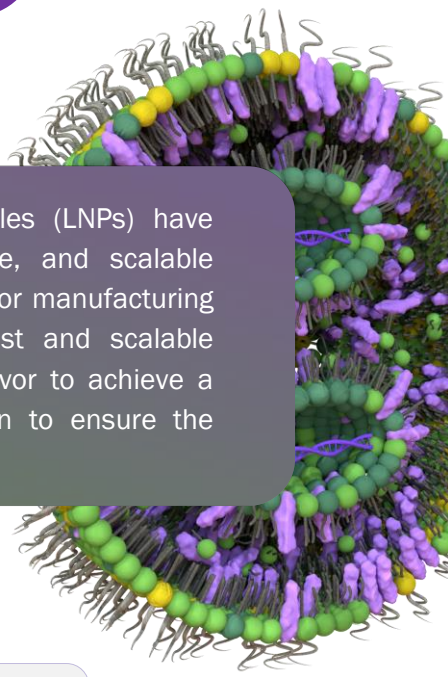
FORMULATION PROCESS APPLICATION NOTE

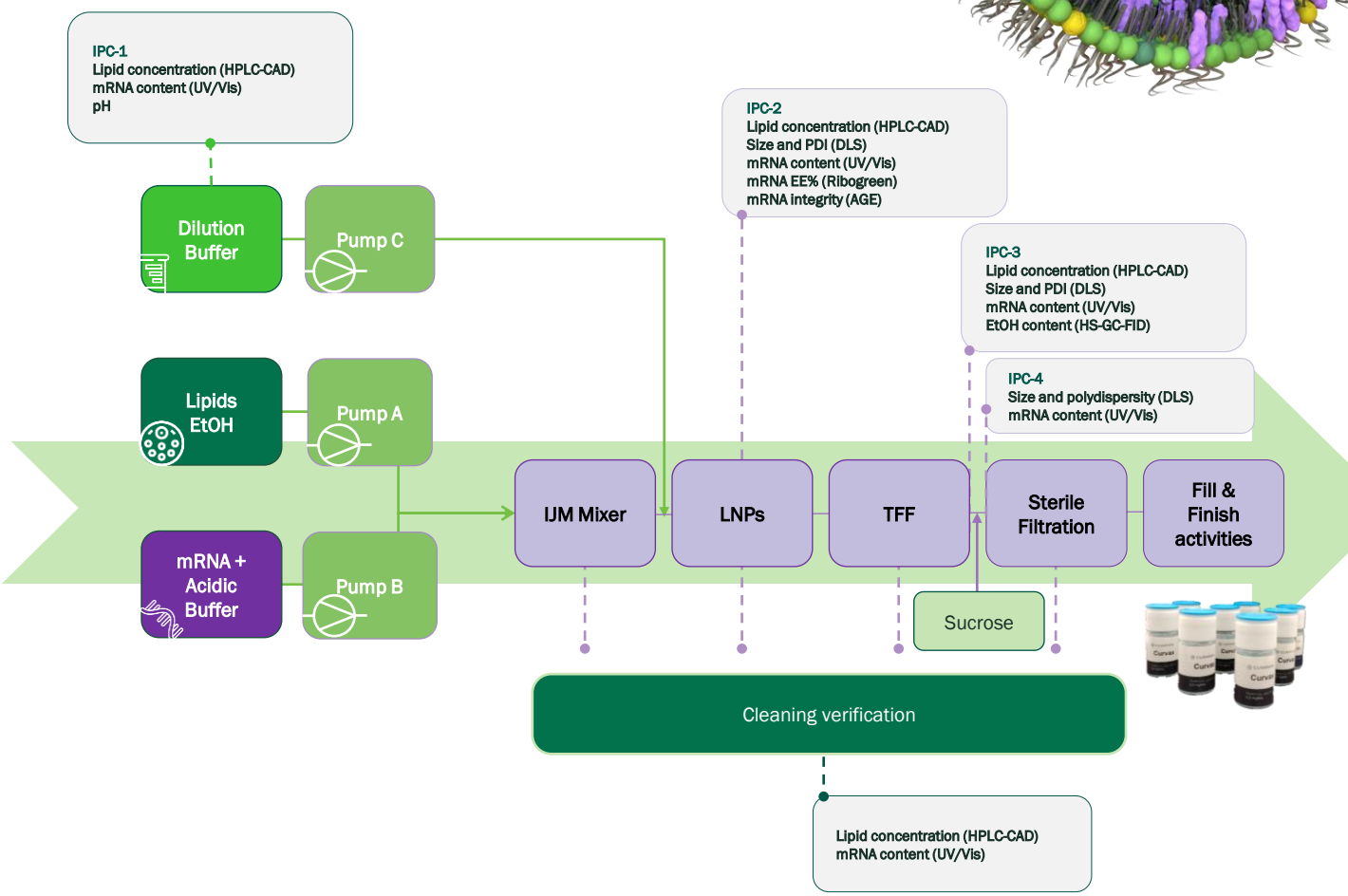
Unlocking the Potential of Lipid Nanoparticles: A Journey Towards GMP-Compliant Formulation Development

CURAPATH



ABSTRACT

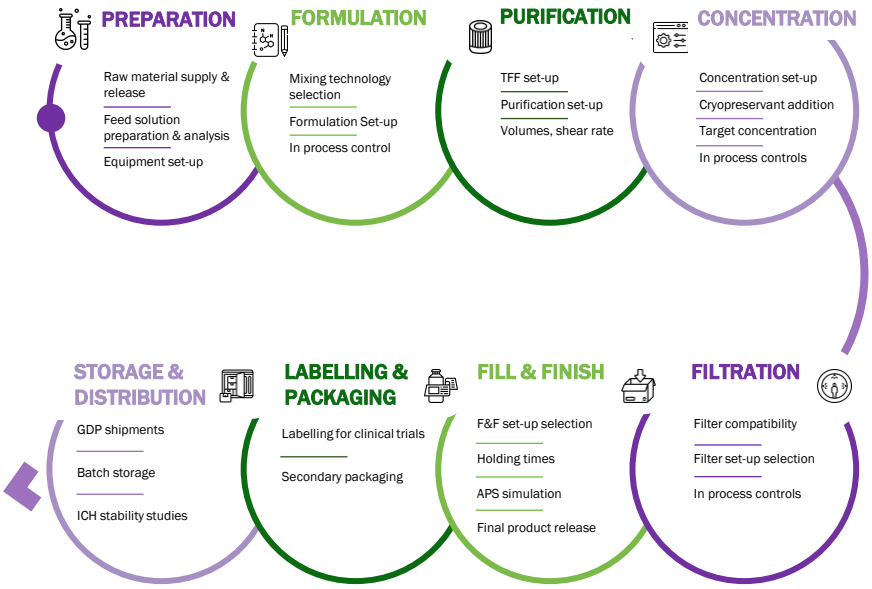
In the current landscape of mRNA-based therapies, lipid nanoparticles (LNPs) have emerged as the foremost delivery system. Their rapid, reproducible, and scalable manufacturing capabilities are unparalleled. Yet, the sensitivity of LNPs for manufacturing and purification processes necessitates the development of a robust and scalable approach. This application note provides insight into Curapath's endeavor to achieve a GMP-compliant LNP formulation, demonstrating the critical path taken to ensure the production of clinically accepted drug products.



The remarkable achievements of the SARS-CoV-2 vaccines, BNT162b2 ("Comirnaty" by BioNTech/Pfizer) and mRNA-1273 ("Spikevax" by Moderna), have spurred pharmaceutical companies to emphasize the utilization of LNPs for delivering nucleic acids. The manufacture of LNPs is a complex process that must be robust, scalable, and reproducible, in which the mastering of each operation unit plays a critical role.

Lipid Nanoparticle Production: Curapath's Journey from Lab to Large-Scale Manufacturing

As illustrated above (Scheme 1), the production of LNPs unfolds in four distinct steps: Microfluidics Formulation, Tangential Flow Filtration (TFF) Purification, Sterile Filtration, and Fill & Finish Activities. Navigating the transition from research to clinical production is a pivotal phase in any pharmaceutical endeavor.



Scheme 1. Operative units for LNP clinical batch.

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)			

Table 1. LNP Batch composition & basic definition.

Figure 1. Lipid Nanoparticle structure and finished Drug Product formulation.

Illustrated in Figure 2 below is the streamlined trajectory adopted at Curapath, scaling up from 1 ml to 1L GMP batches. Throughout this journey, Knauer's NanoScaler, leveraging Impingement Jets Mixing (IJM) technology, served as the cornerstone for formulating all batches. This system exhibited remarkable proficiency in generating LNPs of optimal size and Polydispersity Index (PDI) between 80 and 120 nm, with high reproducibility, during the Process Development stage, allowing the creation of a robust protocol for the following GMP tech transfer. Notably, KNAUER's IJM technology has demonstrated its efficacy in formulating LNPs for large-scale production of SARS-CoV-2 vaccines, earning it the prestigious accolade of a Pandemic Proven formulation platform.

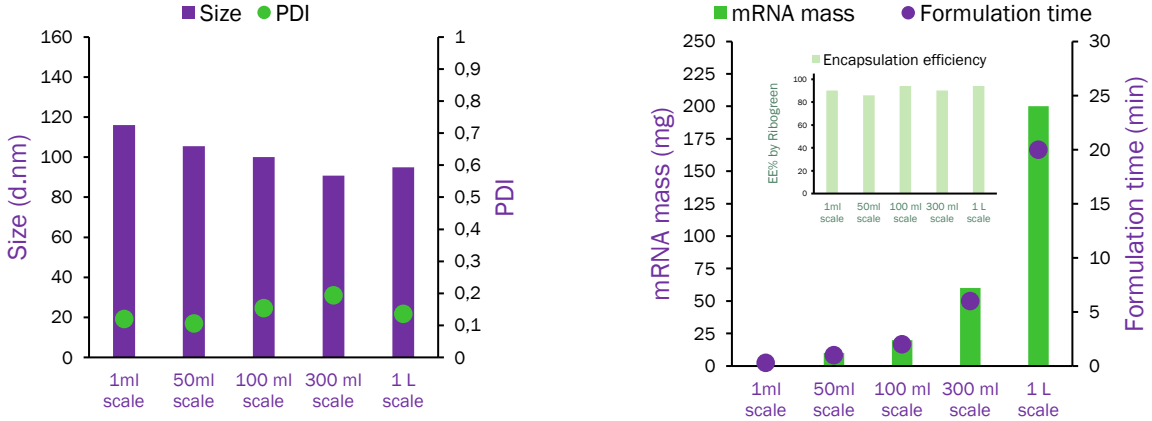


Figure 2. A) Hydrodynamic diameter and PDI of LNPs formulated during scale up to 1L GMP batch scale. B) Batch size and formulation time. Inset: Encapsulation efficiency (EE%) of LNP batches during scale up process up to 1L GMP batch scale.

Streamlining LNP Purification: the Versatile TFF Solution

In the purification process of LNPs, TFF cassettes play a crucial role. TFF facilitates the efficient separation of LNPs from impurities and ethanol, ensuring high purity and quality of the final product. The cassettes used in this process had a 100KDa MWCO and offered advanced filtration capabilities, enabling precise control and optimization of purification parameters, final formulation volume and target API concentration.

The required diafiltration volume was determined to be 5-fold for ethanol removal. Furthermore, TFF enables uninterrupted processing, minimizing downtime and enhancing efficiency. Its adaptable nature makes it suitable for both small-scale development and large-scale manufacturing, providing flexibility in LNP purification methodologies.

Efficient Sterilization & Precision Filling: Curapath's LNPs Formulation Expertise

After the addition of sucrose as a cryopreservative, our LNPs formulation undergoes rigorous sterile filtration in our advanced DP GMP facilities. Equipped with double sterilizing filters of 0.20 µm, our sterilizing filtration transfer set ensures unparalleled sterility, purity, and quality. By integrating customized sterile single use fluid paths into the aseptic filling equipment, we guarantee precise control and optimization at every step. The sterilized product is then transferred to a sterile single-use filling kit and filled into 10 mL glass vials within a Grade A laminar flow cabinet under aseptic conditions.

CURAPATH

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

Certificate of Analysis

Product Name: Curvax
Product Number: 1368
Storage temperature: -25ºC to -15ºC
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)

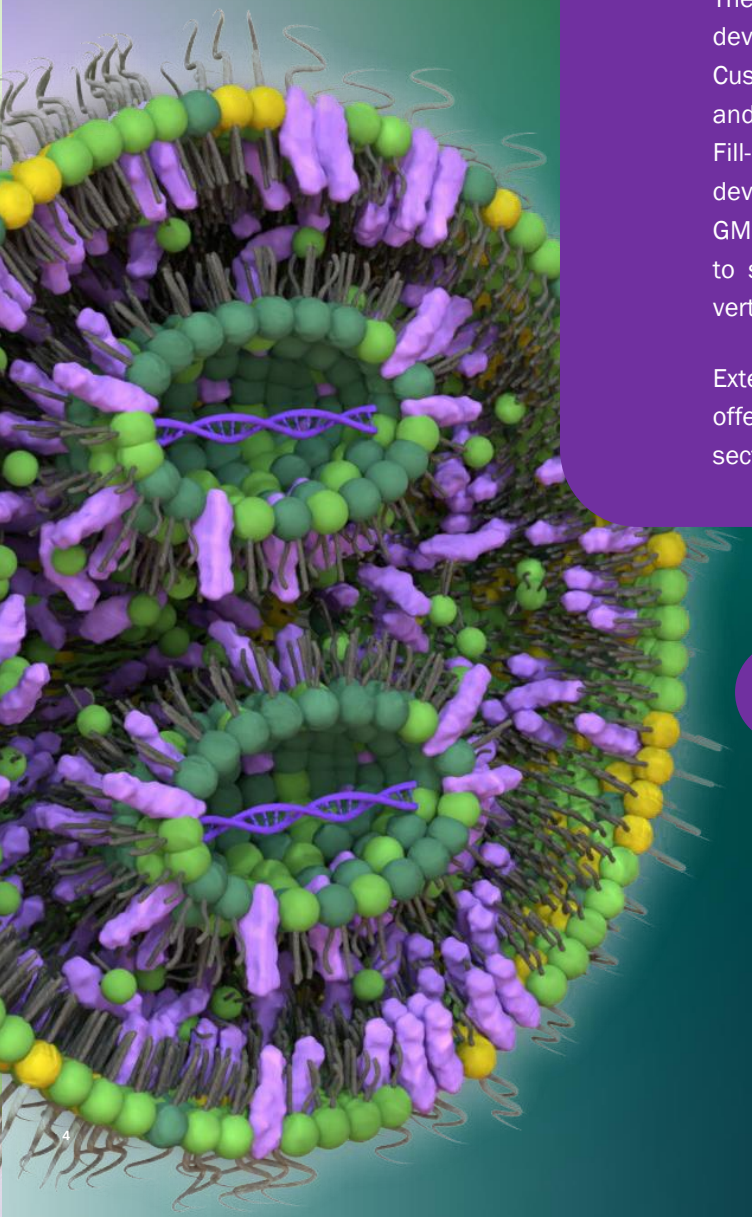
Curapath warrants that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. Purchaser must determine the suitability of the product for its particular use. Product can only be used for research purposes.

Finally, at **Curapath**, we have strong expertise on requirements for labeling Drug Products for **clinical trials, adapting** to **customer needs.** Our commitment extends to offering personalized regulatory support and labeling services, specifically designed to meet individual needs, including provisions for blinded clinical trials.

CURAPATH

For pricing and availability or custom synthesis requests, please inquire at:

ORDER | info@curapath.com



At Curapath we support your Non-Viral Gene Therapy program from preclinical through clinical development to commercial authorization. Specialized in Custom Synthesis of Lipid and Polymer excipients, Lipid and Polymer Nanoparticle Drug Product formulation and Fill-Finish, we provide seamless Process and Test Method development, characterization and scale-up services to GMP supply of Clinical Trial, Process and Method Validation to support your Commercial launch through a seamless vertically integrated manufacturing approach.

Extended details to supplementary services and products offered by Curapath are available in the ***News & Resources*** section of our website:

WWW | WWW.CURAPATH.COM

Scan to discover more