spINSIGHTS

from the lab

In this issue: Density gradient ultracentrifugation can be used to purify fractions of loaded lipid nanoparticles

Issue: 012

How can I develop a deeper understanding of my lipid nanoparticle heterogeneity?

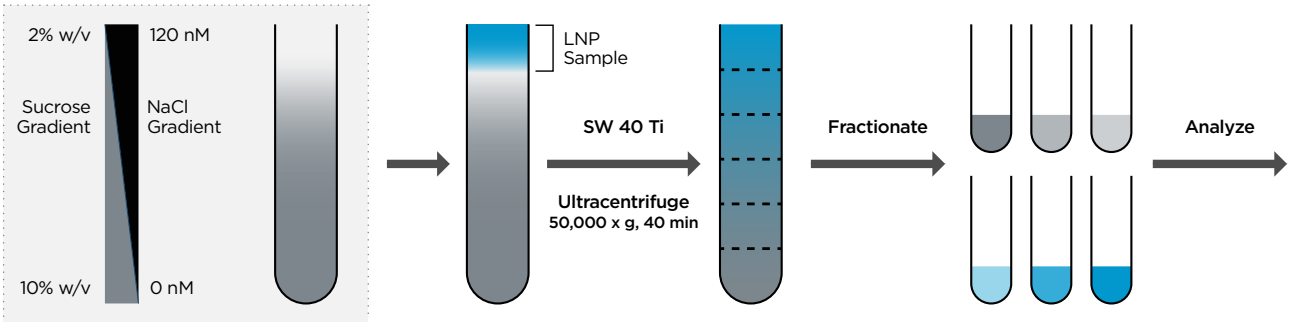
Lipid nanoparticles (LNPs) have emerged as pivotal tools in the therapeutic industry, facilitating the delivery of therapeutic cargo such as mRNA, as demonstrated in some COVID vaccines. However, LNP formulations often exhibit significant heterogeneity which can lead to challenges such as heightened immunogenicity, decreased stability, and increased production costs. Assessing the heterogeneity of an LNP formulation poses difficulties due to variations in the biophysical and functional parameters within the population. Notably, density gradient ultracentrifugation (DGUC) presents a viable method for fractionating LNPs, enabling more in-depth analytical investigations. In a study by Patel et al., DGUC was utilized to evaluate LNP heterogeneity, providing valuable insights into the composition and characteristics of these nanoparticles.[1]

Global Commercial Product Manager
Shawn Sternisha, PhD

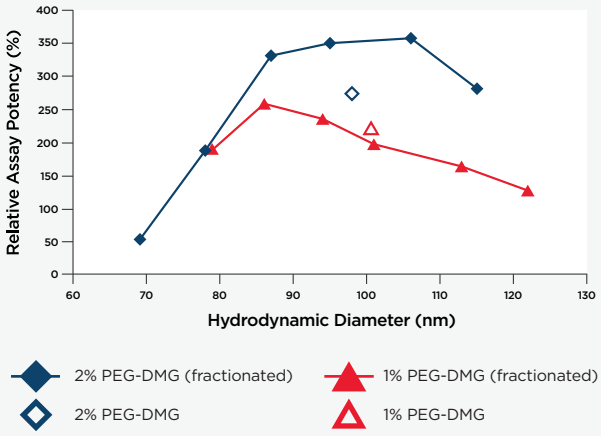
Advantages of DGUC for LNP characterization

Implementing DGUC in LNP workflows

Patel et al. employed a rate zonal DGUC technique to partition their LNP sample into discrete subpopulations. This separation method involves dispensing a continuous sucrose gradient (and opposite NaCl gradient to maintain osmolality) and layering a small sample volume on top. As the sample is subjected to centrifugal force, its particles migrate towards the tube's bottom at varying rates determined by their sedimentation coefficients. For rate zonal separations, swinging-bucket rotors are advantaged due to their long pathlength, which improves resolution. Following separation, six fractions were recovered and characterized to assess their physical and functional attributes.



DGUC reveals the nature of heterogeneity in multiple LNP formulations



Patel et al. prepared two LNP formulations at a composition of 1% or 2% 1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol (PEG-DMG). By modulating the T-mixing ratios, they were able to generate a pair of samples with nearly equal mean diameter (101 and 98 nm) and mean relative potency (234 and 274%) (open symbols). Using DGUC, they purified these samples into six distinct fractions and then determined each fraction's hydrodynamic diameter and potency using dynamic light scattering (DLS) and transfection, respectively. DGUC revealed that both LNP formulations were highly heterogeneous with respect to hydrodynamic diameter and potency (closed symbols).

Summary

Although the mean diameter and transfection efficiency of each LNP preparation were nearly identical, a high degree of heterogeneity was observed after fractionation using rate zonal DGUC. This underscores the critical need to accurately discern the composition of LNP formulations for ensuring patient safety. DGUC represents an optimal approach for both preparative and analytical sample purification. To learn how you can improve your LNP workflow using ultracentrifugation, please visit **beckman.com/centrifuges/ultracentrifuges**.

1. Patel, et al. DOI: 10.3390/vaccines11071224, Vaccines© 2023, https://creativecommons.org/licenses/by/4.0/, images were not altered.

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