

Quality-by-Design (QbD) optimization of lyophilization parameters for siRNA-loaded LNPs using sequential Design of Experiments (DoE)

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Introduction

Lipid nanoparticles (LNPs) enable nucleic-acid delivery, including small interfering RNA (siRNA), but aqueous formulations remain vulnerable to aggregation, cargo leakage and degradation, creating cold-chain constraints.

Lyophilization may improve handling and storage by reducing molecular mobility, yet freezing and drying can also destabilize LNPs. A Quality-by-Design (QbD) approach was therefore used to optimize Critical Process Parameters (CPPs), including cryoprotectant type, concentration, addition mode, freezing time and primary-drying time, while preserving key Critical Quality Attributes (CQAs) after reconstitution.

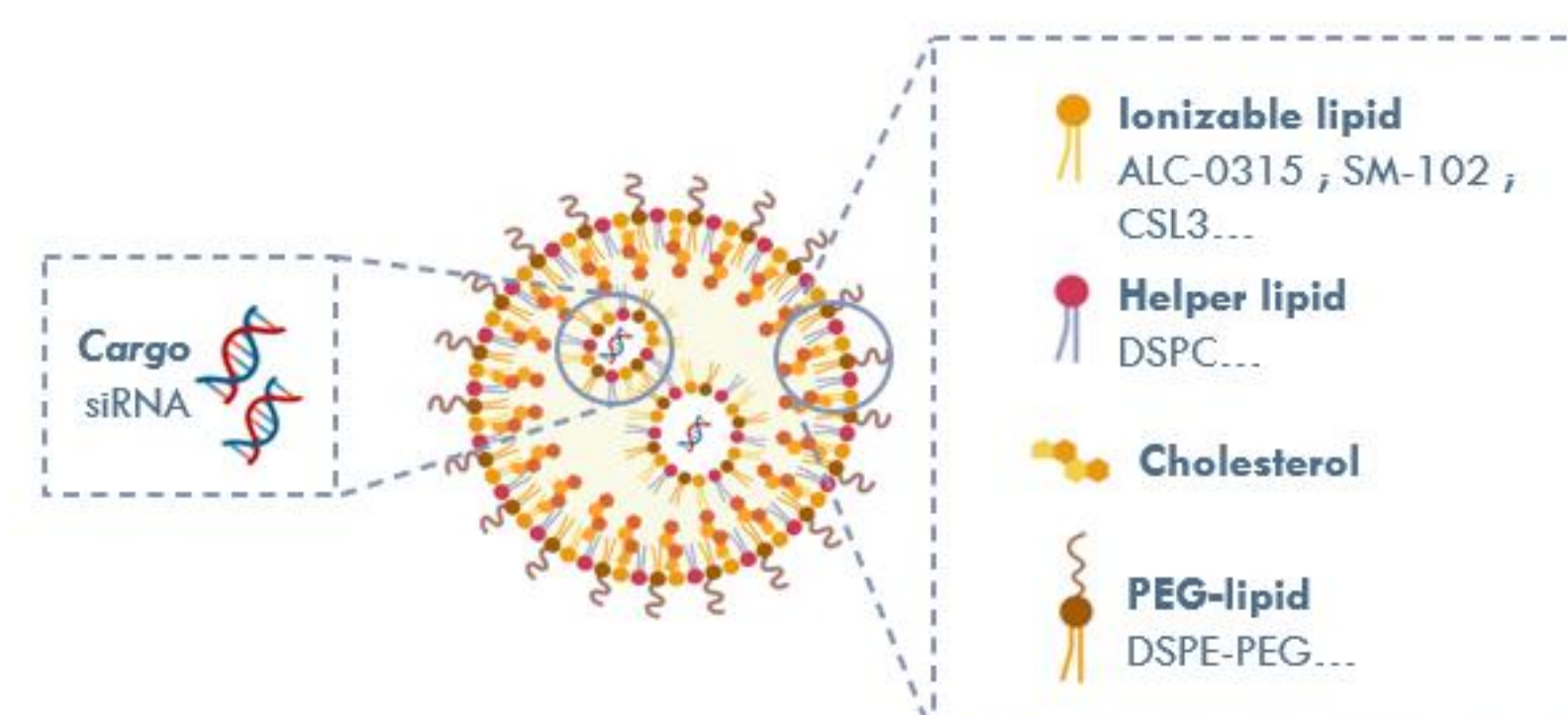


Figure 1. Schematic structure and composition of siRNA-loaded lipid nanoparticles (LNPs).

Objectives

- 1) Identify formulation and process variables that govern the preservation of siRNA-loaded LNPs during lyophilization.
- 2) Validate a QbD-derived lyophilization condition using physicochemical, morphological and functional CQAs.
- 3) Assess stability of lyophilized LNPs stored at 4 °C and –30 °C for up to 11 weeks.
- 4) Determine whether the optimized process can be transferred across different ionizable lipids and PEG-lipid anchors.

Materials and methods

I. LNP-siRNA formulation and Transferability panel

Reference formulation: CSL3/DSPE-PEG₂₀₀₀ (50:10:37.5:2.5 mol%)

Cargo: siGFP

For transferability assessment, seven siRNA-LNP formulations were compared by varying the ionizable lipid (CSL3, ALC-0315 or SM-102) and the PEG-lipid anchor (DSPE-PEG₂₀₀₀ or DMG-PEG₂₀₀₀) under the optimized lyophilization condition.

II. LNPs Production

siRNA-loaded LNPs were produced by rapid mixing using an aqueous siRNA/sucrose phase in Tris buffer (pH 7.4) or acetate buffer (pH 4.0 for ALC-0315 and SM-102 formulations), using a 3:1 aqueous-to-organic flow-rate ratio and a total flow rate of 12 mL/min, followed by overnight dialysis against Tris buffer (pH 7.4).

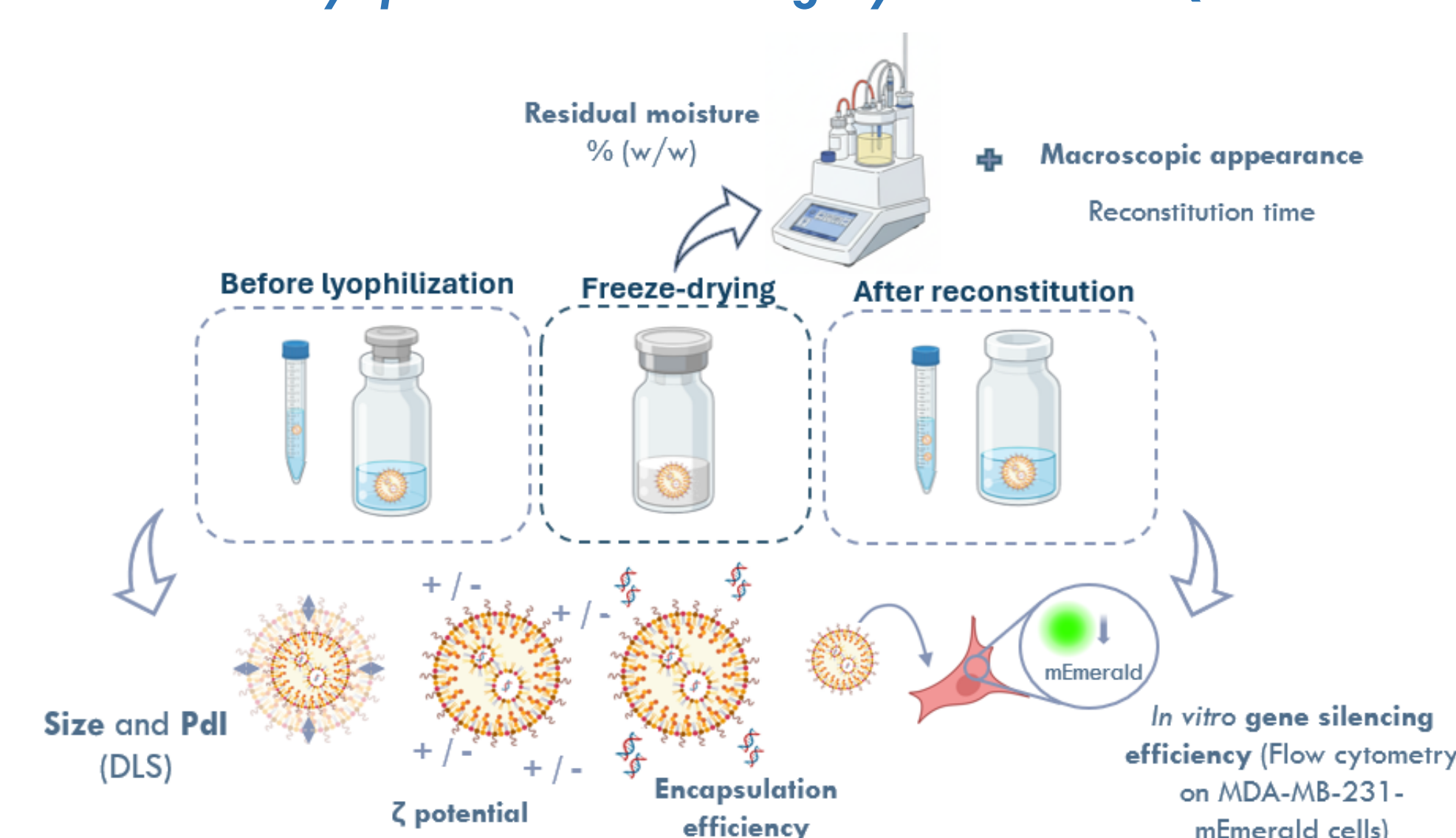
III. Quality by Design (QbD) approach

A response surface A-optimal design (JMP Pro 18, SAS) was used to evaluate the effect of sugar type (trehalose vs. sucrose), concentration (%w/v), mode of addition (internal vs. external), freezing time, and primary drying time. Based on these results, a second I-optimal design was performed with sucrose as internal cryoprotectant and an expanded concentration range.

IV. Freeze-drying process

When added externally, sucrose or trehalose were mixed at the target concentration (between 5-20%) with LNP sample prior to freeze-drying. Freezing (65-326 min) and primary drying (300-1440 min) durations were set according to the DoE.

V. Lyophilized Cake Integrity and LNP CQAs



Results and discussion

I. Quality by Design (QbD) approach

DoE-1 showed high variability, particularly with trehalose. Sucrose and internal addition better preserved LNP integrity. DoE-2 identified 20% sucrose with longer process times as optimal for CQAs; however, 17% sucrose and reduced primary drying time from 1440 to 775 min improved cake appearance (Figure 2). This condition preserved size, Pdl, siRNA encapsulation and ~90% gene silencing.

II. Stability at 4 and –30 °C over 11 weeks

The optimized CSL3/DSPE-PEG₂₀₀₀ LNPs remained stable for 7 weeks at 4 °C and 11 weeks at –30 °C (Figure 3). At 4 °C, week 11 caused cake collapse, increased size and Pdl, slower reconstitution and reduced encapsulation. In contrast, –30 °C preserved cake integrity, Pdl below 0.3, ~90% encapsulation and high gene-silencing activity.

III. Transferability of Optimal Condition

The optimized 17% sucrose condition was evaluated across LNPs containing different ionizable lipids and PEG-lipid anchors. Lyophilization caused a more pronounced increase in particle size for CSL3-based LNPs than for ALC-0315- or SM-102-based formulations, whereas PEG-lipid substitution had a limited effect. Pdl and siRNA encapsulation were preserved (Figure 4). Overall, transferability was mainly governed by ionizable-lipid identity rather than PEG-lipid structure, highlighting the formulation-dependent response of LNPs to lyophilization.

Profiler DoE-2

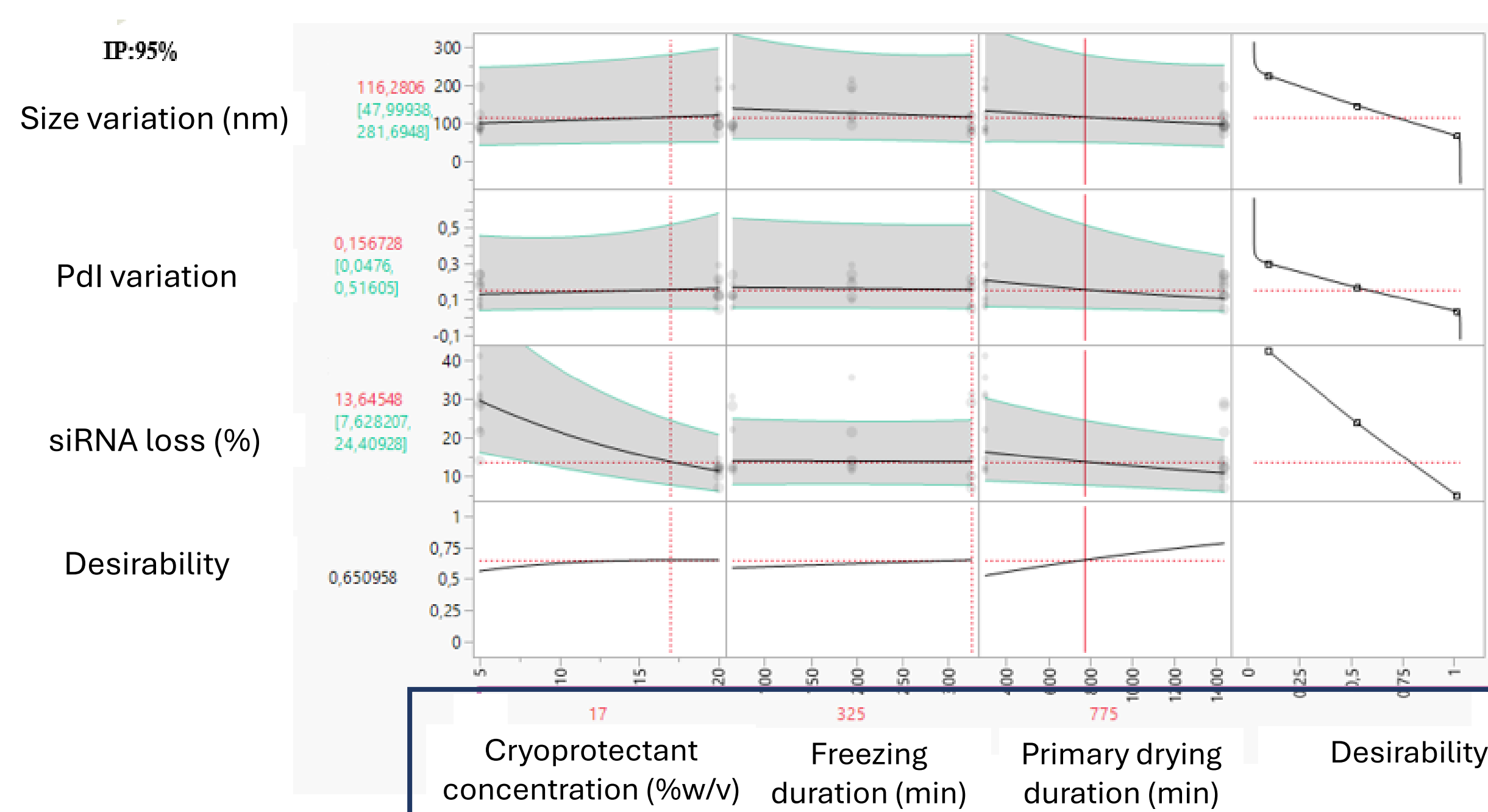


Figure 2. Profiler plot for the condition predicted as optimal by DoE. 17% sucrose condition: predicted effects on physicochemical responses. The 17% condition yielded powdery products and was selected for further validation.

Stability

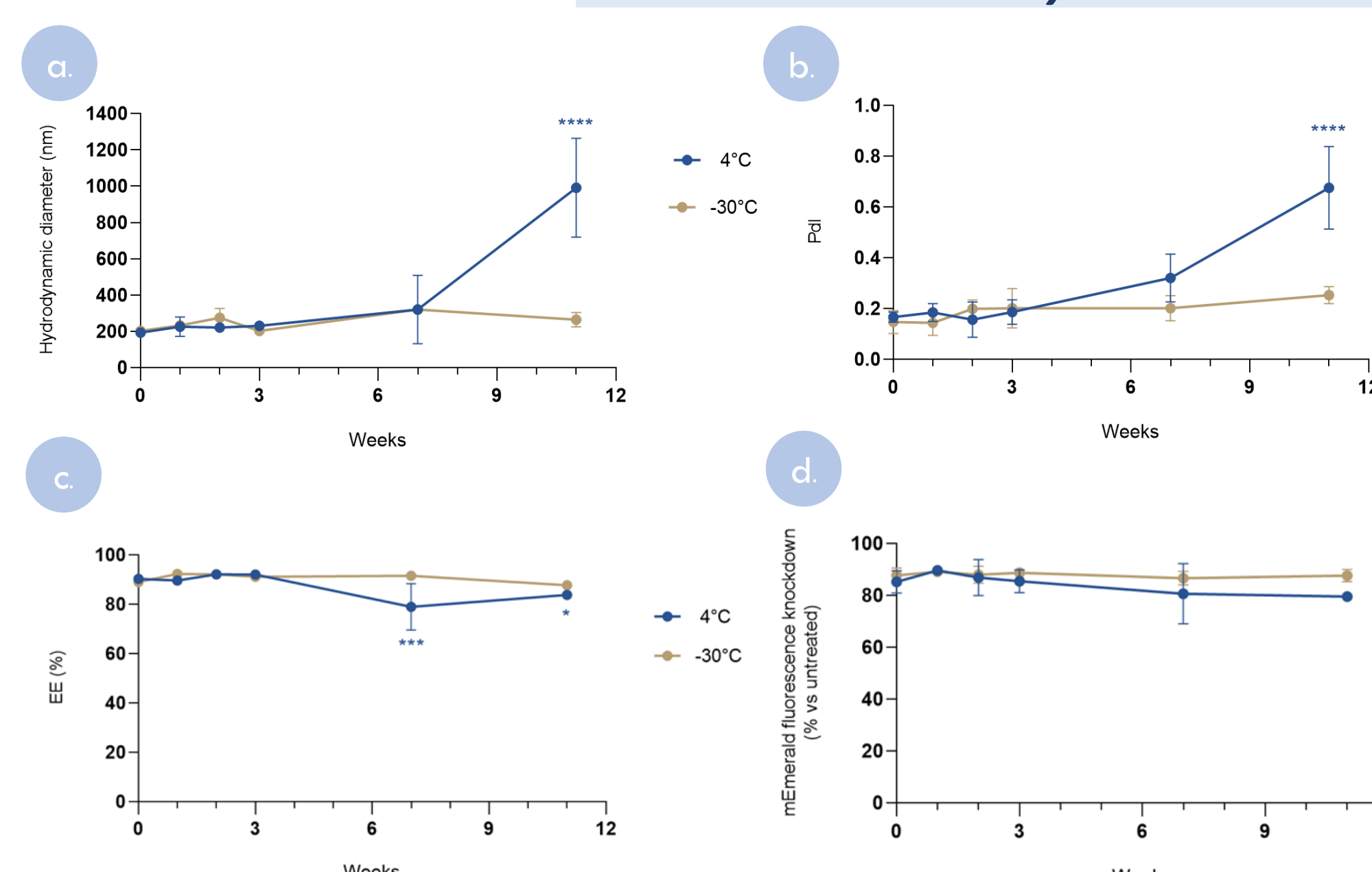


Figure 3. Stability of reconstituted siGFP-loaded CSL3/DSPE-PEG₂₀₀₀ LNPs during 11-week storage at 4 °C and –30 °C, assessed by size (a), Pdl (b), siRNA encapsulation efficiency (c) and mEmerald knockdown (d).

Process Transferability

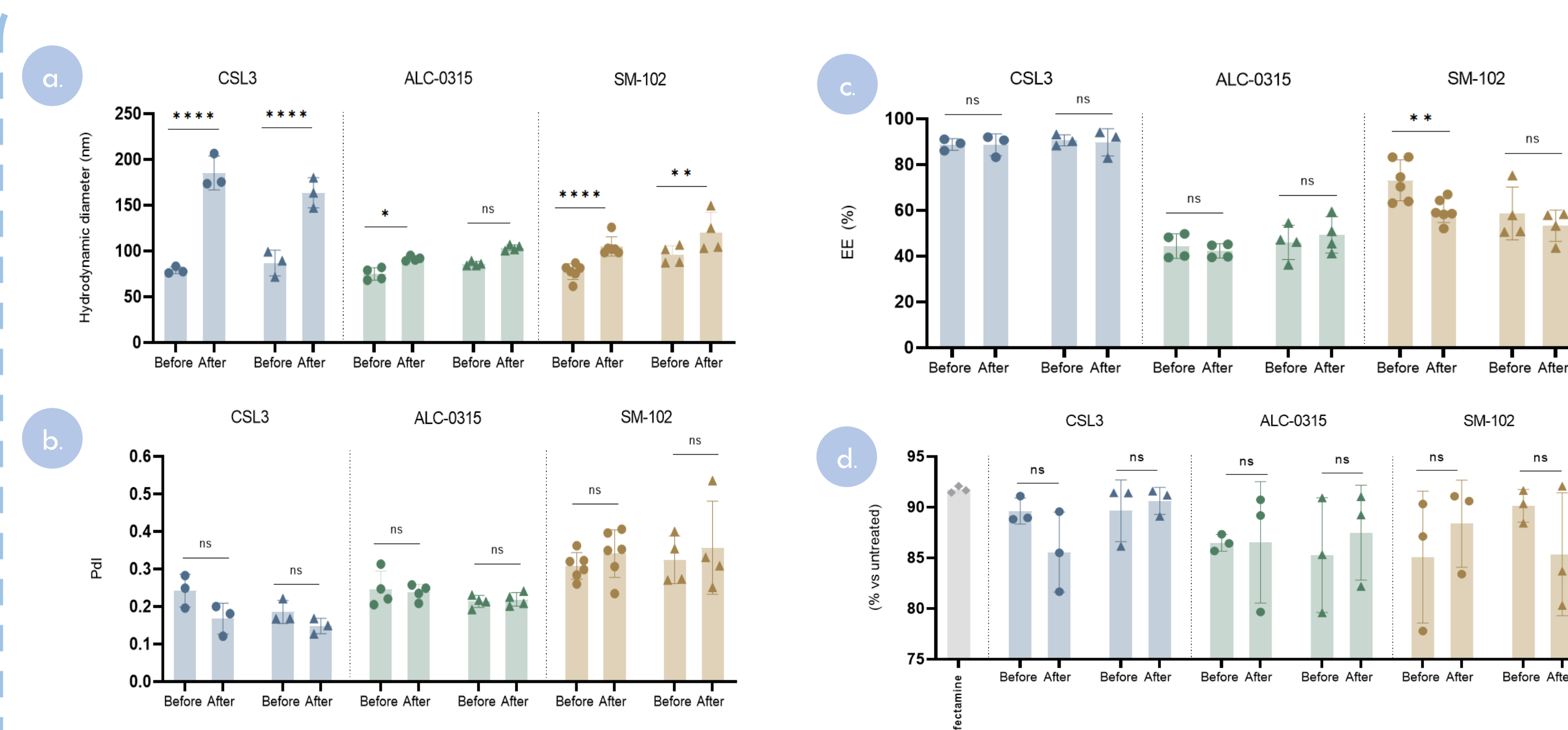


Figure 4. Characterization of LNPs before and after lyophilization. Size (a), Pdl (b), siRNA encapsulation efficiency (c) and mEmerald knockdown efficiency (d) of siGFP-loaded CSL3-, ALC-0315- and SM-102-based LNPs formulated with either DSPE-PEG₂₀₀₀ or DMG-PEG₂₀₀₀.

Conclusion

A QbD-guided strategy identified internal addition of 17% w/v sucrose, combined with 325 min freezing and 775 min primary drying, as an optimal lyophilization condition for siRNA-loaded LNPs. This condition preserved cake integrity, particle properties, siRNA encapsulation and gene-silencing activity after reconstitution. The reference CSL3/DSPE-PEG₂₀₀₀ formulation remained stable for approximately 7 weeks at 4 °C and at least 11 weeks at –30 °C. However, transferability depended mainly on ionizable-lipid identity, demonstrating that the lyophilization process must be tailored to each LNP composition.

References

This work is based on a previously published study:

Manon Degey, Laura Meloni, Berfin Dede, Inês Guerreiro, Noémie Penoy, Pierre-Yves Sacré, Manon Berger, Louise Conrard, Erika Hendrickx, Erik Maquoi, Jeanne Leblond Chain, Brigitte Evrard, Géraldine Piel, Quality-by-Design (QbD) optimization of lyophilization parameters for siRNA-loaded LNPs using sequential Design of Experiments (DoE), International Journal of Pharmaceutics, Volume 700, 2026, 127032, ISSN 0378-5173, <https://doi.org/10.1016/j.ijpharm.2026.127032>.