CHARACTERIZING LIPID NANOPARTICLE VACCINES USING CDMS AND FFF-MALS: A MULTI-DIMENSIONAL APPROACH

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Introduction

Lipid nanoparticle (LNP) systems have become essential delivery vehicles for nucleic acid–based therapeutics, including mRNA vaccines. Their performance is closely tied to critical quality attributes such as particle size, mass, loading efficiency, and heterogeneity. However, the inherent structural complexity and polydispersity of LNP formulations make comprehensive characterization challenging. Traditional ensemble-based analytical methods often provide only limited insights, overlooking low-abundance or structurally distinct subpopulations that may influence potency, stability, or manufacturability.

To address these challenges, we applied a multidimensional analytical workflow that integrates single-particle Charge Detection Mass Spectrometry (CDMS) with solution-phase Field-Flow Fractionation coupled to Multi-Angle Light Scattering (FFF-MALS). CDMS enables direct, high-resolution mass measurements of individual nanoparticles, revealing fine structural distinctions and low-abundance species that ensemble methods may miss. In parallel, FFF-MALS provides orthogonal measurements of size, molar mass, and concentration under native conditions, offering a complementary view of LNP architecture at the population level.

Together, these technologies deliver a more complete characterization of LNP vaccine formulations, strengthening analytical workflows for next-generation nucleic acid therapeutics

Methods

Charge Detection Mass Spectrometry: Xevo CDMS

LNP samples were buffer-exchanged into 20 mM ammonium acetate, pH 7.4 using Slide-A-Lyzer™ MINI Dialysis Devices (Thermo Scientific) for 30 minutes at 4 ° C. Following buffer exchange, samples were introduced by nano-electrospray ionization into a benchtop Xevo™ CDMS Instrument (Waters). CDMS data were processed and reviewed using the CDMS Toolkit within the waters_connect™ Software. Statistical analysis was scripted using Python. CDMS mass histograms were generated using identical logarithmic bins spanning 1 × 10^7 to 3 × 10^8 Da for all samples.

B)

A)

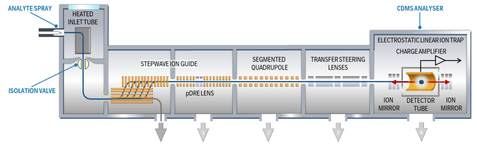

Figure 1: A) Instrument diagram, B) Picture of Xevo CDMS

Methods

Field Flow Fractionation (FFF)- Multi-Angle Light Scattering (MALS)

FFF separation was performed using neat samples with a separation method optimized for LNP-mRNAs on an Eclipse™ FFF instrument with a 350 µm fixed-height short channel connected to an HPLC pump and autosampler. PBS was used as the mobile phase. A DAWN™ MALS instrument, an Optilab™ differential refractometer, and a UV detector set to 260 nm wavelength were used for online detection. The FFF system was controlled by VISION™ software. Reported uncertainties are the standard deviations from triplicate measurements. Data acquisition and analysis were performed using the ASTRA™ software.

Figure 2:Arc HPLC, Eclipse™FFF system, DAWN MALS detector, Optilab dRI detector

Dynamic Light Scattering Data

DLS data was sourced from literature or manufacturers' certificates of analysis.

Spikevax and Comirnaty LNP Analysis by Xevo CDMS, DLS and FFF-MALS

CDMS reveals that Spikevax and Comirnaty differ primarily in their typical single-particle mass and absolute heterogeneity, while maintaining similar distribution shapes, underscoring formulation-dependent loading rather than rare extreme species.

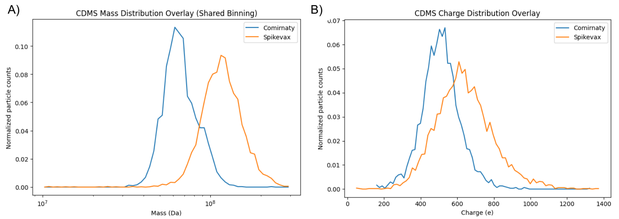

Figure 3: A) Mass distribution and B) charge distribution overlays for Comirnaty (blue) and Spikevax (orange)[1]

Despite sharing the same mRNA-LNP formulation paradigm, Spikevax and Comirnaty exhibit distinct single-particle mass distributions, highlighting formulation-dependent heterogeneity.

Sample	Median Mass	Mass IQR	DLS radius (nm)	PDI
Spikevax	1.17e+08	4.11e+07	75.4	0.24
Comirnaty	6.49e+07	1.99e+07	38.4	0.26

DLS indicates larger hydrodynamic size for Spikevax LNPs, while CDMS reveals correspondingly higher single-particle mass and absolute heterogeneity, highlighting formulation-specific particle composition beyond ensemble size measurements.

Empty and Full Lipid Nanoparticles by Xevo CDMS and DLS

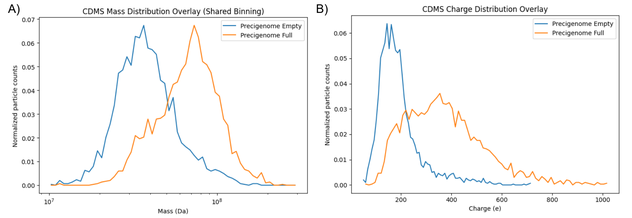

Figure 4: A) Mass distribution and B) charge distribution overlays for Empty (blue) and Full (orange) LNPs

CDMS reveals a pronounced mass shift and increased heterogeneity upon payload loading, resolving differences not observable by ensemble size measurements.

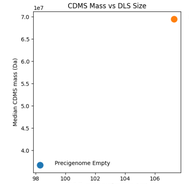

Figure 7: CDMS Mass vs DLS size for empty and full LNPs

Payload loading nearly doubles single-particle mass and increases mass heterogeneity while producing only a modest change in hydrodynamic size, demonstrating that internal composition—not size—is the dominant effect revealed by CDMS.

CDMS reveals that payload loading nearly doubles the median mass of the full LNPs and substantially increases mass heterogeneity, while DLS reports only a modest increase in hydrodynamic diameter and a slightly reduced PDI.

These results indicate that loading primarily affects internal particle composition rather than overall size, and that ensemble size measurements alone mask significant single-particle heterogeneity resolved by CDMS.

Sample	Median Mass	Mass IQR	DLS radius (nm)	PDI
Empty LNP	3.67e+07	1.89e+07	49.2	0.146
Full LNP	6.95e+07	3.75e+07	53.7	0.112

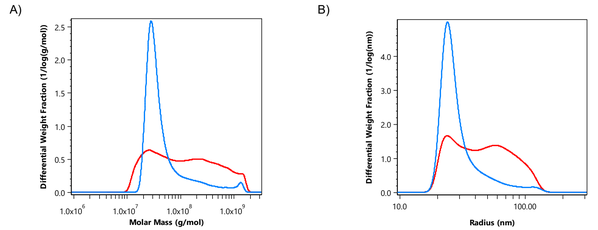

Figure 6: Differential weight fraction vs A) molar mass and B) radius (nm) for Comirnaty (blue) and Spikevax (red)[2]

FFF-MALS provides ensemble-averaged size and mass distributions under native conditions, while CDMS resolves single-particle mass and charge, enabling detection of rare or heterogeneous LNP populations that are masked in ensemble measurements.

Conclusions

- Combining CDMS and FFF-MALS provides complementary single-particle and ensemble insight into LNP vaccine structure.
- CDMS uniquely reveals loading-dependent mass increases and heterogeneity that are largely masked by ensemble size measurements.
- Payload loading significantly alters internal composition with only modest changes in hydrodynamic size.
- Spikevax and Comirnaty exhibit distinct single-particle mass distributions, reflecting formulation-dependent differences despite similar size profiles.
- Single-particle mass and heterogeneity emerge as critical quality attributes for LNP characterization.

Future Directions

- Correlate CDMS-derived mass distributions with encapsulation efficiency, potency, and stability.
- Apply the CDMS–FFF-MALS workflow to process development and batch comparison.
- Extend analyses to additional LNP formulations and nucleic acid payloads.
- Incorporate charge-resolved CDMS metrics to further probe particle architecture.

References

1. D'Esposito et al.. High-Resolution Characterization of Lipid Nanoparticles Using Xevo™ CDMS. Application Note, 2026.
2. Kurnik, M.I.. Multi-Attribute Quantification of LNP-mRNA Therapeutics by FFF-MALS and DLS. Application Note AN2615, 2023.

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