Effect of Purification Methods on the Properties of Minicircle DNA-Loaded Lipid Nanoparticles

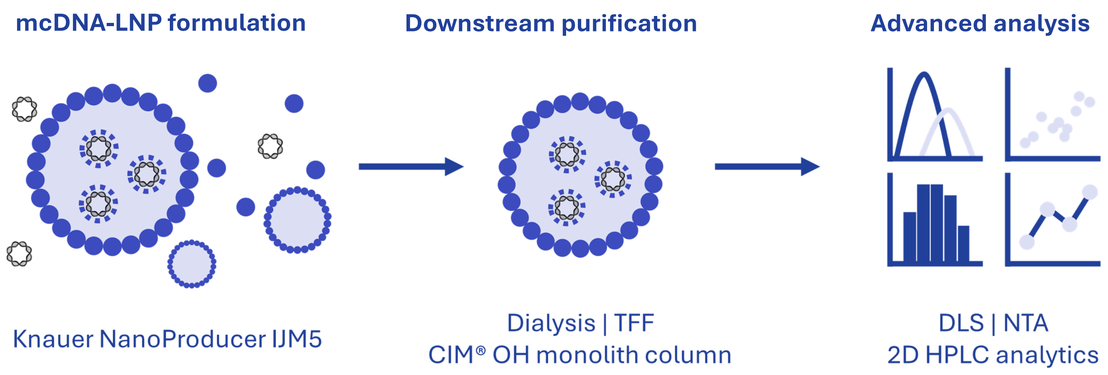
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In collaboration with BIA separations, inc

Introduction and Experimental Concept

Lipid nanoparticles (LNPs) represent a **leading non-viral platform** for gene delivery, where encapsulating minicircle DNA (mcDNA) enables sustained and durable gene expression. **Downstream processing (DSP) can significantly alter critical quality attributes**, including LNP size, encapsulation efficiency, and overall mcDNA integrity. **An integrated analytical screening strategy is essential** to rapidly identify process-induced changes and optimize purification conditions that preserve LNP properties and cargo quality.

Objective: Evaluate and compare the impact of three LNP purification methods on **LNP particle size**, **polydispersity index** (PDI), **encapsulation efficiency** (EE%), **adduct formation**, and **morphological profile**.



- **mcDNA-LNP formulation:** Flow Rate Ratio (FRR) 3:1; Total Flow Rate (TFR) 80 mL/min; N/P ratio 6:1.
- **DSP was compared using three methods:** small-scale dialysis, industry-standard TFF, and CIM OH monolithic chromatographic purification with elution fractionation.
- **LNP physicochemical properties, structure, and cargo quality were evaluated** via a multi-method analytical approach: PATfix Switcher (2D chromatographic system), Zetasizer (Dynamic Light Scattering - DLS), ZetaView Evolution (Nanoparticle Tracking Analyzer - NTA), and Cryo-Transmission Electron Microscopy (Cryo-TEM) analysis.

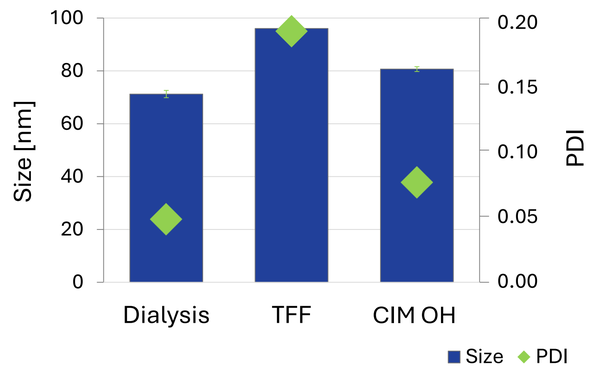
Results

TFF Increases LNP Size and PDI

DLS and NTA size measurements were highly comparable (<15% variance), consistently showing a larger mean hydrodynamic diameter following TFF purification (70 to 96.1 nm).

TFF increased the PDI (0.09 to 0.19), whereas dialysis and CIM OH maintained the baseline size distribution and polydispersity.

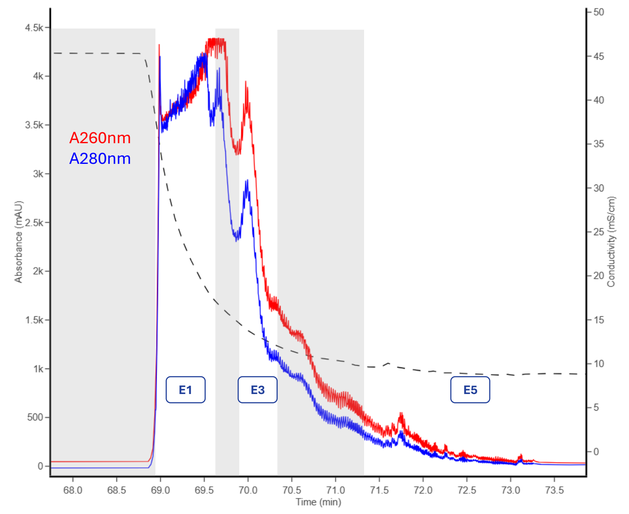
Size and PDI analysis on DLS

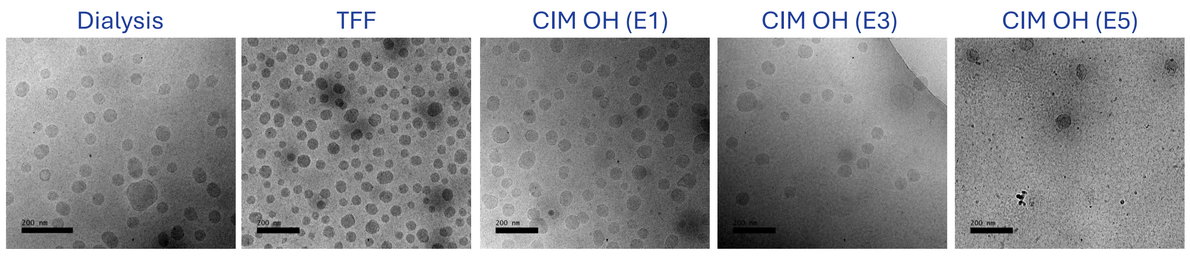


Cryo-TEM Reveals Improved Morphological Homogeneity Following CIM OH Purification

CIM OH chromatographic elution profile

- Zoomed-in view of the CIM OH elution chromatogram is presented.
- **Step-elution was applied** to achieve LNP recovery.
- **The elution peak was fractionated (E1–E5)** for separate subsequent analysis.

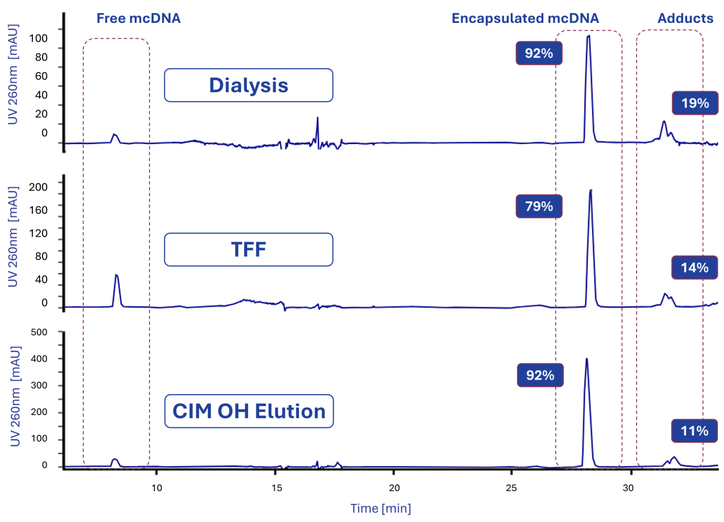




- **TFF induced morphological heterogeneity**, whereas dialysis predominantly preserved intact, spherical LNPs.
- **CIM OH chromatography fractionated distinct LNP populations**, separating larger, low-electron-density particles (potentially empty LNPs) from aggregated or structurally compromised species.
- **Cryo-TEM of individual OH fractions confirmed peak enrichment of intact, homogeneous LNPs in E1**, while larger and compromised particles partitioned into fractions E3 and E5.

Reduced Encapsulation Efficiency Following TFF Purification

PATfix Switcher chromatograms



CIM OH and dialysis enriched encapsulated LNPs to 92% EE%, whereas TFF reached only 79% EE%, potentially due to shear-induced particle instability.

Adduct levels remained under 20% across all evaluated experimental batches.

Conclusions

- **TFF negatively impacts LNP integrity**, potentially due to shear stress, resulting in lower purification efficiency (EE% remaining lower than with other methods) and structural changes such as aggregation (higher DLS mean values).
- **CIM OH chromatography and dialysis both served as gentler processing approaches,** maintaining high EE%. CIM OH additionally concentrates the sample and enriches intact, homogeneous particles for an improved morphological profile.
- **2D HPLC enabled sensitive monitoring of EE% and adduct formation**, allowing detection of process-induced changes.
- **Complementary analytical screening** using 2D chromatography, DLS, and NTA supports rapid DSP evaluation, process monitoring and selection of the most suitable purification strategy.

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