CloudSpec: Scatter-free absorbance for rapid payload-quantification of intact LNPs

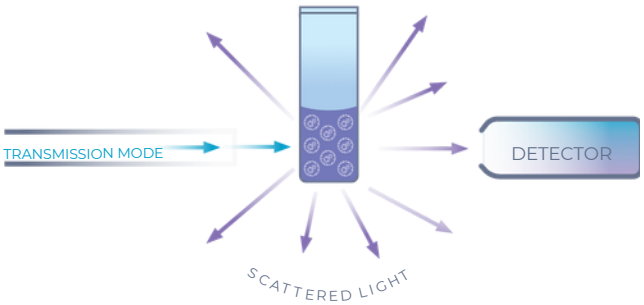
ROBUST TECHNOLOGY | NO DYES REQUIRED | NO PARTICLE DISRUPTION | 15 SECONDS MEASUREMENT | +/– 2% PRECISION

Introduction

CloudSpec™ by Marama Labs enables rapid, non-disruptive quantification of drug loading in nano-formulations, such as RNA or DNA in lipid nanoparticles (LNPs), without fluorescent dye assays. This note explains how CloudSpec's scatter-free absorbance (SFA) technology quantifies the payload in LNPs in seconds. SFA works on intact LNPs, needs no dyes, and involves only a simple dilution step.

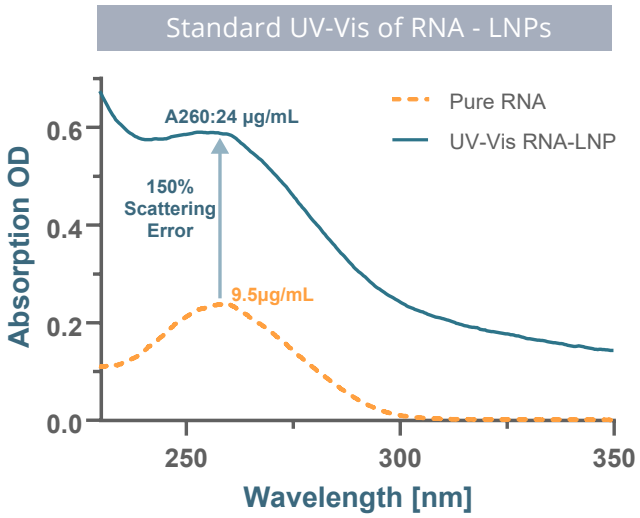
Why UV/Vis doesn't work for LNPs

UV/Vis spectroscopy is commonly used for RNA, DNA and protein quantification using absorption features at around 260-280nm. In traditional UV/Vis, light scattering reduces the amount of light reaching the detector, as illustrated below. This introduces a scattering component into the measured transmission spectrum.



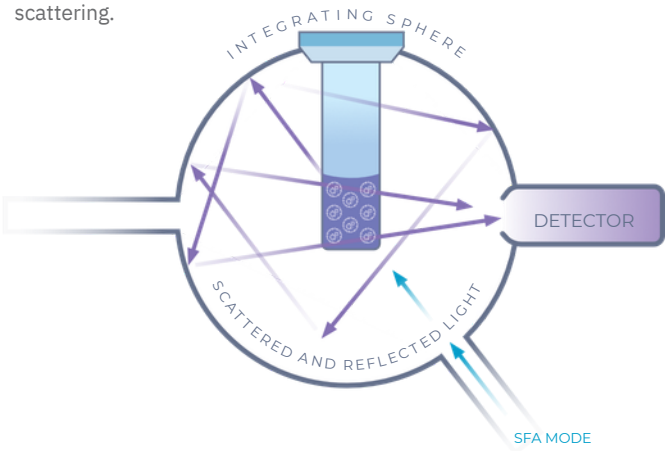
For lipid nanoparticles (LNPs), particle scattering affects the spectrum, confounding quantification by UV/Vis, making it inaccurate or necessitating a separation step. This is a major bottleneck in RNA-LNP research as there are currently no methods that both quickly and reliably quantify RNA content [1].

Lipid Nanoparticles (LNPs) are typically around 60-120 nm in size, which causes them to scatter light significantly at UV wavelengths - right where RNA and other biological molecules tend to absorb light. This causes an unpredictable overestimation of the RNA assay, as shown below.



Scatter-Free Absorbance Spectroscopy

To overcome this problem, CloudSpec uses Scatter-Free Absorbance (SFA) spectroscopy. This works by placing the sample inside a reflective sphere that bounces light until it's absorbed or detected. By capturing the entire transmitted signal, regardless of scattering, SFA enables precise measurement of the absorbance spectrum - and thus the concentration - without interference from scattering.

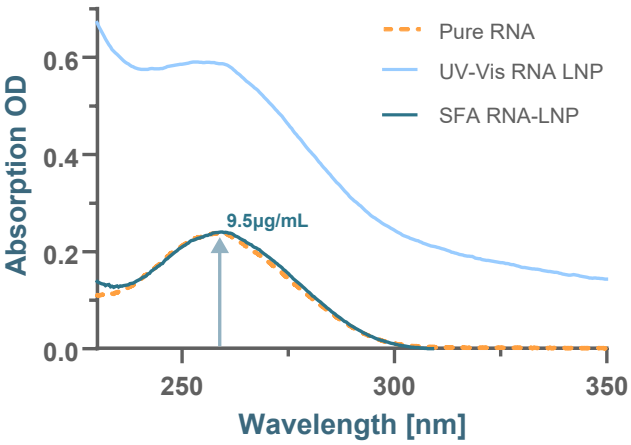


TL;DR: SFA measures true absorbance by capturing light scattered in all directions.
True Absorbance → True Concentration → Accurate Payload

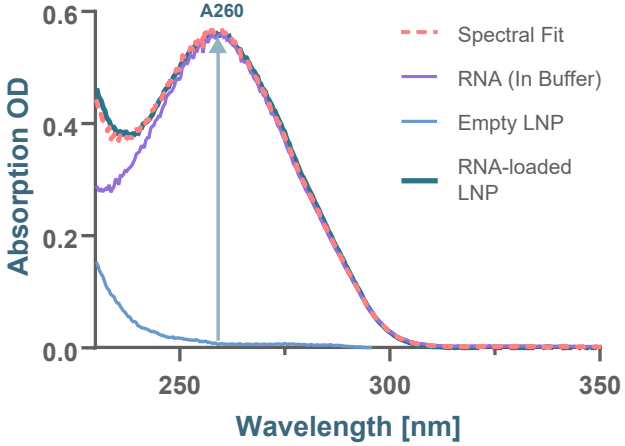
Quantifying drug-load in intact LNPs with SFA

CloudSpec offers formulators a straightforward and convenientmethod for quantifying RNA in LNPs. By measuring UV absorption independent of scattering, SFA accurately determines the total RNA concentration in the sample without the need to disrupt the particles with a surfactant. It provides a clear concentration measurement based on the familiar A260 RNA absorption feature. Using SFA, the RNA spectrum of an RNA-LNP sample can be measured (below left) without interference from LNP scattering. For a standard SM-102 formulation (below right), the RNA concentration can be measured directly by the absorbance at 260nm.

SFA vs Standard UV-Vis



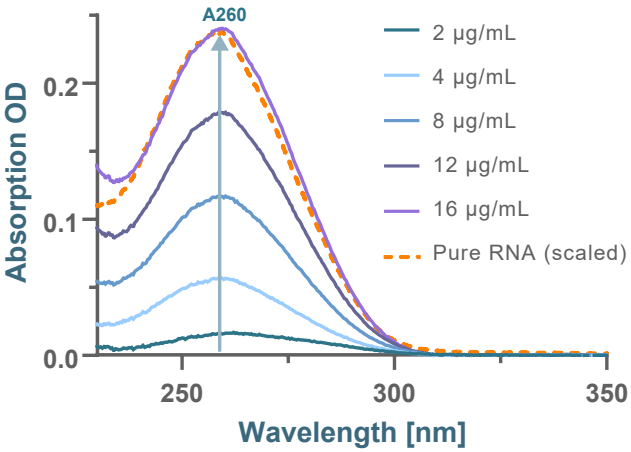
SFA Spectra of SM102 LNPs



RNA-LNP concentration RiboGreen and SFA comparison

Quantifying RNAin LNPs commonly usesthe fluorescence-based RiboGreen assay. However, this method is time-intensive and is prone to systematic errors[1] and operator variance. We compared SFA to RiboGreen for an SM-102 formulation. As shown below, SFA demonstrates an excellent fit with the RNA reference spectrum, a high linearity (R^2 = 0.9995) and achieves triplicate measurement repeat precision of less than 1%. In contrast, RiboGreen exhibits much higher triplicate variance and a bias in concentration estimates, which is consistent with less than 100% RNA recovery in the extraction process.

SFA RNA-LNP Dillution Series Spectra



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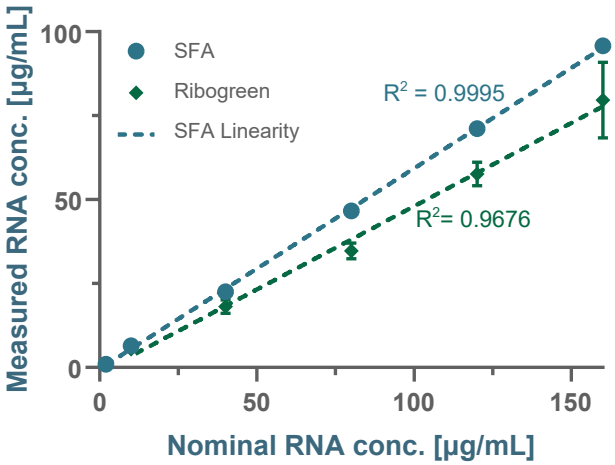
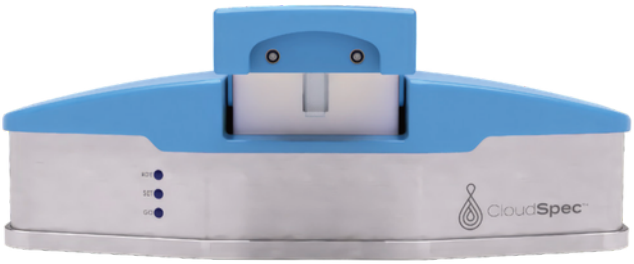


Summary

CloudSpecenables simple and easy-to-use RNA-LNP concentration measurements with almost no sample prep. It has a user-friendly workflow and gives fast and precise answers for RNA quantification. By eliminating the complexities of fluorescence assays and the inaccuracies of conventional UV-Vis techniques, CloudSpec accelerates your development and analysis in lipid nanoparticle formulations.



CloudSpec™

[1] Blenke, E.O., Evers, et. al. 2018. International journal of pharmaceutics, 548(2), pp.793-802.

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