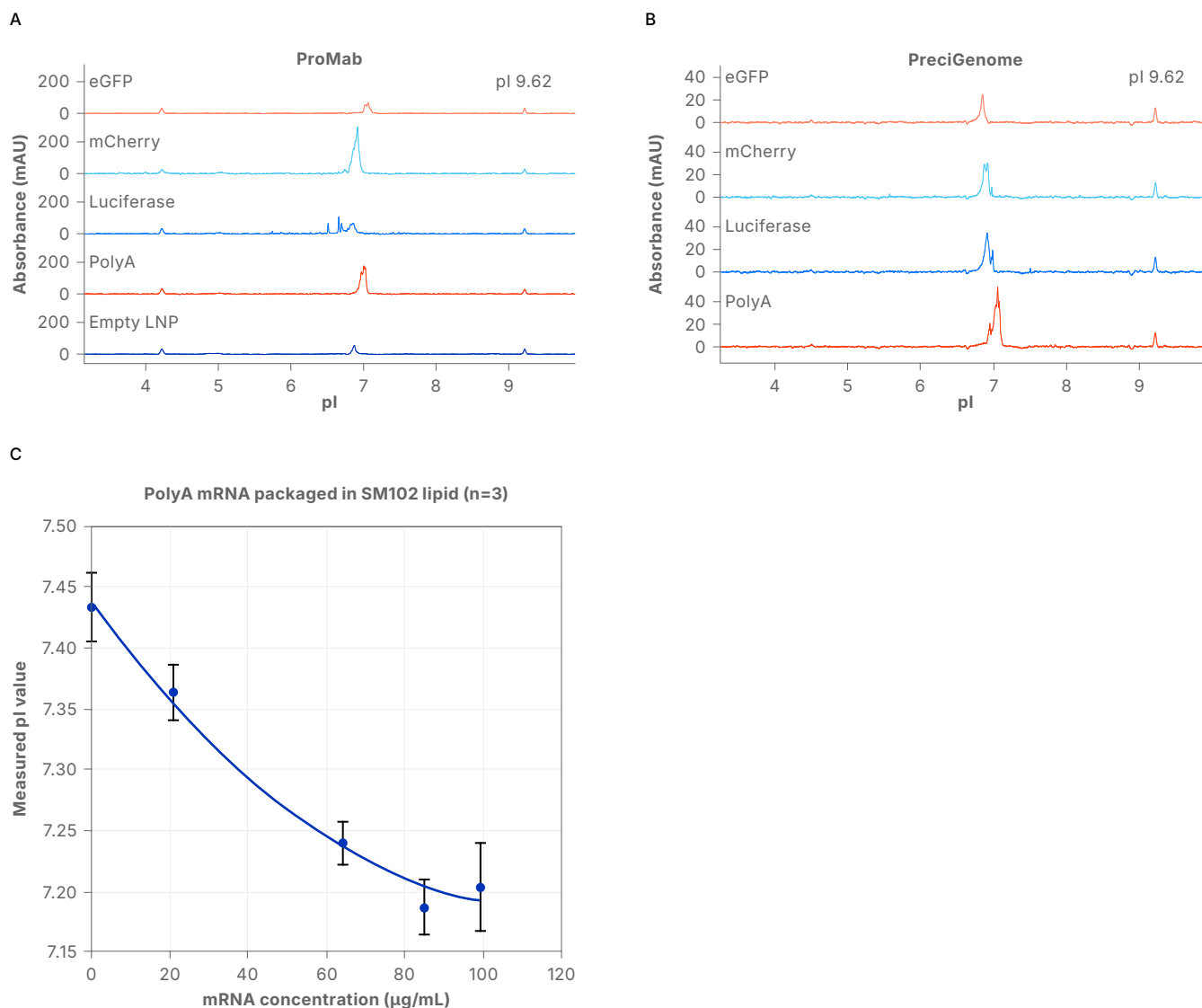


Figure 1. LNP surface charge profiles reflect differences in mRNA payload and formulation composition. (A-B) Representative icIEF electropherograms show distinct charge profiles for LNPs containing different mRNA payloads and originating from different formulations. (C) Increasing encapsulated mRNA concentration results in measurable pI shifts, demonstrating that payload content influences LNP surface charge. These charge-dependent differences enable discrimination of LNP formulations using a single icIEF analysis.

LNPs from different vendors and formulations were readily distinguishable based on their electropherogram profiles, as shown in **Figures 1A** and **1B**, demonstrating the sensitivity of icIEF to the mRNA content. Changes in encapsulated mRNA content also resulted in measurable pI shifts, indicating that both lipid composition and payload contribute to overall particle charge. Furthermore, increasing encapsulated mRNA concentration was associated with a measurable decrease in pI, as shown in **Figure 1C**, indicating that payload content contributes to overall LNP charge. icIEF was also able to distinguish between different lipid formulations, as shown in Figure 2. These results establish charge heterogeneity as a reliable method of assessing LNP identity and formulation variability.

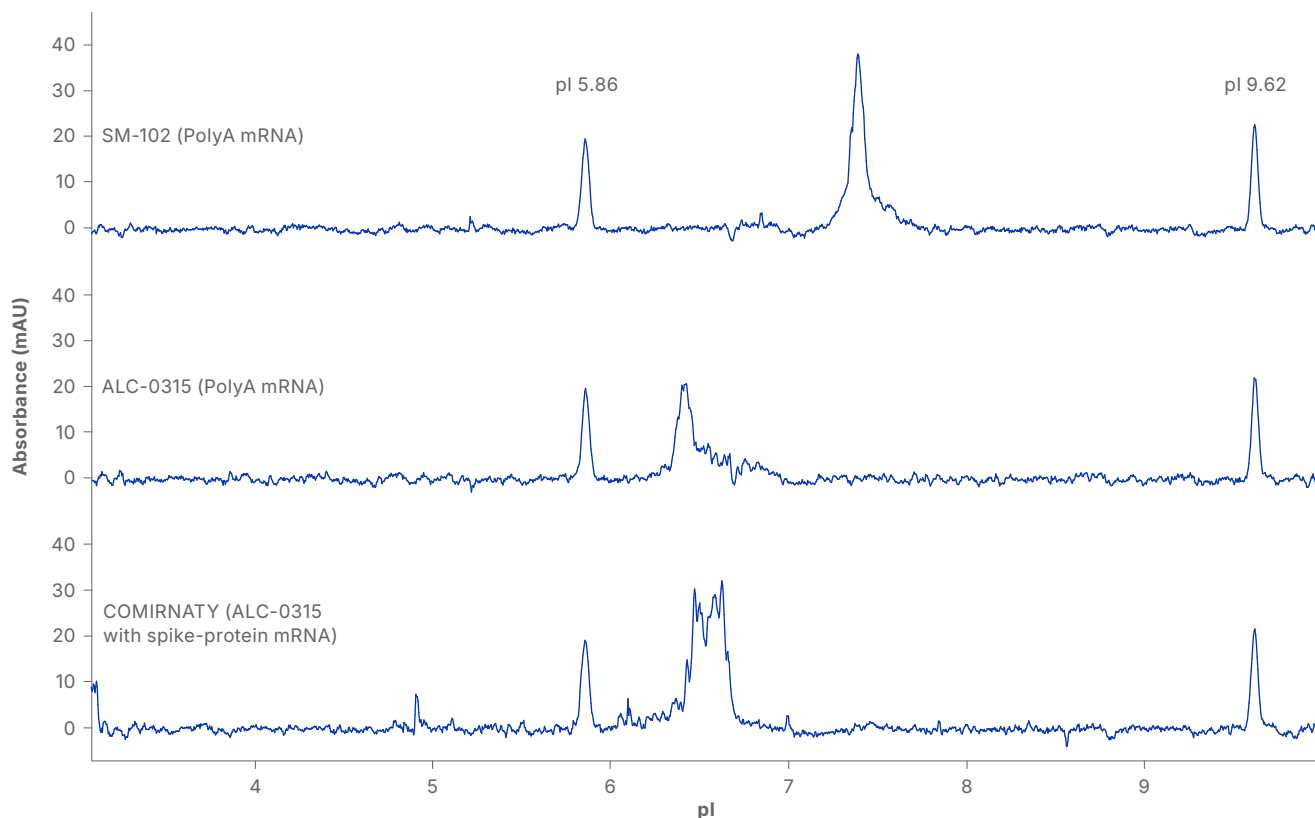


Figure 2. LNPs formulated with different lipids generate distinct icIEF charge profiles. Representative electropherograms show formulation-dependent differences in peak profile and pI distribution for SM-102 polyA mRNA LNPs, ALC-0315 polyA mRNA LNPs, and COMIRNATY®, supporting the use of surface charge profiling for LNP identity assessment.

SYTO 41 Dye Enables Fluorescence-based Detection of Encapsulated mRNA Without Altering icIEF Separation

icIEF method robustness for detecting encapsulated mRNA was evaluated using SYTO 41, a cell-permeable blue-fluorescent cyanine nucleic acid stain. Analysis of COMIRNATY samples demonstrated that addition of SYTO 41 produced minimal changes in electropherogram profiles and measured pI values with absorbance detection, indicating that the dye does not significantly impact charge-based separation by icIEF (**Figure 3A**). Notably, fluorescence signal intensity increased with increasing mRNA concentration, demonstrating that SYTO 41 provides a concentration-dependent readout of encapsulated payload, as shown in **Figures 3B** and **3C**. By combining absorbance detection of LNP particles with fluorescence detection of SYTO 41 labeled encapsulated mRNA, icIEF on Maurice provides complementary information on both particle- and payload-associated attributes within a single characterization workflow.

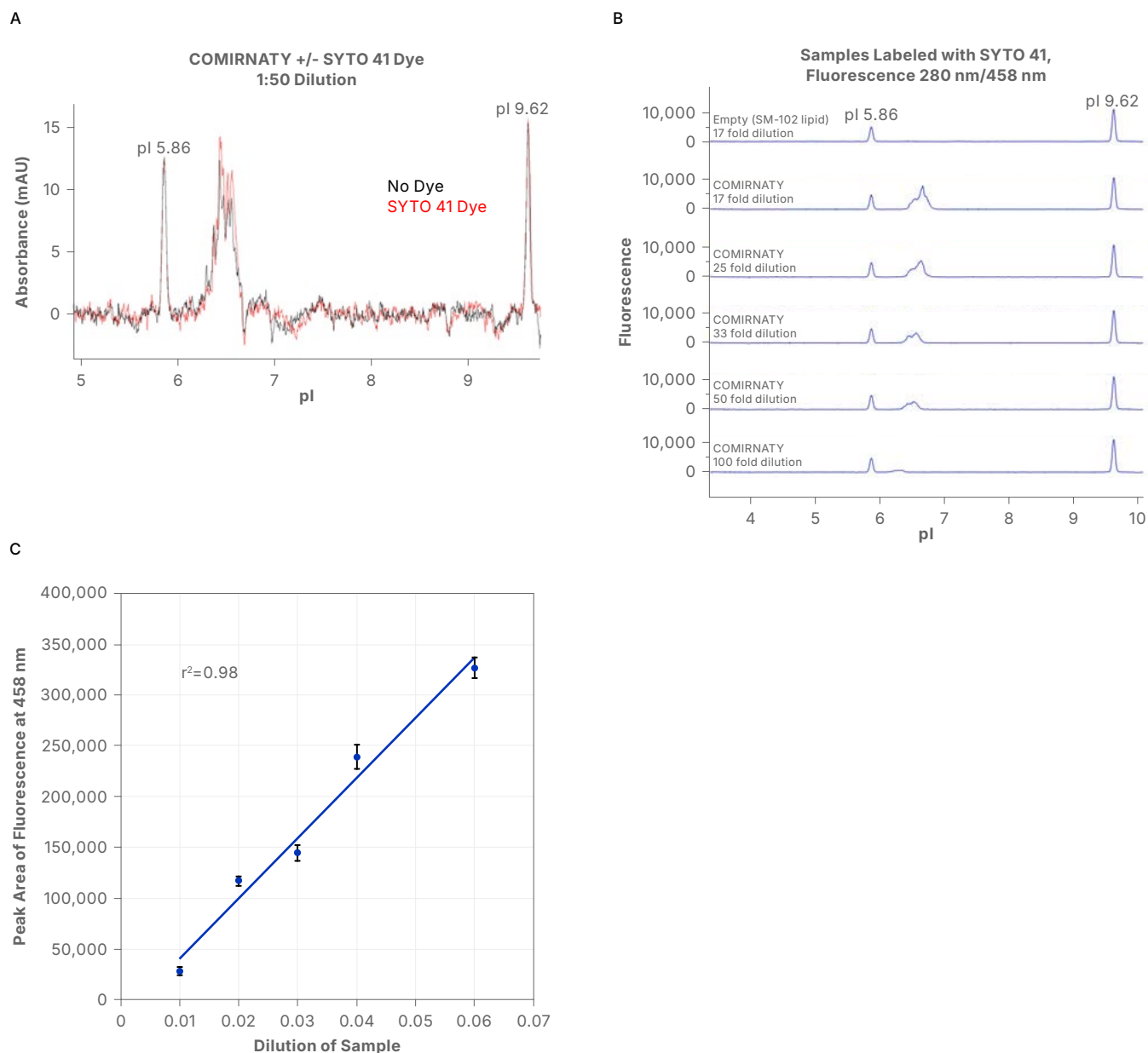


Figure 3. SYTO 41 enables fluorescence-based detection of encapsulated mRNA without altering icIEF separation. (A) Representative absorbance electropherograms of COMIRNATY LNPs analyzed with and without SYTO 41 demonstrate minimal impact of the dye on peak profiles and measured pI values, indicating that using the dye does not significantly affect charge-based separation. **(B)** Fluorescence electropherograms of COMIRNATY samples at multiple dilutions show concentration-dependent decreases in signal intensity, while an empty SM-102 lipid control produces minimal fluorescence signal. **(C)** Peak fluorescence intensity correlates with sample concentration ($R^2 = 0.98$), demonstrating that SYTO 41 provides a quantitative fluorescence readout of encapsulated mRNA. Together, these results establish SYTO 41 as a suitable probe for monitoring encapsulated mRNA during icIEF analysis.

Shifts in Charge Profiles Confirm Stress-induced LNP Degradation

Thermal stress produced substantial changes in both absorbance and fluorescence profiles, suggesting loss of particle integrity and release of encapsulated mRNA. However, below-freezing temperatures didn't appear to degrade LNPs, and only seemed to have a minor impact on particle concentration, as evidenced by the reduced peak intensity (**Figure 4A**). Particle degradation was also observed following exposure to acidic conditions, suggesting disruption of the LNP structure and payload leakage. In contrast, oxidative stress primarily produced shifts in charge profiles with comparatively smaller changes in fluorescence signal, indicating that lipid modification may occur without extensive loss of encapsulated mRNA. Together, these results demonstrate icIEF can detect different modes of LNP degradation within a single workflow.

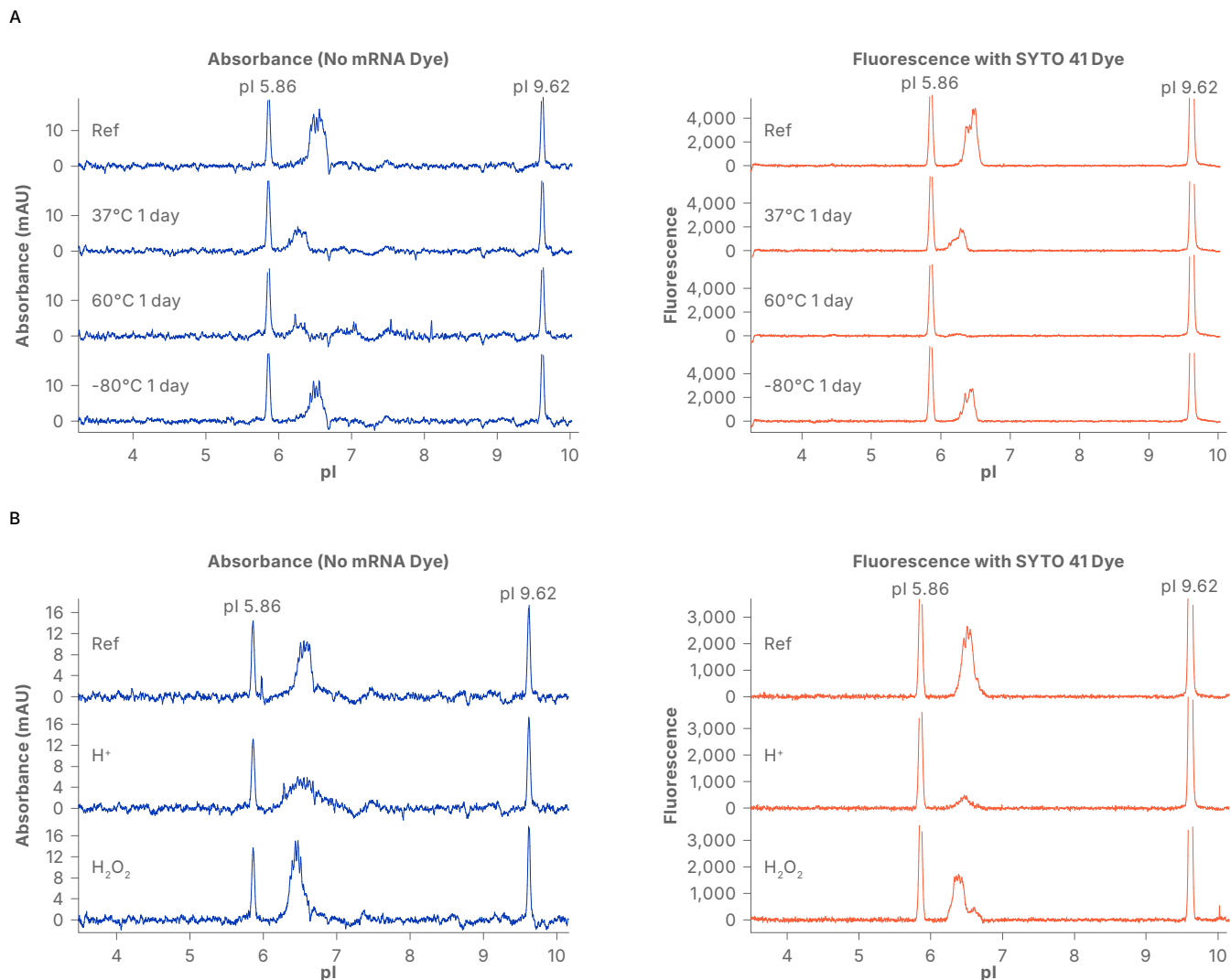


Figure 4. Distinct stress conditions generate unique charge and payload signatures in LNP formulations. Representative absorbance (left) and fluorescence (right) electropherograms of COMIRNATY LNPs following exposure to thermal (**A**) and chemical (**B**) stress. Elevated temperature produced substantial changes in both charge profiles and fluorescence signal, consistent with disruption of LNP integrity and loss of encapsulated mRNA. Acidic conditions resulted in similar changes to both absorbance and fluorescence profiles, suggesting particle destabilization and payload release. In contrast, oxidative stress primarily altered charge profiles while largely preserving fluorescence signal, indicating changes to LNP surface characteristics with comparatively limited impact on encapsulated mRNA. Together, these distinct response patterns demonstrate the ability of combined absorbance and fluorescence measurements to differentiate multiple forms of LNP instability.

Takeaways

- A single icIEF workflow assesses multiple LNP critical quality attributes, including identity, integrity, and stability, reducing reliance on multiple orthogonal assays.
- Surface charge profiles provide a sensitive fingerprint of LNP composition, enabling differentiation of formulations containing distinct lipids and mRNA payloads.
- Combined absorbance and fluorescence detection provide complementary insight into LNP particles and encapsulated payload, supporting assessment of LNP integrity.
- Distinct thermal and chemical stress conditions generate unique charge and payload signatures, enabling detection of multiple forms of LNP instability.
- By combining charge profiling with particle- and payload-associated measurements, Maurice simplifies LNP characterization while providing insight into formulation performance.



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REFERENCES

1. Loughney, J.W., Minsker, K., Ha, S. and Rustandi, R.R. (2019), Development of an imaged capillary isoelectric focusing method for characterizing the surface charge of mRNA lipid nanoparticle vaccines. ELECTROPHORESIS, 40: 2602-2609.
<https://doi.org/10.1002/elps.201900063>

Appendix

Materials

Material	Supplier	Catalog #
COMIRNATY® LNPs	Pfizer/BioNTech	N/A
Other LNPs/mRNAs	ProMab PreciGenome	Off-the-shelf
Maurice™ System	R&D Systems	090-158
Maurice cIEF Cartridge		PS-MC02-C
Maurice cIEF Method Development Kit*		PS-MDK01-C
1% Methyl Cellulose Solution		101876
Pharmalytes™ pH 3–10		17045601
pI Marker 5.5	Millipore Sigma	77866
pI Marker 9.5		89268
Glycerol		G5516
SYTO™ 41 Blue Fluorescent Nucleic Acid Stain	ThermoFisher Scientific	S11352

*The Maurice cIEF Method Development Kit contains the reagents required for icIEF analysis, including carrier ampholytes and associated reagents.

Methods

A master mix containing 20% glycerol, 0.7% methylcellulose, 4% Pharmalyte 3–10, and pI markers 5.86 and 9.62 was prepared and divided into separate tubes based on the number of samples. For fluorescence detection of encapsulated mRNA, 130 µM SYTO 41 was added to the designated preparation before addition of the LNP sample. Samples were vortexed for approximately 10 seconds and centrifuged at 10,000 × g for 3–5 minutes after dye addition and again after LNP addition. SYTO 41-containing samples were incubated on ice for 30 minutes before loading onto Maurice.

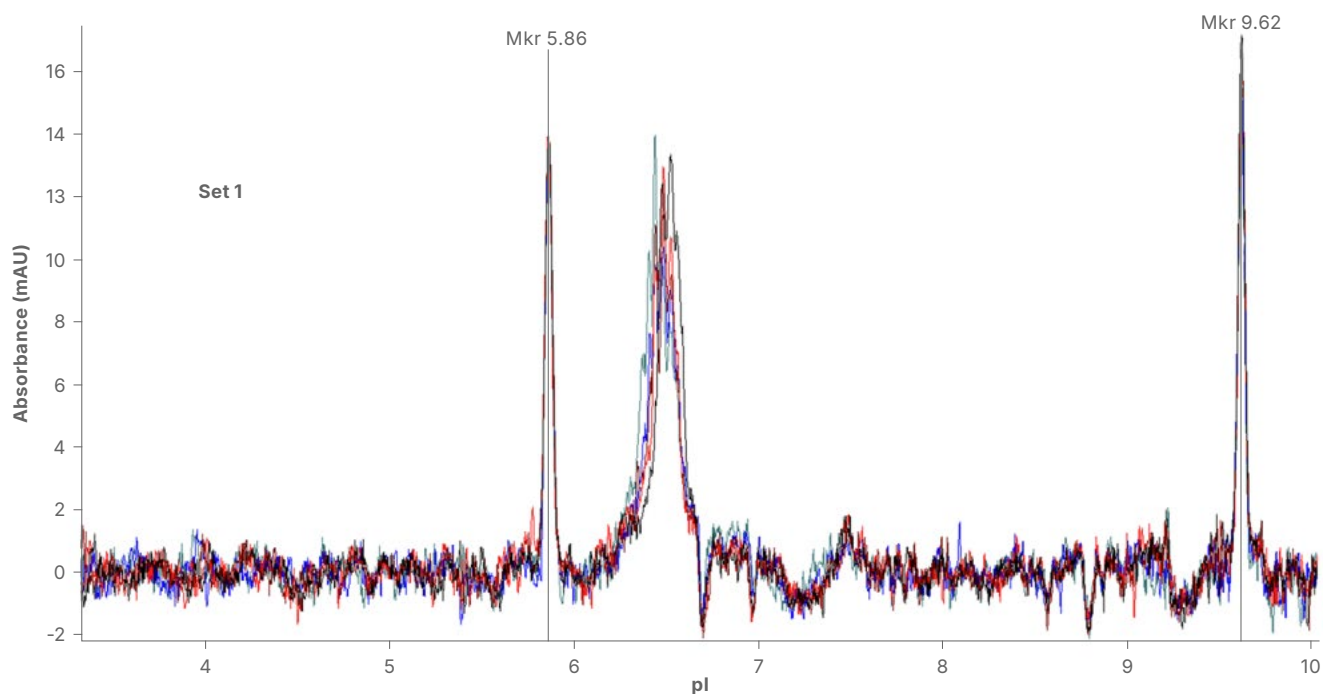
Samples were focused at 1.5 kV for 1 minute followed by 3 kV for 6 minutes and analyzed using either 280 nm absorbance or 458 nm fluorescence. A 1% methylcellulose wash was performed after every five sample injections. All data were analyzed using Compass for iCE software.

*pI markers from Millipore Sigma were used due to their flexibility for use in both icIEF and icIEF fractionation applications. Minor deviations between observed and nominal marker pI values are expected and reflect calibration within the carrier ampholyte-generated pH gradient.

Method Reproducibility

COMIRNATY® LNPs were analyzed at a 1:50 dilution using two replicate sample preparations (**Set 1** and **Set 2**), each injected five times within the same run to evaluate method reproducibility. Consistent electropherogram profiles were observed across all injections, with average pI values of 6.50 and 6.51 for Rep 1 and Rep 2, respectively. The corresponding pI % RSD values remained below 0.5%, while peak area reproducibility was 5.1% and 5.8% RSD for the two replicate sets. These results demonstrate reproducible charge profiling and pI determination across both replicate preparations and repeated injections.

A1



A2

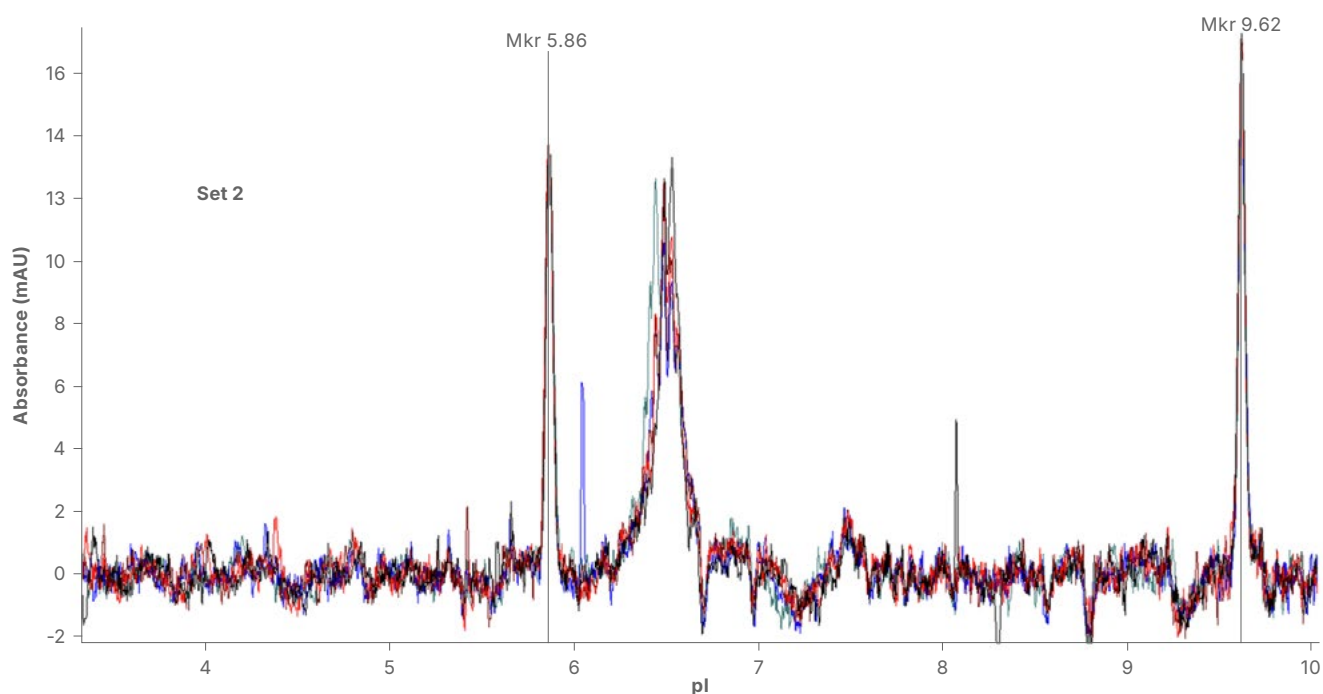


Figure A1-A2. icIEF on Maurice enables reproducible analysis of COMIRNATY LNPs across replicate sample preparations and injections. Representative electropherograms from two independent replicate preparations of COMIRNATY analyzed at a 1:50 dilution are shown for Set 1 (**Figure A1**) and Set 2 (**Figure A2**). Each preparation was injected five times within the same run. Consistent peak profiles and pI marker alignment were observed across all injections, with average pI values of 6.50 and 6.51 for Set 1 and Set 2, respectively. pI reproducibility remained below 0.5% RSD, while peak area reproducibility was 5.1% and 5.8% RSD across the two replicate preparations, demonstrating reliable and reproducible icIEF analysis of LNP formulations.

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