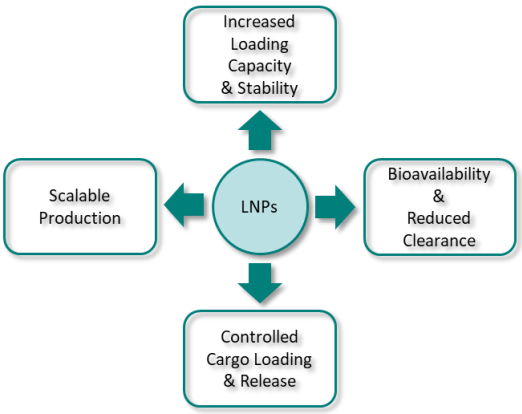
# Lipid-based Drug Product Formulation Development

## Introduction

Biocompatible and biodegradable lipid nanoparticles (LNPs) are being used for the delivery of complex cargos, including nucleic acids of largely varying sizes. LNPs have increased the drug loading capacity, long-term shelf-life and scalability in production and have the advantage to enhance bioavailability. In addition, concerns associated with the toxicity and immunogenicity of cargo vehicles such as virus components are greatly reduced when using LNPs.




Those *solid lipid nanoparticles* (SLNs), *liposomes or nanostructured lipid carriers* (NLCs) are small in size (approx. 100 nm) and exploit lipid features such as their geometry (intentional distortion, nanocompartment formation), headgroup functionality (charge induction by protonation/pH) and phase behavior (phase separation, solid phases, hexagonal phase induction, $H_{II}$) to improve the drug loading capacity and cargo binding (e.g. via use of cationic lipids) as well as the particle stability (PEGylated LNPs), and drug release performance (endosomal escape) (Fig. 1).

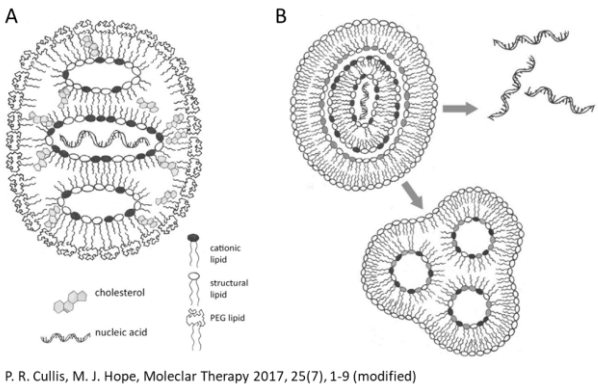



**Fig. 1: Schematic of a nucleic acid LNP system composed of functional lipid components (A) to promote endosomal release by triggered membrane fusion (B).**

Small, colloidal particles, in the range of 100-200 nm, rarely undergo reticuloendothelial blood clearance and are able to penetrate and accumulate in tumor neovasculature and tissue via the "enhanced penetration and retention effect" (EPR).

## Formulation and Processing Parameters

For industrial manufacturing, LNPs and Liposomes can be prepared by a variety of processes such as high-pressure homogenization (HPH); the most *applicable techniques* for feasibility stage development and formulation development are:

| SLNs/NLCs | Liposomes (LUV) |
|---|---|
| 1) Microemulsification (oil-in-water) → Homogenization/ Ultra-sonication<br>2) Solvent emulsification/ evaporation | 1) Multi-lamellar vesicles by evaporation/re-hydration → Large unilamellar vesicles (LUVs) by extrusion<br>2) Microfluidics processing |

Critical *formulation parameters* that determine the drug product properties are the lipid composition, lipid chain length and saturation (phase behavior, curvature) the use of rigidifying and space-filling molecules such as cholesterol, the lipid head group composition (PEGylation and ionizability, cargo interaction). *Process parameters* that determine particle size and encapsulation efficiency are i) processing temperature and pressure ii) organic solvent properties, iii) vacuum settings, iii) stirring speed/sonication amplitude and iv) extrusion pore size.

## Lipid Nanoparticle Characterization

HPLC/UPLC methods are employed to determine the concentration of lipid components in the drug product lipoplex as well as the *lipid-to-cargo ratio*. Since a chromophore is not commonly present in lipid components, an evaporative light scattering detector is often employed. *Lipid stability and degradation* such as hydrolysis and enzymatic degradation can be tracked by employing UPLC-ELSD methods, which enables simultaneous monitoring of lipid components that have high structural similarity (Fig. 2).

The cargo loading capacity of LNPs e.g. for nucleic acids can be determined using in-situ fluorescent labeling of the cargo by using *intercalative dyes*. In conjunction with a reliable *deformulation process* to assess total cargo concentration and ensuring matrix compatibility, the encapsulation efficiency and monitoring of cargo leakage are accessible.

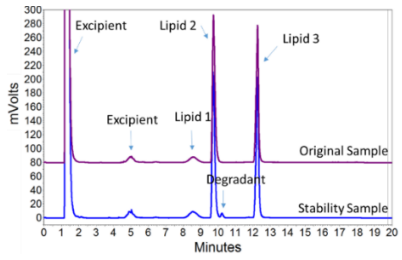



**Fig. 2: UPLC-ELSD method for lipid content and purity analysis (simultaneous analysis of LNP mixture).**

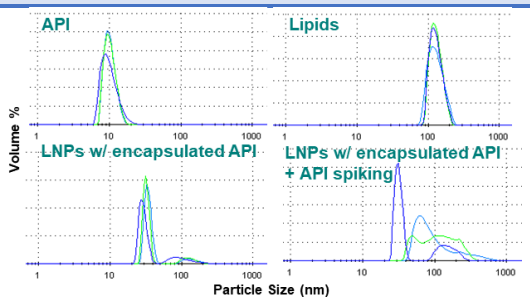
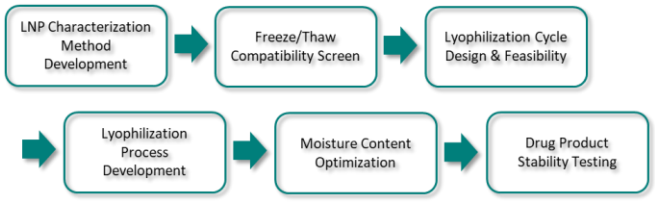



**Fig. 3: Characterization of loaded and non-loaded LNPs (liposomes) by DLS to determine: particle size and polydispersity (triplicate analysis).**

As quality control methods, and to determine *physical stability* and *LNP integrity*, light scattering techniques such as dynamic light scattering (DLS, Fig. 3) or static light scattering (SEC-MALS) can be employed to determine particle parameters and elucidate higher order structures. Aggregation propensity and particle stability can be routinely assessed via Zeta Potential determination.

## Lyophilization of Lipid Nanoparticles

To increase the stability and the shelf-life on LNP formulation, LNPs may be lyophilized. A typical lyophilization work-flow is displayed below.




In order to screen multiple formulation parameters and to limit resources and de-risk the approach, a freeze-thaw compatibility assessment can be employed to define/limit the excipient space and to evaluate applicable freezing/thawing ramp rates to ultimately inform the lyophilization cycle design. Since surface modified LNPs (e.g. PEG moieties) may exhibit slow water vapor release, the cake structure of the lyophilized product and the lyophilization cycle parameters have to be carefully evaluated and optimized during the lyophilization process development stage.

Thermal analysis and freeze-dry microscopy (Fig. 4) are utilized to determine the glass transition temperature ($T_g'$) and the collapse temperature ($T_c$) of the frozen formulation (liquid fill solution) as well as the lipid phase transition temperature ($T_m$), which facilitates data driven and rational lyophilization process development.

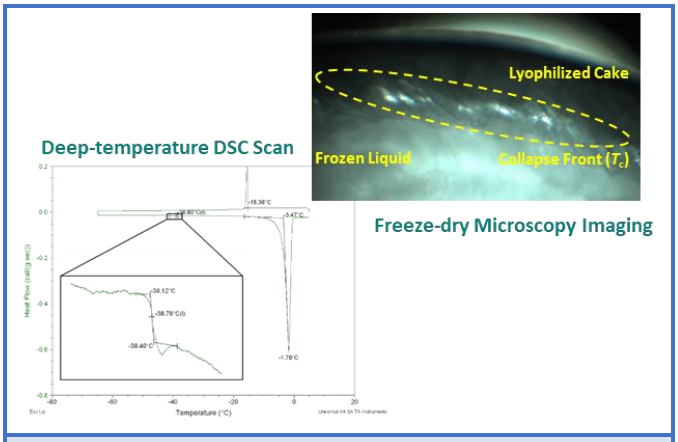



**Fig. 4: Exemplary thermal characterization (deep-temp. scan) by DSC (lower left) and freeze-dry micrograph (upper right) to inform lyophilization cycle design.**

Lyophilization cycle monitoring using drug product temperature probes as well as monitoring the water-vapor release (Pirani gauge) enable lyophilization cycle development with a minimal number of iterations (Fig. 5). To determine the secondary drying time and temperature as well as the most appropriate residual moisture content (Karl-Fischer Titration and TGA) the reconstitution times and accelerated drug product stability must be evaluated. Sampling during the secondary drying phase using a sample thief is an efficient tool to identify optimal conditions (Fig. 5).

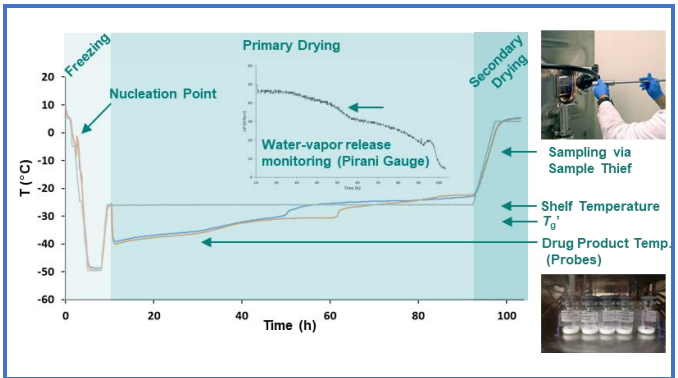



**Fig. 5: Schematic and data for a LNP lyophilization cycle with cycle parameters and drug product monitoring data (temperature probes and water-vapor release)**