

Lipid Nanoparticle Formulation Characterization: Bridging the Gap from R&D to GMP

SERGIO ESTEBAN PÉREZ



Enabling next-generation Non-Viral Gene Therapy through custom design and end-to-end services in manufacturing of polymer and lipid-based therapeutics.

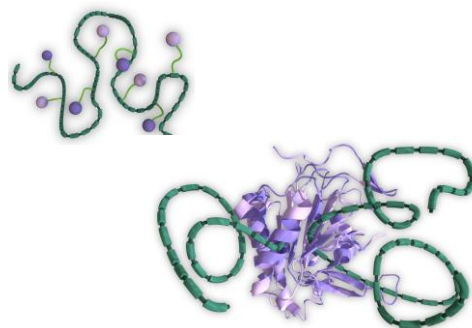
Our product categories include:



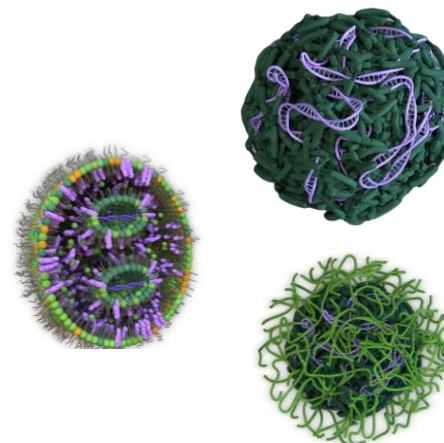
**Polymer & Lipid
Excipients**



**Chemical and
Biological
Conjugation**



**Lipid and Polymer
Nanoparticle
Formulation**



**Aseptic Fill
& Finish**

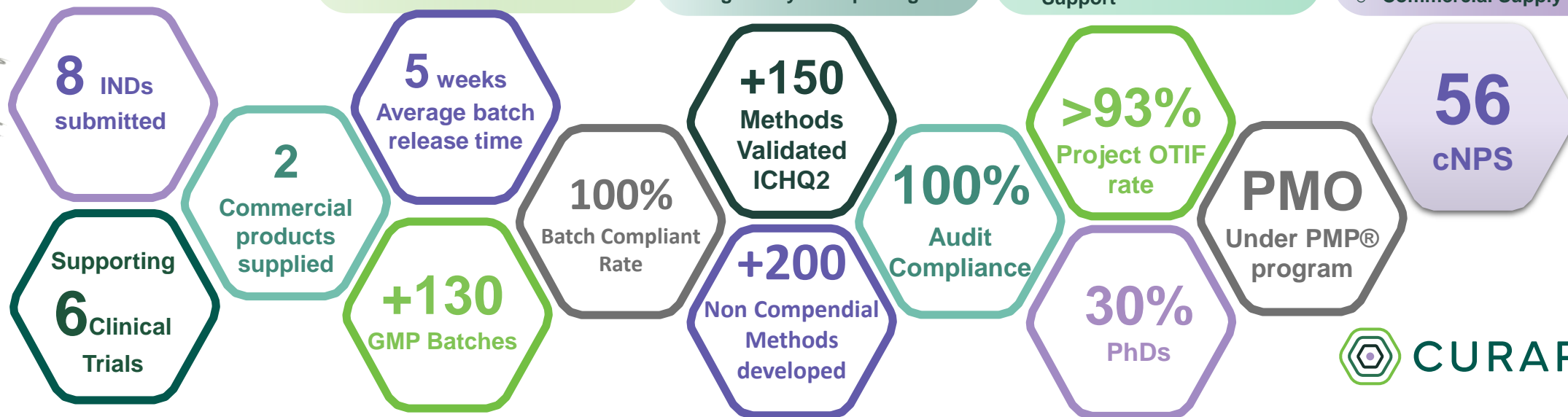


With our integrated partnership style, we support small and large clients and become a strategic partner to solve challenges in development and accelerate your speed to the clinic.



We smoothly navigate your therapeutic product throughout the clinical stages

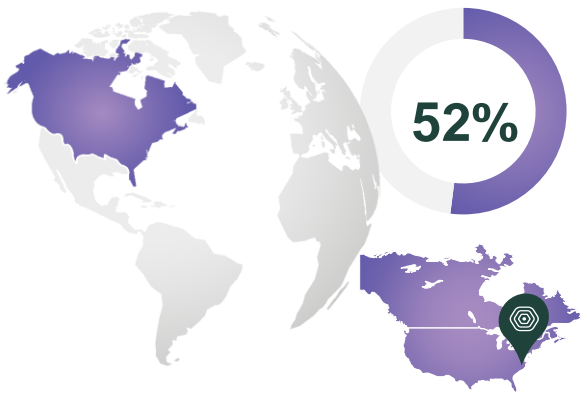
RA Based & Phase Appropriate CMC Development for Novel Excipients & Nanoparticle Drug Products



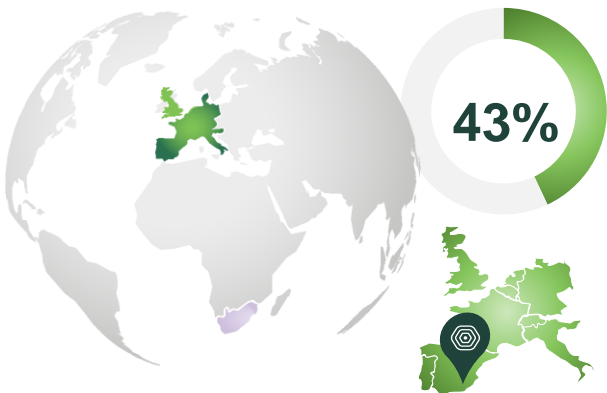
Collaborating with pharma and biotech companies worldwide



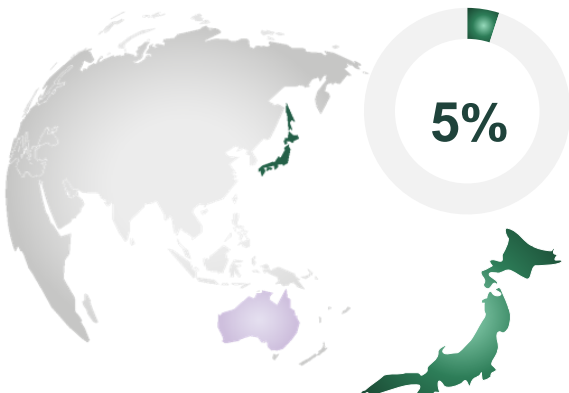
North America



Europe



Japan



Product Type %

PNP	51%
LNP	29%
Polymer Conjugate	17%
Polymer Drug	3%

Payload %

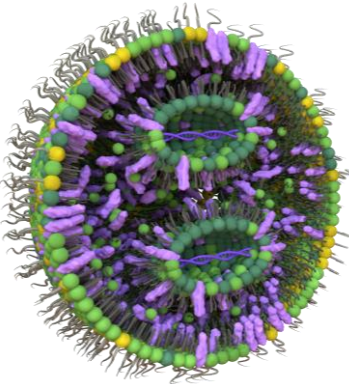
Nucleic Acid	35%
Small molecule	28%
Other	25%
Biologic	12%






Candidate Stage %

Discovery-preclinical	74%
Clinical Stage	12%
Pivotal Stage	8%
Commercial	6%



Beyond PEGylated lipids: Non-immunogenic shielding lipids in vaccines

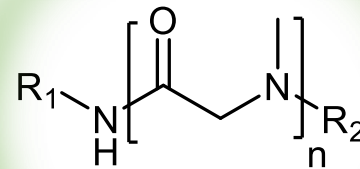


 Ionizable Lipid (<i>neutral/protonated</i>) Aids in the encapsulation of nucleic acids through electrostatic interactions	 Cholesterol Decreases permeability of the LNP and enhances its stability	 Nucleic Acid (<i>e.g. mRNA</i>) Encodes protein of interest
 'Helper' Lipid Improves LNP stability and fusogenicity	 PEG-Lipid Prevents non-specific protein absorption, particle aggregation and controls LNP size	PEG Alternatives PSar, Biocompatible, endogenous building blocks, overcome PEG immunogenicity

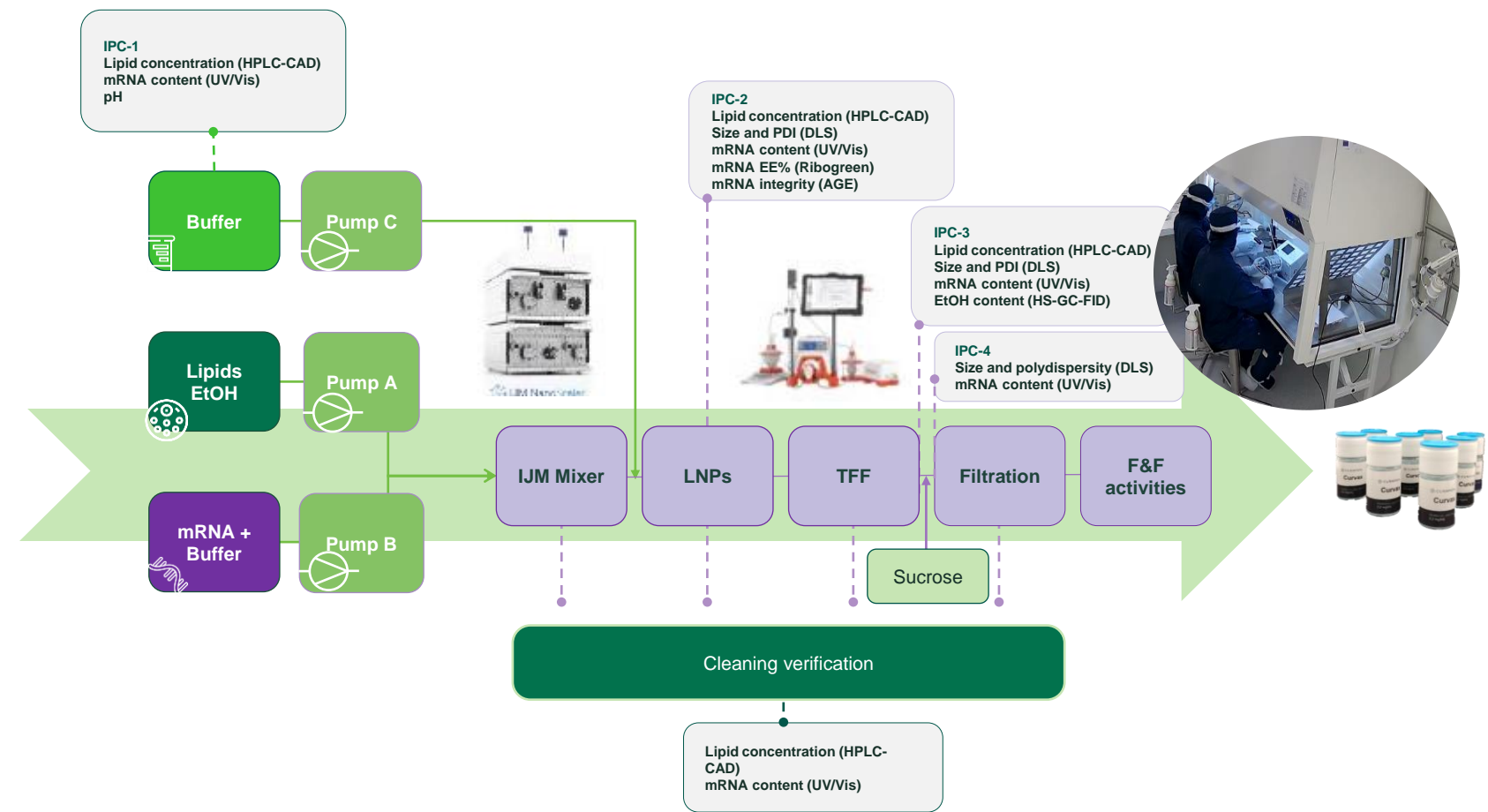
The key challenge is to obtain a scalable and process developed LNPs formulation manufacture



Psar-based Lipid



Integrating Process Development and Scale-Up

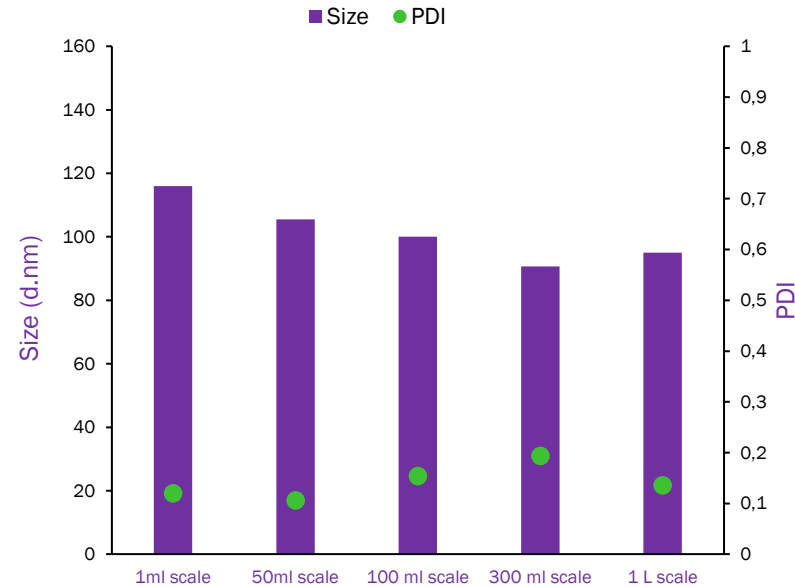


LIPID COMPONENTS	SM-102	DSPC	Cholesterol	DMG-PEG (2000)
mRNA COMPONENT	CatPure™ Firefly Luciferase mRNA			
BUFFER COMPONENT	Tromethamol hydrochloride	Acetic acid	Sodium acetate	Water for injection
CRYOPRESERVANT	Sucrose			
SCALE & API CONCENTRATION	1 L formulation 0.2mg/ml of mRNA (Luc)			
VIAL FORMAT	10R vials 6.3 mL filling volume (160 vials)			

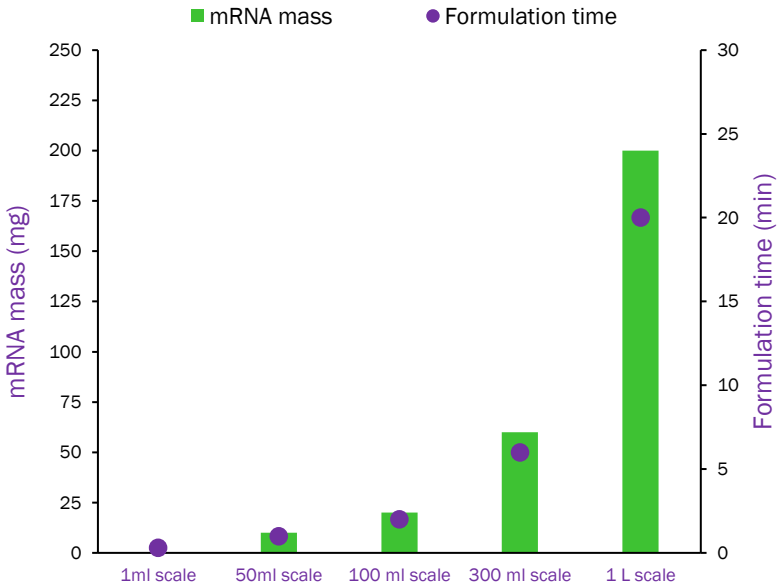


LNPs Formulation Process Development → Scale up process

Particle Size Distribution



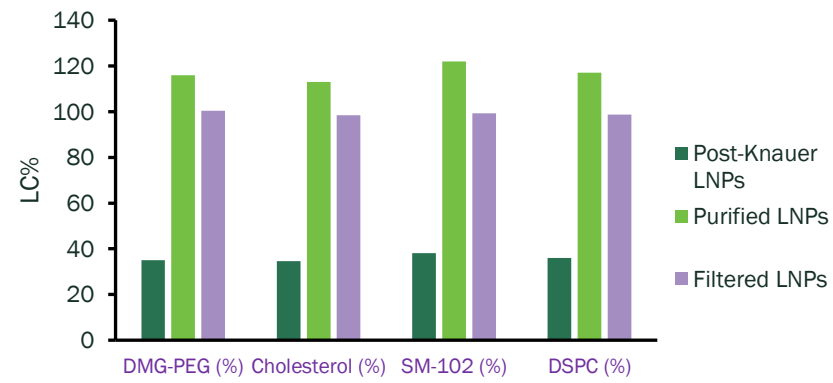
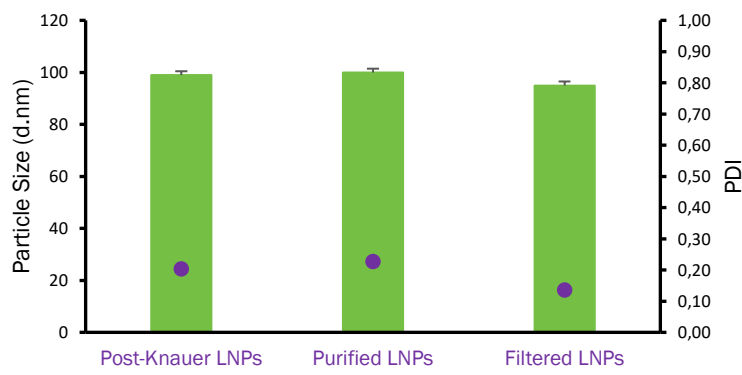
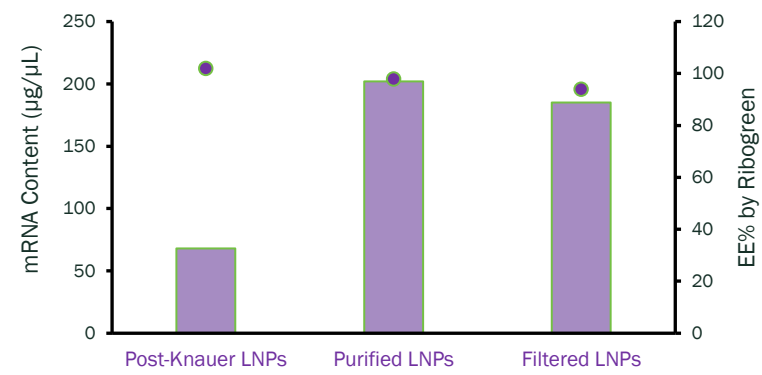
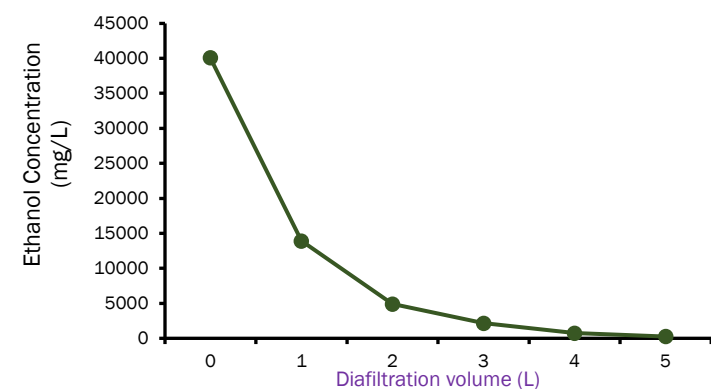
Process Parameters



During the whole process development step the Particle size distribution remained controlled, allowing a scalation of the mRNA amount employed and optimizing the formulation time. The amount of mRNA and its encapsulation efficiency was monitored as well and remained constant



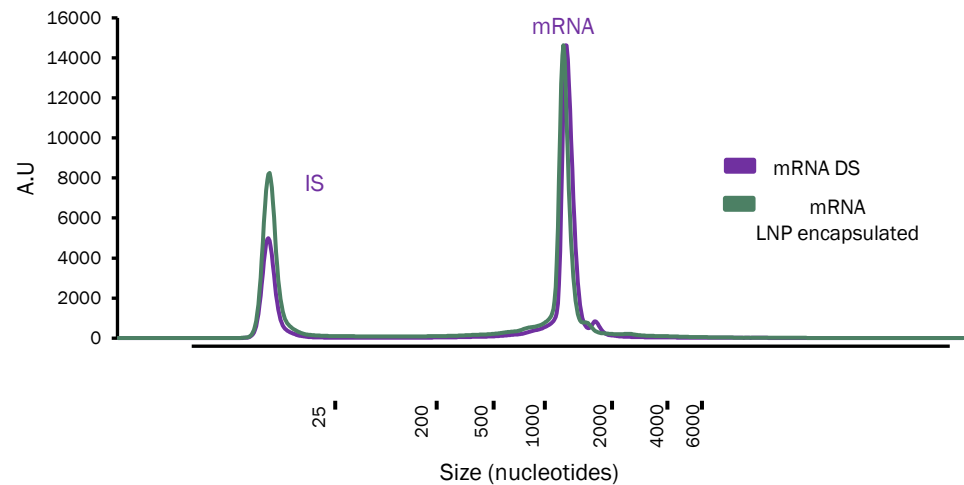
LNPs Formulation GMP Batch Preparation Critical Quality Parameters monitorization



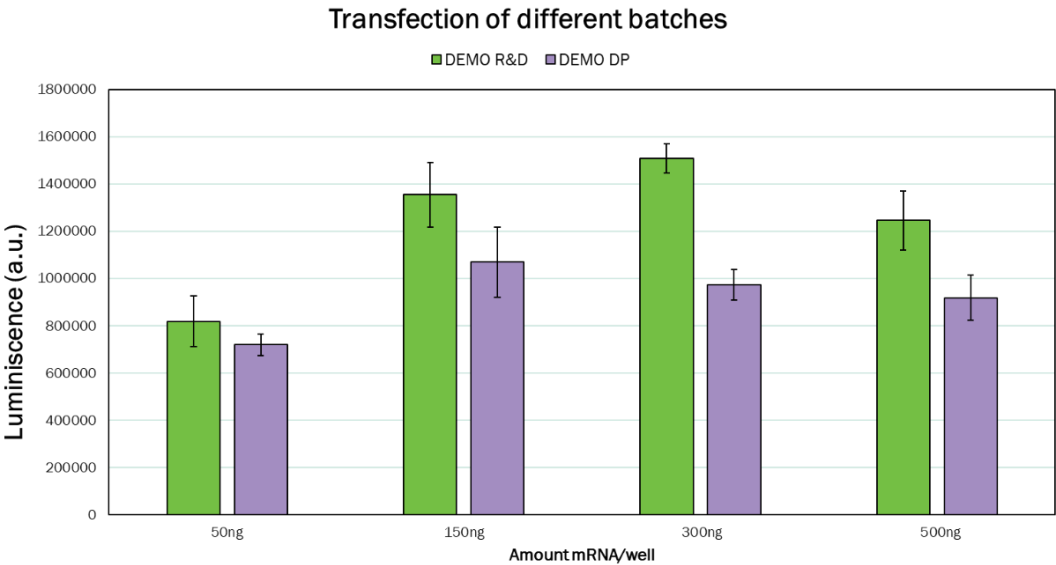
The developed LNPs maintained their CQAs throughout the process and did not suffer alteration during the concentration and purification process



LNPs Formulation GMP compared with R&D Batch *In Vitro* efficiency



The mRNA integrity was retained after the encapsulation process

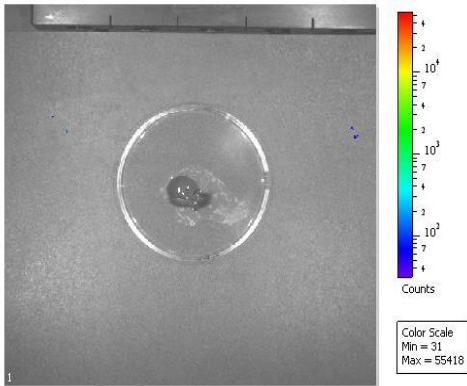


Both formulations presented the ability to transfect *in vitro*

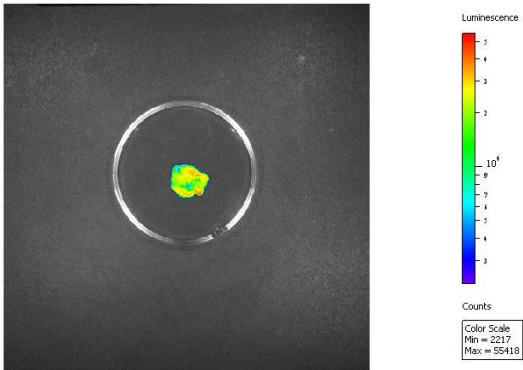


LNPs Formulation GMP compared with R&D Batch *In Vivo* efficiency

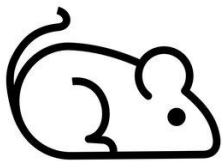
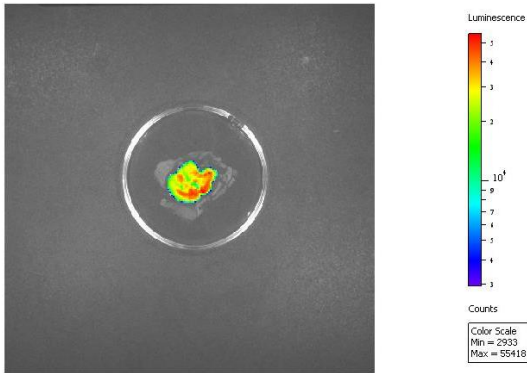
Control (PBS)



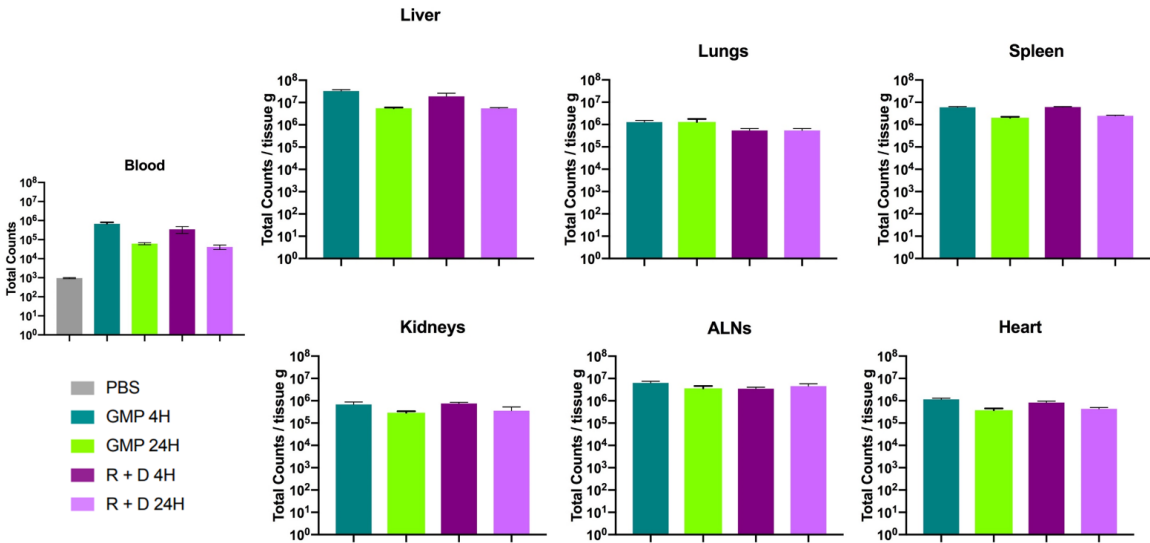
GMP LNPs



R&D LNPs



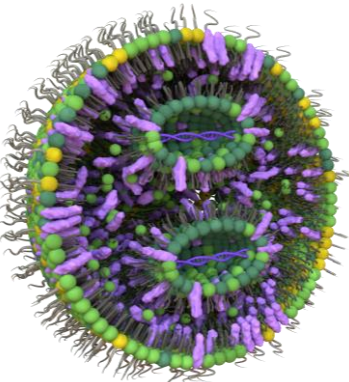
i.v.



Equivalent 5mL R&D vs 1L GMP batch *In Vivo* Expression



LNPs Formulation GMP final product Release



Curapath
Parque Tecnológico
Av Benjamin Franklin 19, 46980 Paterna, Valencia (Spain)

Certificate of Analysis

Product Name: Curvax
Product Number: 1368
Storage temperature: -25°C to -15°C
Batch: 23-006
Manufacturing date: 1/Nov/2023
Analysis date: 30/Nov/2023

Parameter	Method	Limits	Results
Appearance	Visual inspection	White to off-white dispersion	White dispersion
Visible particles	USP <790>	Not detected	Not detected
pH	Ph.Eur. 2.2.3.	7.0-8.0	7.4
Osmolality	Ph.Eur. 2.2.35.	280-320	300
Extractable volume	USP <698>	Conforms	Conforms
Container closure integrity	USP <1207>	Conforms	Conforms
Size (nm)	DLS	90-110	95
Polydispersity index	DLS	< 0.20	0.14
DMG-PEG content (mg/mL)	HPLC-CAD	0.14-0.22	0.18 (100 %LC)
Cholesterol content (mg/mL)	HPLC-CAD	0.56-0.84	0.69 (98 %LC)
SM-102 content (mg/mL)	HPLC-CAD	1.34-2.01	1.66 (99 %LC)
DSPC content (mg/mL)	HPLC-CAD	0.30-0.45	0.37 (99 %LC)
Total RNA content (µg/µL)	UV-Assay	0.18-0.22	0.19 (95 %LC)
RNA encapsulated (%) w/w	Fluorescence	> 70	94
Total unknown impurities (%) w/w	HPLC-CAD	< 10.0	2.4
Residual ethanol (mg/L)	GC-FID	< 5000	< 5000
In vitro expression	Assay	>750000 RLU	Conforms
Endotoxin content	Ph.Eur. 2.6.14.	<100 EU/ml	Conforms
Sterility	USP <71>	Conforms	Conforms

Date and Place: 30/NOV/2023, Paterna (Valencia)

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- The monitorization of the CQAs led to the obtention of a scalable LNPs manufacturing process
- The CQAs has remained unaltered during the whole process development stage
- The R&D and GMP formulation has provided similar performance *in vitro* and *in vivo*


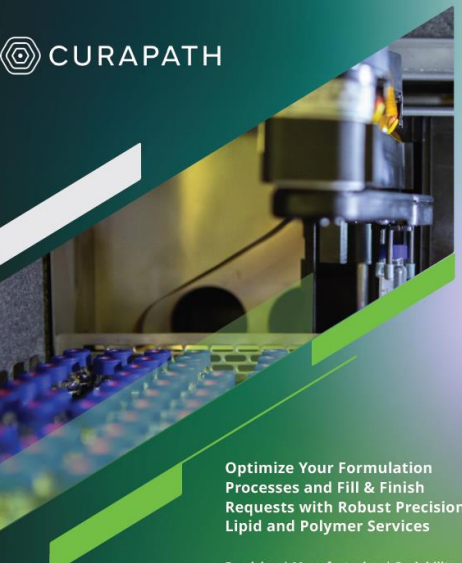


Annual Meeting
AND Exposition
JULY 8-12, 2024 • BOLOGNA, ITALY

INTEGRATING
Delivery Science
ACROSS DISCIPLINES




Brochures, Product Catalogue, Case Studies, Product Highlights, App Notes, Posters.....

Optimize Your Formulation Processes and Fill & Finish Requests with Robust Precision Lipid and Polymer Services

Precision | Manufacturing | Scalability

White Paper



Polysarcosine (pSar)—a safer, more effective alternative to poly-ethylene glycol (PEG)



ABSTRACT

Conjugation of polyethylene glycol (PEG) to therapeutic molecules can increase drug half-life, solubility, and therapeutic potency. However, an increasing number of healthy individuals develop anti-PEG antibodies. PEG immunogenicity can cause anaphylactic shock and dramatically reduce the efficacy of the treatment. Here we describe a potential solution to the problem. Polyaminoacid-based polymers such as polysarcosine (pSar), provide a non-immunogenic alternative to PEG. Non-toxic, biodegradable polymers are not recognized by the immune system and provide equal or better solubility and therapeutic potency compared to PEG. Polysarcosine, along with other polyaminoacid-based delivery systems, have been successfully developed & manufactured at Curapath cGMP manufacturing facility in Valencia, Spain.

Introduction

Polyethylene glycol is a hydrophilic polymer that has been frequently used in everyday products, including paints, cosmetics, food, and medicine. The PEG market reached 4.15 billion dollars in 2019 and is expected to grow at a CAGR of 10.8% from 2020 to 2026, primarily driven by strong demand from the pharmaceutical industry (<https://www.emisinsights.com/industry-analysis/polyethylene-egcol-market>). In the pharmaceutical industry, polymers are widely used as a gold standard of bioconjugation to protect biologic circulation in the blood, increase solubility and improve potency. Many PEGylated products, including peptides, proteins, small interfering RNAs, and even small molecules, were successfully tested in clinical trials over the past two decades. Sales of the two most successful products, Pegasys and Neulasta, exceeded \$5 billion in 2011 (1, 2). PEG polymer use grew steadily in the pharmaceutical, cosmeceutical, and food industries making it one of the most abundantly manufactured polymers. With the successes observed for lipid-based nanoparticle delivery systems, it was not surprising that the two leading vaccine-producing companies, Moderna and BioNTech/Pfizer, incorporated PEGylated lipids as part of the mRNA delivery of nanoparticles, in their race to stop COVID-19 pandemic. Both companies went on to manufacture and use hundreds of millions of doses to eradicate the coronavirus pandemic that claimed over 6 million human lives globally. That dramatic increase in the polymer use exposed a critical problem: PEG is immunogenic and should not be used for individuals with severe allergic reactions (3,4,5,6).

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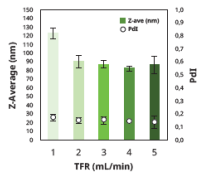
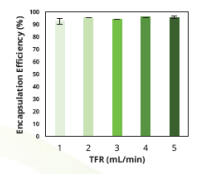
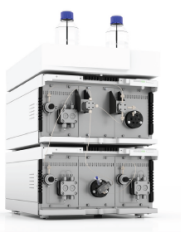
LNP Formulation and Analytical Services

Lipid nanoparticles (LNPs) are biocompatible, biodegradable, non-viral vectors that show high encapsulation efficiency. LNPs are used to deliver nucleic acids in clinical applications. Most notably, LNPs are being used as mRNA-delivery vehicles in the SARS-CoV-2 vaccines developed by BioNTech/Pfizer and Moderna.

The manufacture of mRNA-LNPs is a complex process in which the micromixer plays the critical role of mixing the organic phase containing lipids and the aqueous phase containing the nucleic acids. Tightly controlling the micromixer's operating parameters allows the system to produce mRNA-LNPs of reproducibly defined physical and functional properties.

Using the IJM NanoScaler, we have optimized key operating parameters, such as total flow rate (TFR).

As part of our end-to-end services, we at Curapath develop and optimize LNP formulations during pre-clinical R&D.







The NanoScaler, KNAUER's new benchtop Impingement Jets Mixing (IJM) micromixer, is designed for low sample consumption to minimize waste, and can be used to produce from one to hundreds of milliliters of lipid-encapsulated nucleotides.

Encapsulation efficiency is greater than 90% across all conditions tested.

With the NanoScaler, we are able to formulate batches 50 times larger than R&D batches, obtaining mRNA-LNPs of similarly adequate characteristics. Pre-clinical studies can therefore be performed with the use of the NanoScaler, as several hundred mL of LNPs can be produced in a few hours.

Case Study

Development of a Highly Efficient, Biodegradable, Polymeric, Non-viral Vector (NVV) Platform for Nucleic Acid Delivery

Emerging Biotechnology

Drug Candidate and Development Status

An emerging biotechnology company sought help in advancing novel nucleic-acid constructs to the market via an orphan drug designation pathway. This client approached Curapath to design and develop a suitable vehicle based on polymeric nanoparticles to bring its technology from R&D to the clinic as rapidly as possible. Moreover, the customer intention was to pave the way towards developing a novel polymeric platform for treating other genetic diseases in the future.

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... and more to come

Booth #61



GMP production site



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46980 Paterna, Valencia

Innovation & Development site



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46980 Paterna, Valencia

Commercial Offices



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