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Application Note

June 2025

Keywords or phrases:
mRNA-LNPs, SIMCA®-online, continuous manufacturing, process intensification, single pass tangential flow filtration, lipid nanoparticle, SIMCA®-online, digital twin, AI ecosystem

Advancements in Lipid Nanoparticle Production: A Joint Exploration Between Sartorius and DIANT® Pharma

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Abstract

This study evaluated the integration of DIANT® Pharma and Sartorius technologies for continuous mRNA-lipid nanoparticle (mRNA-LNP) production. The DIANT® PILOT platform, incorporating DIANT® JET technology and single-pass tangential flow filtration, was combined with Sartorius consumables, including Flexsafe® bags, Flexboy® bags, Sartocon® Hydrosart®, and Sartocon® PES TFF cassettes. Inline analytics, powered by SIMCA® software, enhanced process control.

The technical work focused on three key milestones:
1. Optimizing flow conditions for concentrated nanoparticle production
2. Developing potent mRNA-LNP formulations, using Onpattro® as the benchmark formulation[1,2]
3. Evaluating the quality and efficacy of the resulting mRNA-LNPs[3]

Encapsulation efficiency exceeded 95%, with controlled particle size and concentration. In vivo efficacy was confirmed through animal studies using the mRNA-LNP Onpattro® formulation. Freeze-thaw studies demonstrated particle stability, with minimal variations in particle size, polydispersity index, and zeta potential. mRNA integrity remained intact post-filtration and across freeze-thaw cycles.

The collaboration successfully demonstrated mRNA-LNP production exclusively on DIANT® and Sartorius platforms, providing a viable solution for scalable and continuous therapeutic mRNA manufacturing.

Introduction

The rapid advancement of mRNA technology, particularly in the realm of vaccines and therapeutics, has necessitated the development of efficient and scalable production methods for mRNA-lipid nanoparticles (mRNA-LNPs). This application note describes the collaborative efforts between Sartorius and DIANT® Pharma to demonstrate continuous mRNA-LNP production using advanced technologies. In this context, Sartorius Flexsafe®, Flexboy®, and Celsius® bags were used for holding or capturing raw materials, process intermediates, and final formulations. Sartocon® tangential flow filtration (TFF) cassettes were used for concentration and dilution, Sartopore® 2 XLG filters were used to sterilize the solution, SIMCA®-online was installed on DIANT® PILOT to enrich process analytics, and Sartorius provided the Fluc plasmid mRNA. DIANT® Pharma executed the study using the DIANT® PILOT and sourced the remaining raw materials and solutions.

DIANT® PILOT

The DIANT® PILOT is an advanced continuous production platform designed specifically for lipid nanoparticle (LNP) production. It uniquely integrates proprietary turbulent jet mixing technology (DIANT® Jet), allowing precise and rapid mixing of components. This results in highly uniform nanoparticles with tightly controlled particle size distribution and excellent encapsulation efficiency.[4,5] Unlike traditional batch or microfluidic-based approaches, this platform enables continuous, scalable, and consistent production.

The DIANT® PILOT platform includes inline monitoring and process analytical technologies (PATs), ensuring real-time control and adjustment of critical process parameters. Its single-pass, closed-system design significantly reduces the overall production footprint, decreases waste generation by minimizing dead space and material losses, and reduces energy consumption compared to traditional batch processes. The sustainability aspect of the DIANT® PILOT is especially noteworthy: continuous production methods inherently reduce waste, limit human intervention (thus reducing contamination risks), and streamline the entire manufacturing process, contributing positively to environmental sustainability goals within the pharmaceutical manufacturing industry.

Sartorius' SIMCA® and SIMCA®-online

SIMCA® (model building) and SIMCA®-online (model tracking) are part of the Umetrics® Digital Twin AI Ecosystem. These powerful software tools are designed for multivariate data analysis and are trusted across various industries to enhance process optimization and quality control.

Their primary purpose is to delve into complex datasets, employing sophisticated statistical techniques to uncover patterns, relationships, and trends that might otherwise remain hidden. One of SIMCA®'s standout capabilities is its ability to seamlessly build powerful, easy-to-understand digital twin models to simplify process optimization. By identifying the key variables that influence process outcomes, SIMCA® enables users to make targeted improvements, thereby enhancing efficiency and productivity. In quality control applications, SIMCA®-online plays a crucial role in monitoring and maintaining product quality. It excels at detecting deviations and anomalies, ensuring that products consistently meet the highest standards. Moreover, SIMCA® and SIMCA®-online contribute significantly to improving efficiency. Through comprehensive data analysis, they offer valuable insights into streamlining processes, reducing waste, and boosting overall productivity. This makes them indispensable tools for organizations striving to achieve operational excellence.

Importantly, the use of Umetrics® Digital Twin AI Ecosystem is not limited to Sartorius equipment. It can be seamlessly loaded onto non-Sartorius machines, demonstrating its flexibility and compatibility with various systems and platforms. This integration capability allows SIMCA® and SIMCA®-online to work harmoniously with existing systems, providing insightful analytics that drive process efficiency and innovation.

Sartorius Sartocon® TFF Cassettes

Sartorius TFF cassettes provide high-performance separation for a variety of upstream and downstream bioprocessing applications, including next-generation biologics. Available in a broad range of molecular weight cut-offs (1 – 300 kDa) and pore sizes (0.1 – 0.45 µm), they scale seamlessly from lab to process (50 cm^2 – 3.5 m^2 effective filtration area). Two membrane types were offered — Hydrosart® and polyethersulfone (PES) — in both single-use and reusable formats.

This study employed the Sartocon® Slice Disposable, designed for ease of use with its single-use, holder-free configuration. It offers flexibility to run ultrafiltration and diafiltration as separate steps and features a Hydrosart® membrane, which is hydrophilic, non-protein binding, and virtually non-fouling while remaining stable across a broad pH range. Crucially, the Sartocon® Slice Disposable aligned with the system requirements and molecular weight cut-offs needed for DIANT® mRNA-LNP process.

Materials

However, for continuous TFF applications, the Sartocon® SP (single-pass)—which was recently added to the Sartorius TFF portfolio—is the preferred solution. Delivered as a pre-flushed, irradiated, and closed device, it minimizes biocontamination risks and eliminates the need for cleaning or extended water flushes. This design reduces setup and validation time while maintaining high product yield and sustained flux through its low-protein-binding membrane. With no glues used in construction, it also ensures minimal extractables and leachables. The portfolio is being expanded with additional molecular weight cut-offs to address a wide variety of processes.

Sartorius Flexsafe®, Flexboy®, and Celsius® Bags

Sartorius bags enable robust and safe fluid-handling throughout the production process. Flexsafe® and Flexboy® bags serve as containers for LNP raw materials such as ethanol, naked mRNA, and cationic lipids, facilitating aseptic delivery to DIANT® PILOT®. Their versatility extends to functioning as holding vessels for process intermediates, waste collection, and buffers used during concentration and dilution phases. Sartorius single-use bags incorporate advanced film technology that provides excellent barrier properties, protection against contamination, and preserves the quality of sensitive materials through fully characterized extractable and leachable profiles. The robust construction and high purity make Sartorius bags ideal for achieving streamlined and efficient mRNA-LNP production.

Benefits of Applying Single-Use Technologies

- Buffers can be prepared and stored in advance to simplify setup and accelerate batch turnaround
- The costs and time associated with cleaning multi-use systems are avoided, as users simply swap out to a new and unused fluid pathway
- Material validation costs are reduced through the use of consistent fluid-contact materials at different process scales
- Production volumes can be quickly adjusted by scaling single-use assemblies to meet changing demands
- Multi-product operations are enabled without the risk of cross-contamination, thanks to the quick change-out of fluid-contact surfaces for new, unused materials

The equipment and consumables used in this study are displayed in Tables 1 and 2, respectively.

Table 1: *Equipment Used in This Study*

Equipment	Item Number | Supplier
DIANT® PILOT	DIANT® Pharma
Tubing Transfer Sets	DIANT® Pharma
Flexboy® 2D Bags	FFB103398, FFB101974
Flexsafe® 2D Bags	FLS130040
Flexsafe® 3D Bags for Drums	FIS127861
Drum for Storage	FBC125627
Dolly for Drum	FXA126305
Sartopore® 2 XLG	5445307GS-HH—M
Sartocon® Slice PES Cassette 100 kDa	3061466801E—SW
Sartocon® Slice Hydrosart® Cassette 100 kDa	3061446801E—SW

Table 2: *Solutions and Raw Materials Used in This Study*

Solutions & Raw Materials	Chemical name | Supplier
Fluc Plasmid mRNA	Sartorius BIA Separations
SM-102 Ionizable lipid	8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl ester, Cayman Chemical
DSPC phospholipid	1,2-distearoyl-sn-glycero-3-phosphocholine, Lipoid
Cholesterol	(3β)-cholest-5-en-3-ol, Cayman Chemical
DMG-PEG2000	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000, Avanti Polar Lipids
Citrate buffer (0.5 M, pH 4.0)	Boston BioProducts
Tris-HCl buffer (1 M, pH 7.2)	Boston BioProducts
Sucrose	Fisher Scientific
190 proof ethanol	Pharmco
Nuclease-free, deionized water	Milli-Q® System, Merck

Methods

Project Milestones
The collaboration was structured around three key milestones:
- Establish optimized flow conditions and achieve concentrated nanoparticles meeting LNP specifications
- Develop potent mRNA-LNP formulations as indicated using an in vivo model
- Evaluate the performance of Sartorius consumables using the DIANT® PILOT system

Equipment and Materials
The DIANT® PILOT system was integrated with Sartorius products (Figure 1), including:
- Sartocon® Slice disposable cassettes
- Sartorius Flexboy® 10 L 2D bags (×2) + tubing assembly for A1 phase (dilution)
- Sartorius (1) Flexsafe® 20 L and (2) 50 L 2D bags for concentration and buffer exchange
- Sartorius Flexboy® 1 L 2D Bags for final collection
- Sartorius SIMCA®-online for real-time monitoring, prediction and control
- Sartorius SIMCA® Digital Twin for model building and data interpretation

TFF Consumables
Sartocon® Slice disposable cassettes with a MWCO of 100 kDa and surface area of 0.1 m^2 were tested under two different membrane materials: Hydrosart® and PES.

Preparation of mRNA-LNPs
Stock solutions of ionizable lipid, DSPC, cholesterol, and DMG-PEG2000, were dissolved in ethanol and mixed at a molar ratio of 50:10:38.5:1.5, respectively.[1] mRNA was diluted in 10 mM citrate buffer, pH 4.0, to the target concentration. LNPs were formed via inline mixing of the ethanol-phase lipids with the aqueous mRNA solution. Immediately following particle formation, LNPs were diluted in-line with Tris buffer, pH 7.2, to reduce ethanol content and adjust pH.

The resulting bulk material was then concentrated and buffer exchanged into 8% sucrose in Tris buffer, pH 7.2. Final LNP formulations were sterile filtered through Sartopore® 2 XLG 0.2 µm sterile filters, aliquoted, and stored at – 80 °C.

Experimental Phases

Phase 1: mRNA-LNP Production
The objective of Phase 1 was to assess how various formulation and process parameters affect particle size, polydispersity index (PDI), and encapsulation efficiency of mRNA-LNPs on the DIANT® PILOT while integrating Sartorius single-use consumables.

Variable conditions examined during preparation, particle formation, and TFF stages included:
- N/P (lipid to nucleic acid charge ratio): 3 and 6
- TFF cassette material: Hydrosart® vs. polyethersulfone (PES)
- TFF cassette format: Sartocon® Slice disposable cassettes vs. slice cassettes

Process parameters such as runtime, temperature, and pH were fixed across formulation runs.

Phase 2: In Vivo Assessment of mRNA-LNPs
The primary objective of Phase 2 was to assess the in vivo functionality of mRNA-LNPs by measuring transfection efficiency. A selected LNP formulation in Phase 1 was reproduced under the following conditions: N/P of 6, SM-102 ionizable lipid, and a flow rate ratio of 3:1.

Following production, the formulation was concentrated to reach a final mRNA concentration of 20 µg/mL, and sterile filtered with Sartopore® 2 XLG 0.2 µm sterile filters. Samples were collected and stored at – 80 °C until injection. Mice received 0.2 mg/kg of luciferase-encoding mRNA via tail vein injection. Control animals were injected with empty LNPs (without mRNA) via tail vein injection. Bioluminescence imaging was conducted using the IVIS Spectrum System (Perkin Elmer) at 6- and 24-hours post-injection. At 24 hours, spleen, lung, and liver tissue were harvested for ex vivo imaging to assess organ-specific expression.

Phase 3: Freeze-Thaw Stability

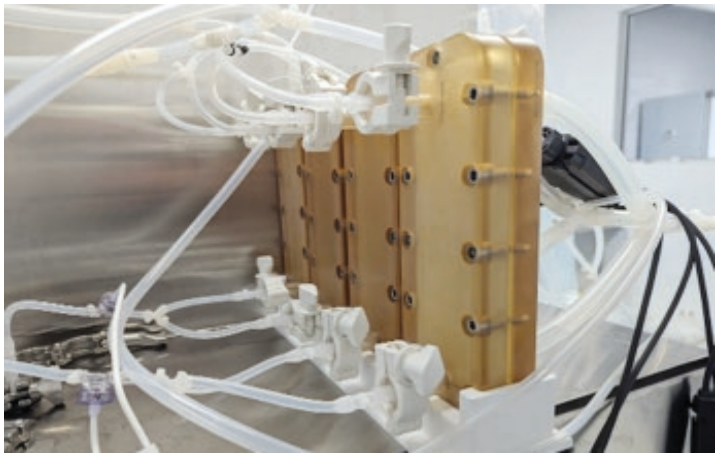
mRNA-LNPs were subjected to three freeze-thaw cycles to assess changes in particle size, PDI, encapsulation efficiency, and mRNA integrity. For each freeze-thaw cycle, samples were removed from −80 °C, thawed at 4 °C, and subsequently refrozen at −80 °C.

mRNA-LNP Characterization

Physicochemical and functional characterization of mRNA-LNP formulations was analyzed for both phases of the study. The quality attributes evaluated in this study are displayed in Table 3.

Figure 1: *DIANT® PILOT with single-pass TFF and Sartorius Products. All-in-One Skid to Produce and Concentrate Nanoparticles*

1. Control software for nanoparticle production
2. **Sartorius Flexboy®** 1 L 2D Bags for final collection
3. **Sartorius Flexboy®** 20 L 2D (×2) + tubing assembly
4. **Sartorius SIMCA®** for online analytics
5. **Sartorius** 20 L 2D Bag (1)
6. **DIANT®** particle formation stage using **DIANT®JET**
7. **Sartorius Flexsafe®** 50 L 2D Bags (×2)



DIANT® single-pass TFF operation using Sartorius Sartocon® Slice disposable cassettes and includes a tubing assembly

Controlled Particle Concentration Using DIANT® Single-Pass TFF

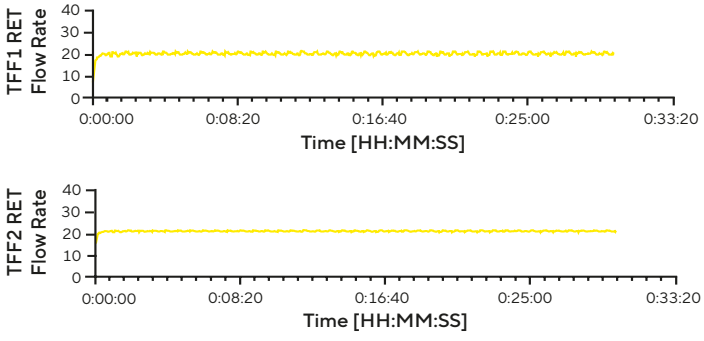


Table 3: *Quality Attributes Assessed in This Study*

Quality Attribute	Technique | Instrument	Target Value	Sample Preparation	Reporting
Particle size diameter [nm]	DLS Zetasizer Ultra Red (Malvern Panalytical Ltd.)	50 – 150	100-fold dilution in Tris buffer, pH 7.2, to 1 mL	Mean values of triplicates with standard deviation
PDI	DLS Zetasizer Ultra Red (Malvern Panalytical Ltd.)	< 0.2	100-fold dilution in Tris buffer, pH 7.2, to 1 mL	Mean values of triplicates with standard deviation
Surface charge [mV]	ELS Zetasizer Ultra Red (Malvern Panalytical Ltd.)	± 10	100-fold dilution in Tris buffer, pH 7.2, to 1 mL	Mean values of triplicates with standard deviation
Encapsulation efficiency	Fluorescence-based Qubit™ RNA High Sensitivity Assay	> 90%	Treated with or without 0.22% Triton-X-100 for quantifying total and free mRNA	Mean values of triplicates with standard deviation
mRNA integrity	RP-IP HPLC	No process- and consumable-specific degradation products. > 90% peak area intact.		
mRNA potency	In vivo imaging (IVIS, PerkinElmer)	Luciferase expression		
Lipid identity and content	HPLC-CAD	Within ± 5% target molar percentage and concentration		

Note. RP-IP HPLC = reversed-phase ion-pair high-performance liquid chromatography, HPLC-CAD = high-performance liquid chromatography with charged aerosol detection

Results

Phase 1: mRNA-LNP Production

The DIANT® PILOT system, integrated with single-pass TFF and Sartorius single-use consumables, enabled scalable and consistent production of mRNA-LNPs, with high encapsulation efficiency (EE%) and tight control over critical quality attributes (CQAs), including particle size, PDI, and zeta potential.

Effect of N/P Ratio
Initial runs included evaluation of N/P ratios of 3 and 6 at a fixed total lipid concentration. N/P 6 formulations resulted in larger particles relative to N/P 3 and improved uniformity with PDI averaging 0.16 ± 0.02 (Figures 2A – 2D).

EE% remained above 90% in both conditions, with a minimal decrease at higher N/P ratio.

TFF Conditions
LNPs achieved 3 – 7x concentration factors across membrane formats, maintaining particle size and encapsulation efficiency within the target range. PDI was significantly lower with Hydrosart® cassette material (0.16 ± 0.02 vs. 0.20 ± 0.03 in PES), and closer to neutral surface charges compared to PES.

Higher N/P ratios improved monodispersion but slightly reduced encapsulation efficiency. Hydrosart® and PES membrane materials supported effective concentration, while Hydrosart® showed superior performance in maintaining lower PDI and target zeta potential.

These data confirm that the DIANT® PILOT system, paired with Sartorius consumables, enables reproducible and controllable mRNA-LNP production under scalable conditions.

Phase 2: mRNA-LNP In Vivo Assessment

mRNA-LNP formulations achieved high encapsulation efficiency (> 95%) with controlled particle size and PDI (Figure 3, B). The post-TFF concentration factor reached a target final API concentration of 20 µg/mL, reflecting effective nanoparticle concentration. In vivo functionality of mRNA-LNPs was validated in BALB/c mice studies. Following IV administration at a dose of 0.2 mg/kg, IVIS imaging was performed at 6- and 24-hours post-injection. Strong bioluminescent signals were detected in the whole body, liver, and lungs, compared to the empty LNPs, which showed minimal signal (Figure 3, A). These findings confirmed the biological activity and transfection efficiency of LNPs produced in Phase 1.

IVIS imaging from mouse studies confirmed good transfection efficiency, with high luminescence intensity in the liver and lungs 6 hours post-injection, followed by a marked reduction at 24 hours, indicating effective mRNA delivery and subsequent clearance (Figure 3).

Phase 3: Assessment of mRNA-LNP Stability With Sartorius Celsius® Bags
SM-102 formulations showed high stability, with minimal changes in particle size and PDI after three freeze-thaw cycles (Figure 4). Encapsulation efficiency remained above 90%, and high-performance liquid chromatography (HPLC) analysis confirmed that mRNA integrity was preserved after repeated cycles.

Figure 2: *Effect of Formulation Parameters on Nanoparticle Characteristics. Average (n=3), Particle Size, PDI, Zeta Potential, and Encapsulation Efficiency Evaluated Under Varying Conditions*

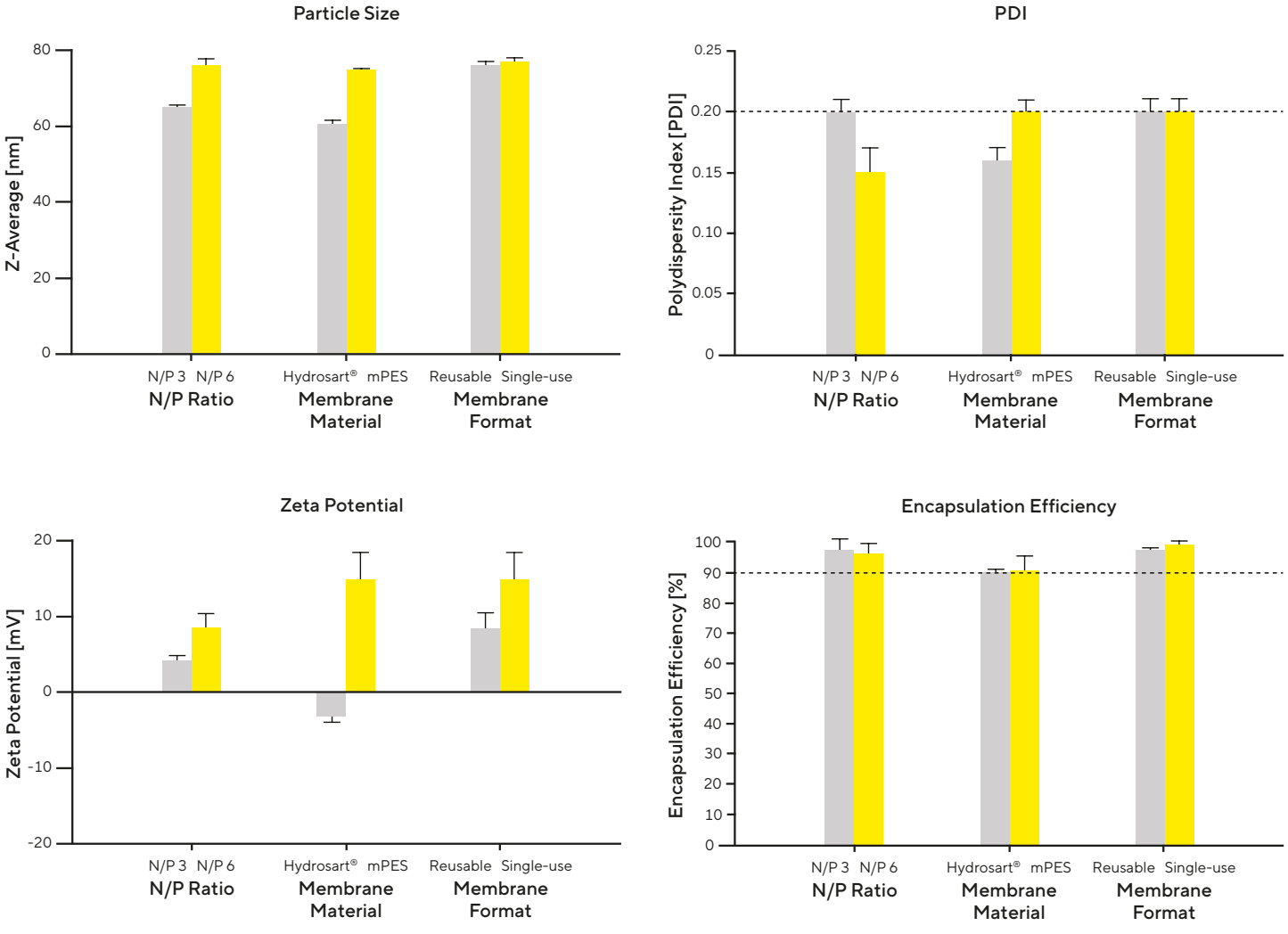
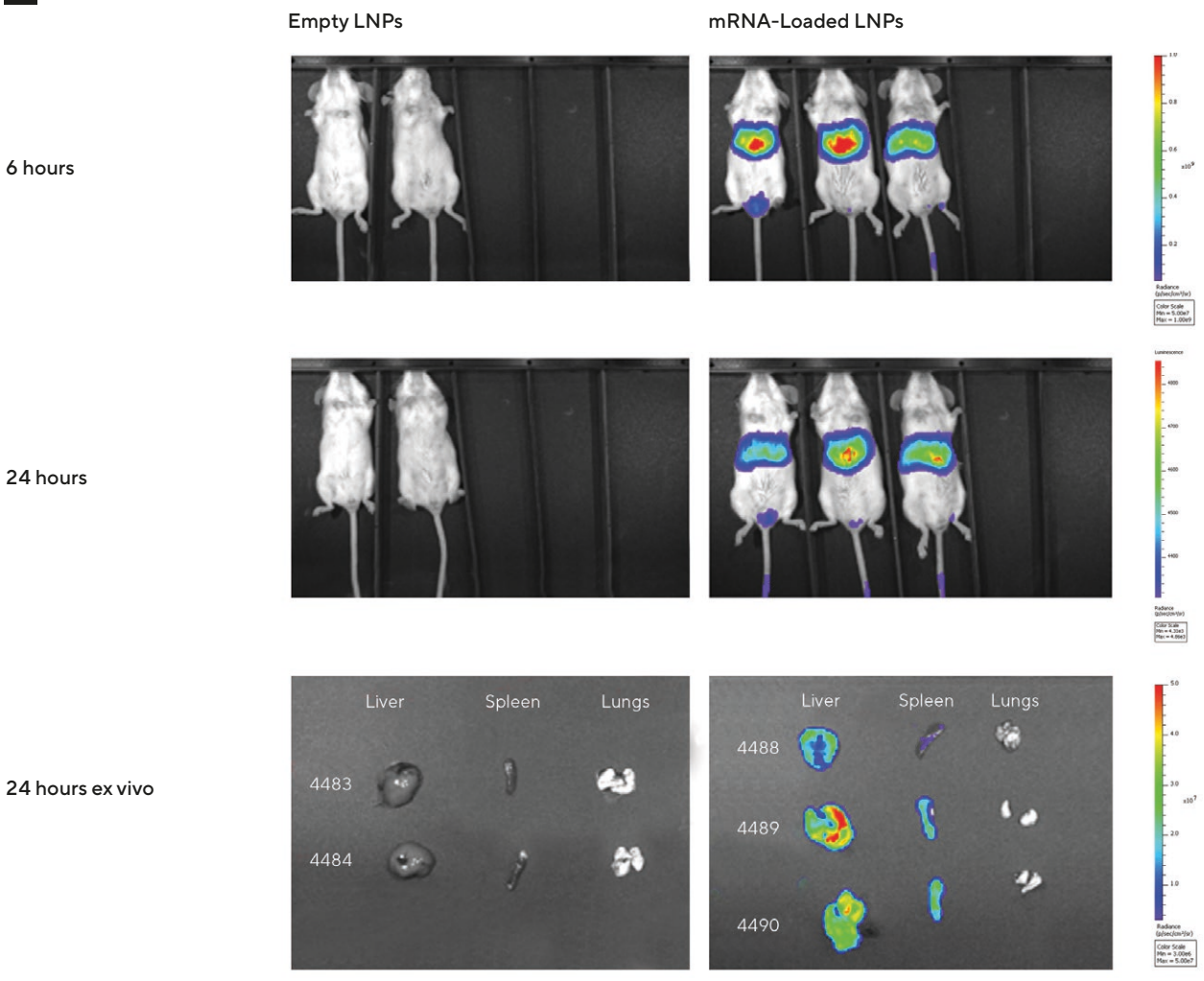


Figure 3: *In Vivo Bioluminescence Imaging* ***(A)*** *and Selected Formulation Results of Empty and mRNA-Loaded LNPs. Particle Characterization* ***(B)***

A



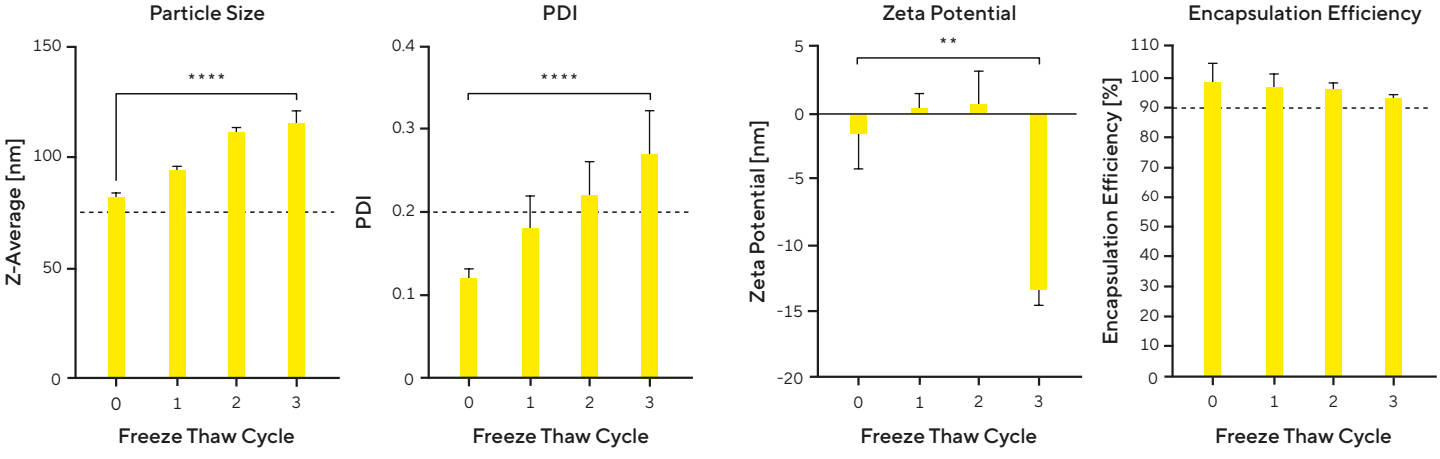
B

N/P	Z-Average [nm]	Cumulant PDI	Zeta Potential [mV]	Encapsulation Efficiency [%]	Initial (mRNA) [µg/mL]	Final (mRNA) [µg/mL]	Concentration Factor
Empty	68.7±0.2	0.10±0.02	0.9±1.1	–	–	–	–
6	82.2±0.8	0.12±0.01	-1.5±2.8	98.7±6.1	3.0	21.3±0.2	7.0±0.2

Table 4: *Summary of Phase 2 Critical Quality Attributes*

CQA	Instrument	Target Value	Actual Value (Mean ± SD, n=3)
Particle size	DLS Zetasizer Ultra Red (Malvern Panalytical Ltd.)	50 - 150 d.nm	82.2 ± 0.8
PDI	DLS Zetasizer Ultra Red (Malvern Panalytical Ltd.)	< 0.2	0.12 ± 0.01
Surface charge	ELS Zetasizer Ultra Red (Malvern Panalytical Ltd.)	± 10 mV	- 1.5 ± 2.8
Encapsulation efficiency	Fluorescence-based Qubit™ RNA High Sensitivity Assay	> 90%	98.7 ± 6.1%
mRNA integrity	RP-IP HPLC	No process- or consumable-specific degradation products. > 90% peak area intact	No process- or consumable-specific degradation. No fragmentation or secondary structure formation
mRNA potency	In vivo imaging (IVIS, Perkin Elmer)	Luciferase expression	Significantly higher signal intensity in whole body, spleen, and liver tissue compared to empty LNPs
Lipid identity and content	HPLC-CAD	Within ± 5% target molar percentage and concentration	SM-102: 50.7 ± 0.4% DSPC: 10.4 ± 0.7% Cholesterol: 37.5 ± 0.4% DMG-PEG2000: 1.5

Figure 4: *Phase 3: Stability of Nanoparticle Formulation After Freeze-Thaw Cycles*



Note. Analysis was performed in triplicate. Values represent the mean. Error bars represent the standard deviation. Means are significantly different when $p < 0.05$ by Tukey's multiple comparison test.

Discussion

Scalability and Process Consistency
The integration of DIANT® PILOT technology with Sartorius SIMCA®-online software and single-use consumables validated a highly controlled, scalable platform for continuous mRNA-LNP production. The system consistently produced LNPs that met CQAs identified with SIMCA®, including particle size, PDI, and encapsulation efficiency, while the SIMCA®-online software monitored, predicted, and controlled process parameters with high reproducibility. The closed, single-pass configuration minimized dead volumes and reduced bioburden risk, offering significant advantages over traditional batch processes.

Efficacy and Performance of mRNA-LNPs
Mouse studies confirmed that SM-102 formulations enabled efficient mRNA transfection, with IVIS imaging demonstrating strong luminescence signals in liver and lung tissues. The high transfection efficiency observed was likely due to the ionizable lipid properties of SM-102, which facilitate endosomal escape and enhance mRNA delivery.[5,6] The significant reduction in luminescence at 24 hours post-injection suggests effective clearance and processing of mRNA-LNPs.

Stability and Integrity Across Freeze-Thaw Cycles
SM-102-based mRNA-LNPs exhibited remarkable stability during freeze-thaw studies, with minimal changes in particle size and PDI, and encapsulation efficiency remaining above 90%. mRNA integrity was preserved across three freeze—thaw cycles, underscoring the formulation's robustness for long-term storage and transport. These results suggest that mRNA-LNP formulations stored in Celsius® bags can withstand handling and storage conditions without compromising quality.

This study successfully validated the integration of Sartorius consumables with the DIANT® PILOT as a scalable platform for high-quality mRNA-LNP production. By leveraging the Umetrics® Digital Twin AI Ecosystem, the system achieved consistent encapsulation efficiency (>95%) and controlled particle size across formulations. The AI-driven insights provided by the ecosystem facilitated optimization and predictive modeling, ensuring robust performance. Mouse studies demonstrated efficient transfection, with effective mRNA delivery to the liver and lungs. Freeze-thaw stability studies confirmed the robustness of the formulations, maintaining mRNA integrity and encapsulation efficiency across multiple cycles. This validated platform, enhanced by the Umetrics® Digital Twin AI Ecosystem, offers a reliable and scalable solution for continuous therapeutic mRNA production, ensuring quality and efficiency for future clinical applications.

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Last modified: 11 | 2025