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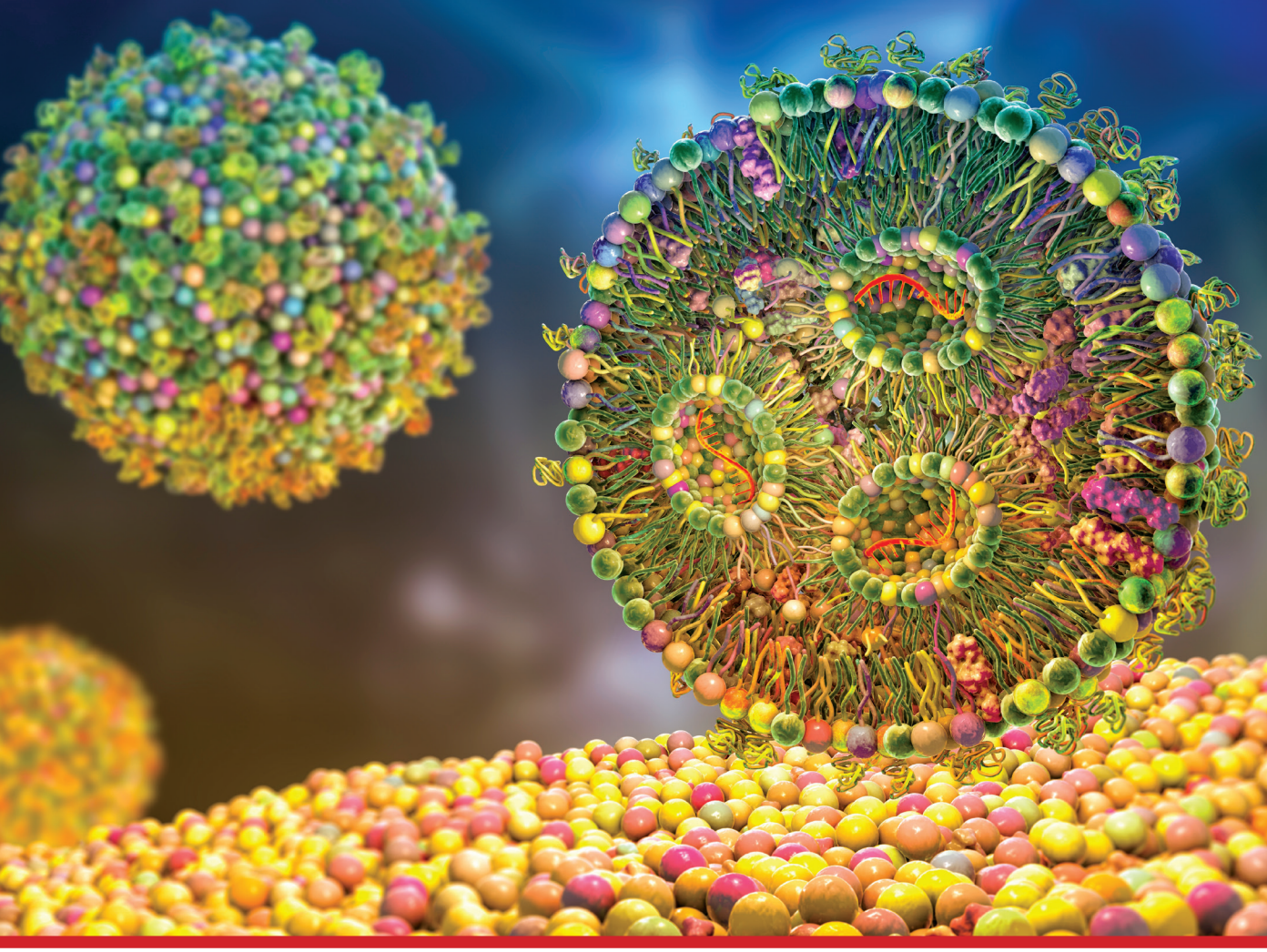
SPECIAL REPORT

Rapid and Nondestructive Total mRNA Quantification with Slope Spectroscopy

for Conventional and UV-Absorbing Lipid Nanoparticles



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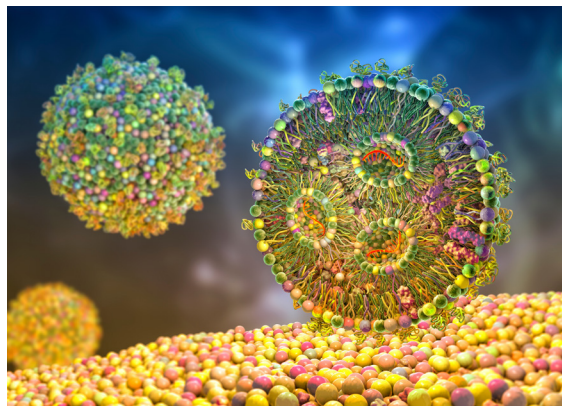
Rapid and Nondestructive Total mRNA Quantification with Slope Spectroscopy

for Conventional and UV-Absorbing Lipid Nanoparticles

Michael Galchenko and Roland Böttger

Abstract: *This report presents a new method for quantifying messenger RNA (mRNA) encapsulated in lipid nanoparticles (LNPs). Traditional ultraviolet (UV) spectroscopy and fluorescence-based assays come with limitations and complications for quantifying mRNA in LNPs, including estimation errors and material loss during sample preparation. The method presented here uses a CTech SoloVPE variable-pathlength spectrophotometer system to measure mRNA concentration. The technology measures UV extinction at multiple pathlengths, eliminating the need for dilution or extraction. Furthermore, the scattering contribution caused by the presence of nanometer-diameter LNPs is corrected to measure the underlying UV absorbance of mRNA accurately and consequently to determine its concentration nondestructively. Herein, we describe method development for conventional mRNA-LNPs and other challenging systems containing UV-absorbing lipids that overlap with the mRNA spectrum. The method is linear, robust (1.4%), and highly accurate compared with reference methods (2%). Thus, the SoloVPE system is a versatile and rapid analytical solution for mRNA-LNP quantification, with the potential to accelerate related formulation development and quality-control work.*



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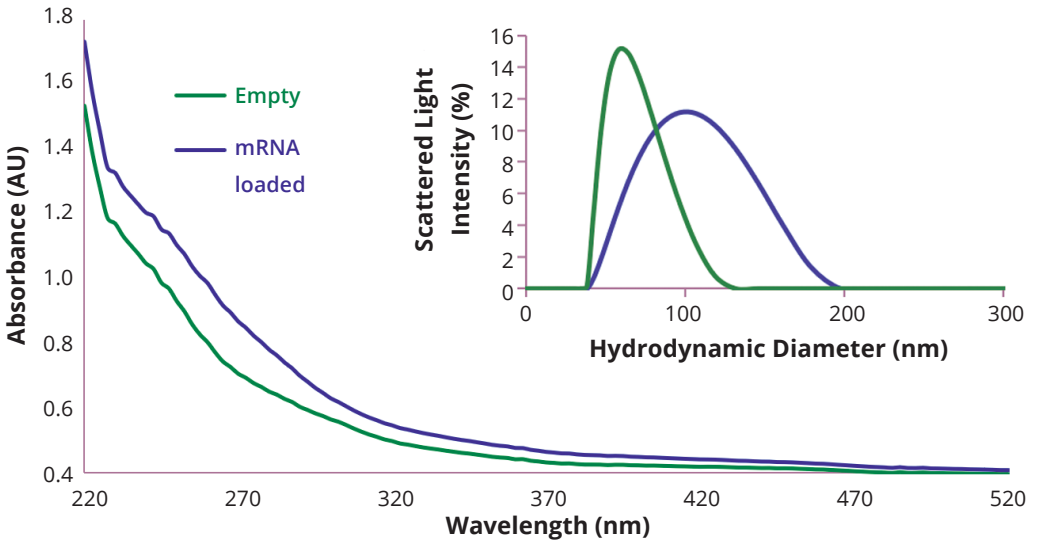
Messenger RNA (mRNA)–based therapeutics are revolutionizing medicine, with several therapies recently receiving clinical approval. The success of such therapies, including mRNA vaccines for COVID-19, relies heavily on lipid nanoparticles (LNPs) to protect nucleic acids from degradation while facilitating cellular uptake. Typically ranging 60–150 nm in size, LNPs are composed of ionizable lipids, polyethylene glycol (PEG)–conjugate lipids, and structural lipids such as cholesterol and phospholipids (**1**). In the pharmaceutical industry, LNPs are particularly valuable for cancer treatment, for which they enhance drug solubility, reduce toxicity, and improve delivery efficiency through the enhanced permeability and retention (EPR) effect (**2**).

Quantification of mRNA in LNPs presents a significant analytical challenge due to the necessity of accurately extracting mRNA and measuring concentration both before and after its encapsulation in LNPs. That dual requirement complicates mRNA drug development, demanding precise methodologies that can ensure the integrity and quantity of the nucleic acid. Traditional measurement techniques for quantifying naked mRNA include UV spectroscopy, reverse-transcription quantitative polymerase chain reaction (RT-qPCR), and reverse-transcription digital polymerase chain reaction (RT-dPCR). Fluorescence-based assays, such as the RiboGreen assay, are used for both naked and total mRNA in LNP-encapsulated mRNA drug products. However, all those methods are limited in application by issues such as insufficient calibration, lack of specificity, and inadequate traceability — all of which further complicate quantification. No uniform approach or shared reference material

Figure 1: Absorbance spectra for empty and mRNA-loaded lipid nanoparticles (LNPs); INSET compares light-scattering results from LNPs with particle hydrodynamic diameters



exists for harmonizing quantification methods, so the mRNA field currently lacks a standardized analytical platform.

Quantification of mRNA-LNP with a RiboGreen assay requires extraction of mRNA from LNPs, a process that adds complexity, variability, and the risk of incomplete disruption, all of which can compromise significantly the accuracy of results (**3**). The RiboGreen assay requires disruption of LNPs using Triton-X-100 surfactant; however, such detergents are prone to quenching fluorescence by up to 8%. Material loss during processing and degradation during sample preparation can lead to underestimation of quantified mRNA or overestimation of encapsulation efficiency (**4**). Furthermore, Triton-X 100 surfactant is harmful to both human health and the environment, so some regulators encourage developers to explore safer and more reliable surfactants for mRNA-LNP disruption (**5**).

Alternatively, high-performance liquid chromatography (HPLC) can be used to separate mRNA from lipids (**6**). However, such separation methods pose inherent challenges such as the coelution of impurities or lipid components and carry-over effects, which can interfere with accurate detection and quantification of mRNA (**7**). HPLC also requires well-characterized standards and calibration curves; preparation and maintenance of those standards can be resource-intensive and time-consuming. Additionally, interpreting elution curves requires significant operator skill, which further complicates routine HPLC analysis.

For quantifying total mRNA content, UV-visible (UV-Vis) light-absorption spectroscopy is a common technique typically applied to extracted mRNA. This method eliminates the signal-quenching issues associated with fluorescence-based assays by using maximum absorption at 260 nm. However, UV-Vis spectroscopy requires disruption of LNPs because of their light-scattering properties and contribution to the total extinction signal of an mRNA-LNP formulation (**8**). Light scattering is an inherent physical property of LNPs often described with Rayleigh approximation: Once a particle diameter is much smaller than the incident-light wavelength, light-scattering is proportional to wavelength λ^{-4} and particle radius a^6 (**9**). For accurate, absorption-based mRNA quantification in intact LNPs, the contribution of light-scattering to apparent absorption must be estimated and subtracted from the extinction signal. Scatter correction is a standard practice for protein-based biopharmaceuticals (**10**) and commonly applied to genome- and capsid-titer quantifications of viral-vector formulations (**11**).

Herein, we demonstrate a UV-Vis absorption-based method for quantifying mRNA within intact LNPs using the CTech SoloVPE system and an enabling scatter-correction algorithm. The SoloVPE System uses variable-pathlength technology (VPT) to measure relative changes in absorbance over multiple pathlengths instead of relying on a single

Figure 2: Absorption spectra for mRNA lipid nanoparticles (LNPs) with extrapolated correction values using either 305 and 320 nm or 320 and 350 nm as scatter-correction wavelengths

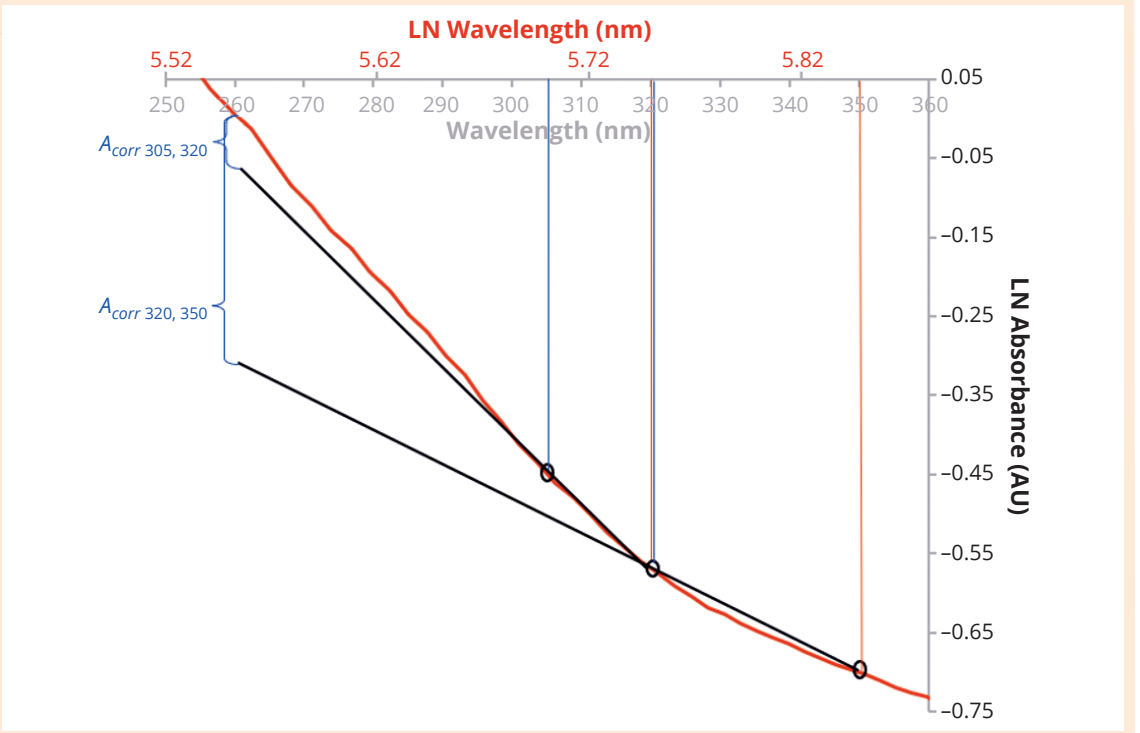


Table 1: Scatter-corrected mRNA concentrations using different correction wavelengths

Scatter-Correction Wavelength	mRNA Concentration
300 nm, 320 nm	0.361 mg/mL
300 nm, 325 nm	0.379 mg/mL
300 nm, 330 nm	0.388 mg/mL
300 nm, 335 nm	0.395 mg/mL
300 nm, 340 nm	0.408 mg/mL
305 nm, 310 nm	0.374 mg/mL
305 nm, 315 nm	0.403 mg/mL
305 nm, 320 nm	0.426 mg/mL
310 nm, 320 nm	0.453 mg/mL
320 nm, 350 nm	0.486 mg/mL

absorbance reading. VPT is highly accurate and requires no baseline or buffer correction, sample preparation, or dilution steps, thus enabling its seamless use as an analytical tool in mRNA-based drug development and manufacturing. The method is nondestructive and does not alter sample composition, allowing subsequent use of samples. In addition to conventional LNPs without inherent UV-absorbing properties, this approach extends to UV-absorbing lipid compositions with a spectral overlap with mRNA.

Materials and Methods

Reagents: CureVac SE (Tübingen, Germany) provided mRNA, formulated in conventional LNP, based on SM102. All chemicals were purchased from commercial suppliers at purities of ≥98% and used as received without further purification. Pluronic F-127, Tris-HCl (1 M, pH 7.4), and ammonium hydroxide (≥25% in water) were purchased from Merck KGaA (Darmstadt, Germany). Heparin-Natrium-5000 was purchased from Ratiopharm GmbH (Ulm, Germany). Triton X-100 (10%, w/v) was purchased from G-Biosciences (St. Louis, MO). Quant-iT RiboGreen mRNA reagent and 20x Tris-EDTA (TE) buffer were purchased from Life Technologies Corporation (Thermo Fisher Scientific) in Eugene, OR. mRNANaCl (5 M) was purchased from Lonza Ltd (Basel, Switzerland). Sodium citrate (100 mM, pH 4) was purchased from Alpha Teknova Inc. (Hollister, CA). Isopropanol was purchased from VWR International SAS (Briare, France). Ethanol (absolute) was purchased from AppliChem GmbH (Darmstadt, Germany).

mRNA-LNP Extraction: mRNA-LNPs (100 µL) were mixed with Pluronic F-127 (5 µL, 10% v/v in water) and Heparin (50 µL, 500 I.E. in 10 mM Tris-HCl buffer) and incubated in a ThermoMixer system

Table 2: mRNA concentration results (R^2= 0.9991)

mRNA Concentration, Expected Aliquot 1	mRNA Concentration, SoloVPE Measured	R^2 Slope Regression (SoloVPE)	Deviation
0.392 mg/mL	0.392 mg/mL	0.9998	—
0.196 mg/mL	0.185 mg/mL	0.9998	–5.74%
0.092 mg/mL	0.088 mg/mL	0.9996	–4.28%
0.044 mg/mL	0.044 mg/mL	0.9995	–0.68%
0.022 mg/mL	0.022 mg/mL	0.9993	+0.23%
0.011 mg/mL	0.011 mg/mL	0.9994	–3.64%
0.005 mg/mL	0.005 mg/mL	0.9985	–3.77%

Table 3: Measured mRNA concentration in different mixtures of loaded and empty lipid nanoparticles (LNPs)

Volume of Loaded LNPs	Volume of Empty LNPs	Volume of Buffer	mRNA Concentration	Deviation from 90/0/90 Sample
90 µL	0 µL	90 µL	0.0522 mg/mL	—
90 µL	10 µL	80 µL	0.0524 mg/mL	+0.40%
90 µL	20 µL	70 µL	0.0520 mg/mL	–0.46%
90 µL	30 µL	60 µL	0.0528 mg/mL	+1.21%
90 µL	50 µL	40 µL	0.0529 mg/mL	+1.36%

Table 4: Comparing SoloVPE concentration measurements with Nanodrop microvolume spectrophotometer and Ribogreen assay results

Sample	Extraction + Nanodrop	Extraction + RiboGreen Assay	SoloVPE Measurement	SoloVPE Deviation from Nanodrop Results	SoloVPE Deviation from Ribogreen Results
mRNA-LNP DP	0.378 mg/mL	0.387 mg/mL	0.379 mg/mL	+0.26%	–2.06%

(15 minutes, 45 °C, 1000 rpm, Eppendorf SE, Hamburg, Germany) to disrupt vesicles. NaCl (100 µL, 5M), sodium citrate buffer (100 µL, 50 mM), isopropanol (100 µL), and chilled ethanol (1200 µL, –20 °C) were added and mixed thoroughly to precipitate mRNA. Samples were centrifuged (20 minutes, 4 °C, 13,200 rpm, Centrifuge 5430, Eppendorf SE). The supernatant was removed carefully, and the mRNA-containing pellet was dried in a ThermoMixer system (60 min, 37 °C, 500 rpm). The pellet was resuspended in Tris-HCl buffer (250 µL, 10 mM), and mRNA concentration was measured using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Madison, WI).

RiboGreen Assay: mRNA-LNPs diluted in TE buffer (95 µL, <2 µg/mL mRNA) were added to 96-well black bottom plate (Thermo Fisher Scientific) and either mixed with 5 µL of TE buffer to keep LNPs intact, or with 5 µL of Triton X-100 (10% w/v, G-Biosciences, St. Louis, MO) to release encapsulated mRNA. Quant-iT RiboGreen mRNA reagent (100 µL, 200-fold diluted in TE buffer) was added to each well, and the plate was shaken in a plate reader (TriStar LB949, Berthold Technologies GmbH, Bad Wildbad, Deutschland) for two minutes. The fluorescence intensity (excitation/emission maxima: 500/525 nm) was recorded to detect mRNA. To achieve a linear calibration curve, standard dilutions of equivalent mRNA (0.063–2 µg/mL) were used rather than samples to estimate mRNA concentration.

SoloVPE System: 120 µL of mRNA-LNP or mRNA–UV-absorbing LNP (uvLNP) were pipetted into plastic vessels and placed into the SoloVPE system. Data were acquired at 260 nm, with dual-logarithmic scatter correction enabled at two wavelengths (ranging 300–350 nm). Ten datapoints were acquired with 0.5-second averaging time for regression analysis of three repeated measurements in repeat mode. For comparison of results with those from RiboGreen and Nanodrop assays, a mass-based extinction coefficient of 25 L/(g·cm) at 260 nm was used. Because the TE buffer slope at 260 nm was below 0.01 Abs/mm, no baseline correction was needed.

Logarithmically Scatter-Corrected Absorption of mRNA-LNP

Method Development: Figure 1 presents absorbance spectra of empty and mRNA-loaded

Figure 3: mRNA concentration linearity plot

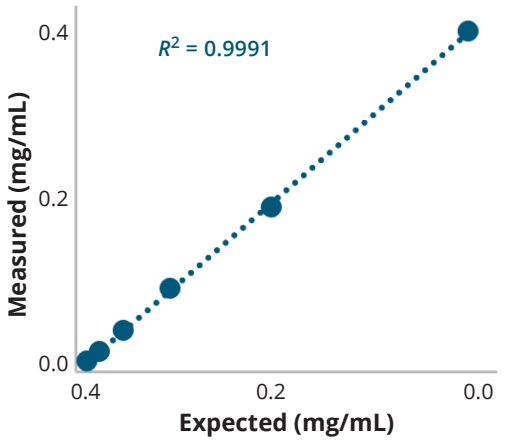


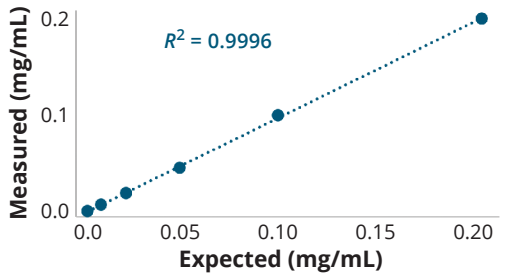
Table 5: Values to calculate $Slope_{Corr}$

	Slope at 260 nm (Abs/mm)	Slope at 280 nm (Abs/mm)
Empty uvLNP	A = 1.588	Y = 3.875
Loaded uvLNP	Z = 2.901	X = 4.441
Corrected slope	$Z - (X \div Y) \times A$ = 1.082 Abs/mm	

LNPs. Both spectra exhibited a constantly increasing absorbance from 500 nm toward lower wavelengths into UV regions, in addition to a 0.4-AU offset attributed to experimental setup. The increase is caused by light-scattering properties of LNPs, which result from their size and can be explained by Rayleigh scattering. When comparing the spectra of empty and mRNA-loaded LNPs, an additional offset in the UV region is apparent. The offset can be attributed to mRNA absorption and additional scattering caused by increased hydrodynamic radius of mRNA-loaded LNPs. Given the overlap of LNP scattering and mRNA absorption, a scatter correction was applied to assess mRNA concentration at 260 nm.

The scatter-correction approach considered in our work is based on a logarithmic-correction method suggested by *United States Pharmacopeia* (USP) Chapter ‹1057› for total protein quantification (**12**). In that approach, natural logs for absorbance and wavelength are plotted for an mRNA-loaded LNP spectrum (see Figure 2). USP Chapter ‹1057› recommends using seven wavelengths of 320–350 nm for regression to an analyte's absorption maximum, but a biopharmaceutical industry standard using two scatter correction wavelengths — 320 and 350 nm — has been established and is applied widely.

Figure 4: Linearity test for serial dilution of a 1:1 mixture of empty and mRNA-loaded LNPs



Porterfield et al. discuss a similar Rayleigh-approximation-based approach for genome- and capsid-titer quantification in viral capsid particles, in which absorption measurements at 340 and 360 nm are used to extrapolate a correction value at the wavelength of interest (260 nm for DNA and 280 nm for capsid titers) (**11**). In that method, corrected absorbance scales with λ^{-4}, consistent with the Rayleigh approximation.

We combined both approaches to quantify LNP-encapsulated mRNA and present a method-development approach tailorable to all LNP sizes and polydispersities (Equation 1):

$$\ln(A_{\text{corr i,j}}) = \ln(A_{\text{raw}}) - C_{\text{i,j}}$$

After plotting the natural log of absorbance versus natural log of wavelength, points corresponding to 305-, 320-, and 350-nm wavelengths are used as reference points for scatter correction. The slope between any two of those wavelengths then is extrapolated to 260 nm, yielding three possible correction values represented by $C_{\text{i,j}}$, in which i and j are the selected wavelengths. $C_{\text{i,j}}$ then is subtracted from raw absorbance (A_{raw}) to correct absorbance ($A_{\text{corr i,j}}$) (Equation 1).

Using the Beer–Lambert law, $A_{\text{corr i,j}}$ values at 260 nm are converted to mRNA concentration (C_{mRNA}) based on a mass-absorption coefficient of 25 L/(g × cm). Table 1 presents a number of scatter-correction wavelength pairs applied to determine the mRNA concentration. As illustrated in Figure 2, the mRNA concentration obtained varies depending on the wavelength combination used for scatter correction. To align with expected mRNA concentration obtained by the commonly applied RiboGreen method (0.380 mg/mL), the wavelength pair showing the smallest deviation — 300 and

Figure 5: Absorbance spectra for empty, UV-absorbing lipid nanoparticles (uvLNPs) in green, mRNA-loaded uvLNPs in blue, and the monomers in orange used in LNP formation; INSET compares LNP light scattering with particle hydrodynamic diameters.

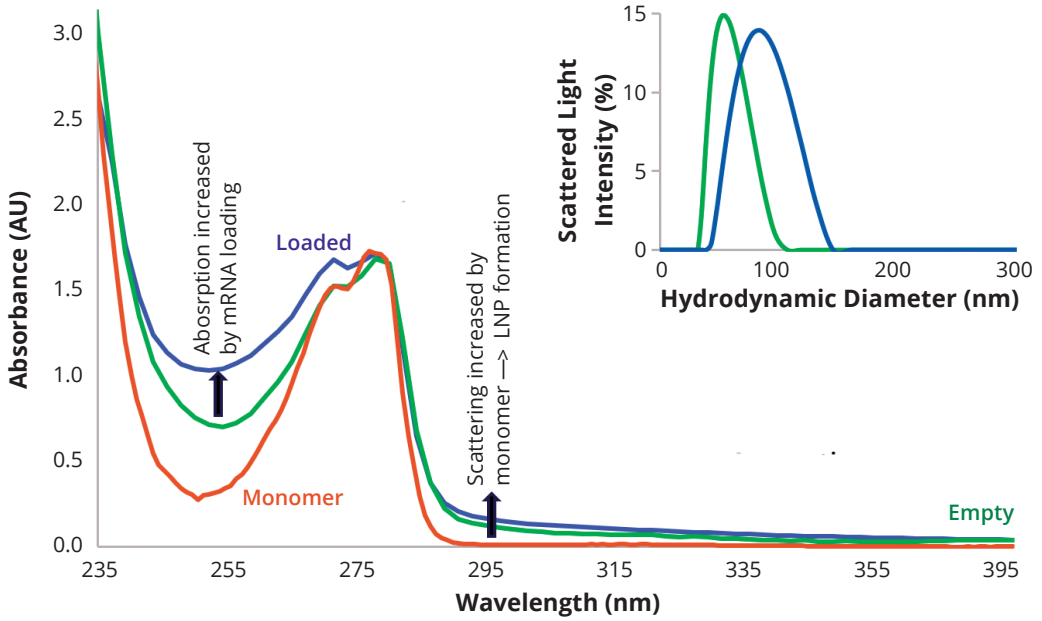


Table 6: Slope ratios at factorization wavelengths of interest

Dilution Ratio	Slope at 260 nm	Slope at 280 nm	Slope Ratio 260/280	Slope at 260 nm	Slope at 320 nm	Slope Ratio 260/320
Undiluted	2.97 Abs/mm	4.66 Abs/mm	0.64	2.87 Abs/mm	0.30 Abs/mm	5.72
1:2	1.52 Abs/mm	2.33 Abs/mm	0.65	1.49 Abs/mm	0.18 Abs/mm	6.08
1:4	0.78 Abs/mm	1.17 Abs/mm	0.67	0.76 Abs/mm	0.10 Abs/mm	6.28
1:8	0.39 Abs/mm	0.58 Abs/mm	0.67	0.39 Abs/mm	0.05 Abs/mm	6.62

325 nm — was selected for this study and LNP formulation. Choice of scatter-correction wavelengths depends on LNP size and polydispersity, necessitating careful evaluation during method development.

The SoloVPE system automatically applies the aforementioned corrections once "Dual LN" scatter correction is enabled and collects 10 scatter-corrected absorbance values at varying pathlengths. Those pathlengths are adjusted dynamically based on sample optical density. The resulting absorbance versus pathlength data are subjected to regression analysis, yielding a slope and a regression coefficient (R^2). Using the Beer–Lambert law, the slope is converted to a concentration value, whereas the R^2 value serves as an acceptance criterion with a threshold of $R^2 > 0.999$, ensuring that pathlengths are optimized so that the photodetector functions within the linear region of the Beer-Lambert law.

Linearity and Serial Dilutions of mRNA-LNPs: To evaluate method linearity and applicability of scatter-correction parameters across an expected mRNA-concentration range of 0.01–0.40 mg/mL, a serial dilution of four aliquots was prepared. Table 2 summarizes the measured mRNA concentrations alongside expected values, which were calculated based on applied dilution factors for aliquot 1 (complete dataset is available in the Appendix Data table). As a result, the deviation between expected and measured mRNA concentrations for the undiluted sample is 0%. For clarity, only three decimal places are shown, and the same data are plotted in Figure 3.

Figure 3 shows how linearity at $R^2 > 0.999$ was achieved across all four dilution series within the tested concentration range. R^2 for the VPT slope analysis was <0.999 for only one of the four aliquots at an mRNA concentration of 0.005 mg/mL.

Table 7: Concentration measurement of serial dilution using 260/280-nm factorization method

Expected	Measured	Deviation
0.428 mg/mL	0.428 mg/mL	—
0.214 mg/mL	0.226 mg/mL	+5.82%
0.113 mg/mL	0.120 mg/mL	+5.72%
0.057 mg/mL	0.062 mg/mL	+8.72%

Table 8: Accuracy of quantified mRNA using different scatter wavelength pairs

Wavelengths	Expected (mg/mL)	Measured (mg/mL)	Deviation
No correction	0.415	0.432	+4.20%
320/350 nm	0.415	0.435	+4.87%
300/320 nm	0.415	0.372	–10.46%
310/335 nm	0.415	0.440	+6.17%
305/335 nm	0.415	0.431	+3.96%
305/320 nm	0.415	0.414	–0.02%

Table 9: Linearity across an mRNA concentration range of 0.02–0.43 mg/mL tested using 305/320 nm

Expected	Measured	Deviation
0.372 mg/mL	0.372 mg/mL	—
0.186 mg/mL	0.190 mg/mL	+2.50%
0.095 mg/mL	0.096 mg/mL	+0.58%
0.048 mg/mL	0.046 mg/mL	–3.55%
0.023 mg/mL	0.023 mg/mL	–2.51%

Table 10: Linearity across an mRNA concentration range of 0.02–0.43 mg/mL tested using 320/350 nm

Expected	Measured	Deviation
0.435 mg/mL	0.435 mg/mL	—
0.218 mg/mL	0.220 mg/mL	+1.13%
0.110 mg/mL	0.109 mg/mL	–0.56%
0.055 mg/mL	0.054 mg/mL	–1.34%
0.027 mg/mL	0.026 mg/mL	–3.20%

That indicates that an mRNA concentration of 0.01 mg/mL represents the lowest concentration at which a reliable slope analysis using VPT and 60–120 µL sample volume can be achieved consistently. To measure lower concentrations and use the complete SoloVPE pathlength range (up to 15 mm), a 2.5-mL sample volume can be prepared in a large vessel, enabling slope analysis to extend beyond 5 mm.

Robustness and Mixing with Empty LNPs: To evaluate the robustness of the scatter-correction method and confirm its applicability across varying LNP:mRNA ratios, mRNA-loaded LNPs were mixed with empty LNPs and buffer. Table 3 summarizes mixture composition and corresponding mRNA concentrations. Particle concentrations for empty LNPs were matched to those of loaded LNPs, which were determined by measuring lipid concentration with HPLC coupled to a charged-aerosol detector (CAD). Furthermore, concentration reading for the mixture containing 90 µL of loaded LNPs and 90 µL of buffer was taken as the expected value, and all mixtures were anticipated to have identical mRNA concentration.

Considering that all tested mixtures deviate up to 1.4% from the expected value, it is evident that the increased scattering from empty LNPs does not impact the method's ability to quantify mRNA concentration accurately. Additionally, to verify method linearity, a serial dilution of a 1:1 mixture of empty and mRNA-loaded LNPs was prepared, with expected and measured mRNA concentrations plotted in Figure 4. Given an R^2 value of 0.9996, concentration linearity reading down to 0.006 mg/mL is evident.

Comparison with Orthogonal Methods: The accuracy of the SoloVPE results was evaluated in comparison with RiboGreen and Nanodrop results using a drug-product mRNA-LNP sample. Note that mRNA had to be extracted from LNPs (see Materials and Methods section) for analysis with Nanodrop and RiboGreen assays, whereas SoloVPE measurements could be conducted directly on undiluted samples without sample preparation. That was feasible because the SoloVPE system can adjust pathlengths between 0.005 to 15 mm, based on optical density at the wavelengths of interest (260, 300, and 325 nm), which allows for reliable absorbance and slope readings even at wavelengths with low absorbance (e.g., 325 nm). After analyzing a well-characterized drug-product sample across all three techniques, the results show an acceptable deviation of up to 2.1% (Table 4).

Factorization Method for UV-Absorbing LNPs

Method Development: Unlike conventional LNPs such as SM102, certain LNP types exhibit absorption in the UV region due to modifications of lipid monomers. Figure 5 presents absorbance spectra of an empty UV-absorbing LNP (uvLNP, green), an mRNA-loaded uvLNP (blue), and the monomer (orange) used in LNP formation. For improved readability, all spectra were normalized to the λ_{max} of the LNP's monomer.

The spectra show that the uvLNP monomer exhibits an absorbance maximum at 289 nm, with no significant absorbance or scattering observed

Table 11: Scatter correction slope for different ratios of lipid nanoparticles (LNPs) to mRNA; SC = scatter correction

Volume of Loaded uvLNPs	Volume of Empty uvLNPs	Volume of Buffer	Slope with SC at 320 and 350 nm	Deviation	Slope with SC at 305 and 320 nm	Deviation
25 µL	0 µL	75 µL	0.108 Abs/mm	—	0.099 Abs/mm	—
25 µL	25 µL	50 µL	0.105 Abs/mm	–3.12%	0.093 Abs/mm	–5.48%
25 µL	50 µL	25 µL	0.107 Abs/mm	–1.24%	0.093 Abs/mm	–5.72%
25 µL	75 µL	0 µL	0.113 Abs/mm	+4.20%	0.099 Abs/mm	–0.02%

Table 12: Comparing mRNA concentration measurements by SoloVPE system, Nanodrop microvolume spectrophotometer, and Ribogreen assay

Sample	Extraction + Nanodrop	Extraction + RiboGreen Assay	SoloVPE Measurement	SoloVPE Deviation from Nanodrop Results	SoloVPE Deviation from Ribogreen Results
mRNA-uvLNP 1	0.386 mg/mL	0.377 mg/mL	0.397 mg/mL	+2.84%	+5.30%
mRNA-uvLNP 2	0.430 mg/mL	0.416 mg/mL	0.414 mg/mL	–3.72%	–0.48%

above 300 nm. The increase in absorbance beyond 300 nm observed during LNP formation therefore can be attributed to the transition from monomers to LNPs, the resulting particle size, and associated scattering effects. Furthermore, when comparing the spectra of empty and mRNA-loaded uvLNPs, a slight increase in absorbance at the scattering wavelengths, as well as a pronounced increase around 260 nm, is evident. Although the increased scattering is due to the larger particle size of mRNA-loaded LNPs, the rise at 260 nm is attributed to mRNA absorption.

Due to the overlap of uvLNP and mRNA absorption, the scatter-correction mechanism introduced above for conventional LNPs does not apply here. Instead, a corrected slope value at 260 nm ($Slope_{Corr}$) is obtained by subtracting the slope values of empty and mRNA-loaded uvLNPs (Table 5). For that approach, particle concentrations of empty and mRNA-loaded uvLNPs must be equal. However, because adjusting particle concentration is impractical for routine analysis, the subtraction is factorized using the slope ratio at 280 nm, following Equation 2:

$$Slope_{Corr} = Z - A(X \div Y)$$

For factorization, 260/280-nm and 260/320-nm wavelength pairs were considered. The slope ratios at these wavelengths are expected to remain within typical measurement uncertainty (less than 5% for SoloVPE) across a dilution series. Table 6 presents the dilution series, the acquired slope values, and their respective slope ratios. It is evident that the 260/320 slope ratios exhibit noticeable drift, likely because of the relatively low slope value at 320 nm and the resulting inaccuracy. In contrast, the 260/280-nm slope ratio deviates by only 4.7%, making the 260/280-nm pair the preferred choice for factorization in this method.

To determine whether mRNA concentration can be calculated linearly across a concentration range, a serial dilution was prepared using the 260/280-nm factorization method. Table 7 presents the corresponding mRNA results. High deviation and drift were observed, indicating that the method requires further improvement.

Therefore, a scatter correction was applied to obtain 260- and 280-nm slope values before factorization. Table 8 summarizes a number of tested wavelength pairs, the acquired mRNA concentration, and the SoloVPE results deviation from the expected value of 0.415 mg/mL (RiboGreen assay). As observed for conventional LNPs (Table 1), the choice of scatter-correction wavelengths affects obtained mRNA concentrations, requiring the adjustment of the method in respect of the expected value. Furthermore, multiple scatter-correction wavelength pairs (305/320 nm and 305/335 nm) can be used to match expected values.

Linearity: To assess whether scatter correction could be combined effectively with the factorization method, linearity across an mRNA-concentration range of 0.02–0.43 mg/mL was tested using 305/320 nm (Table 9) and 320/350 nm (Table 10) as scatter-correction wavelengths. Although the 305/320 nm combination provided an accurate assessment of mRNA concentration, a dilution series using 320/350 nm as scatter correction wavelengths was selected to demonstrate that linear results can

be achieved with a broader range of scatter correction wavelengths. For both scatter correction wavelength pairs, linearity across the tested mRNA concentration range is evident.

Robustness: To evaluate method robustness and to confirm the technology's applicability across different LNP-to-mRNA ratios, mRNA-loaded uvLNPs were mixed with empty uvLNPs and buffer. Table 11 summarizes the mixture composition and corresponding measured mRNA concentrations. Note that the concentration reading for the mixture containing 25 µL of loaded LNPs and 75 µL of buffer represents the expected value and all mixtures are anticipated to have identical mRNA concentration. Considering that all tested mixtures are within ±5.8% of the expected value, it is evident that the increased scattering from empty uvLNPs does not compromise the method's ability to accurately quantify the mRNA concentration.

Comparison with Orthogonal Methods: Finally, the accuracy of SoloVPE results was assessed in comparison to RiboGreen and Nanodrop using two mRNA-uvLNP samples. As Table 12 shows for both mRNA-uvLNP samples, the mRNA concentration measured with SoloVPE deviated by up to 5.3%. Interestingly, for the mRNA-uvLNP sample 2, the SoloVPE result fell between those obtained from Nanodrop and the RiboGreen assays.

Conclusion

Our study demonstrates that the SoloVPE method for quantifying mRNA in LNPs is robust, rapid, and accurate. The results are comparable within ±2.1% to those obtained from conventional methods that use Nanodrop and RiboGreen assays and require extraction of mRNA. The SoloVPE method is robust across many LNP:mRNA ratios and maintains linearity. Furthermore, the SoloVPE instrument's automatic pathlength adjustment enables accurate results over a broad concentration range without the need for dilution, sample preparation, or blanking. That capability mitigates operator-error, material loss, and time required for sample preparation and manual calculations.

A factorization method involving scatter correction for UV-absorbing LNPs was demonstrated for a wide mRNA concentration range. Our method was confirmed to be robust, linear, and accurate compared with the Nanodrop and RiboGreen methods. Both of those assays require method development that identifies scatter correction wavelengths in respect to the LNP size. Additionally, correction wavelengths must be identified for UV-absorbing LNPs. We have presented a workflow for assessing such values.

Overall, the method presented herein is a versatile and rapid tool for mRNA-LNP quantification with the potential to accelerate formulation development and quality control during lipid manufacturing. The technology potentially applies to all types of mRNA-LNPs, including absorbing lipids, functionalized LNPs, antibody conjugates (e.g., LNPs by Capstan Therapeutics), and coformulation with small-molecule immune stimulators that have inherent UV absorbance (e.g., LNPs by Integrated Nanotherapeutics).

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Appendix Data

mRNA Concentration, Expected (Aliquot 1)	mRNA Concentration, Measured	Deviation	mRNA Concentrated, Expected (Aliquot 2)	mRNA Concentration, Measured	Deviation
0.392 mg/mL	0.392 mg/mL	0.00%	0.416 mg/mL	0.416 mg/mL	+0.00%
0.196 mg/mL	0.185 mg/mL	–5.74%	0.208 mg/mL	0.214 mg/mL	+2.90%
0.092 mg/mL	0.088 mg/mL	–4.28%	0.107 mg/mL	0.111 mg/mL	+3.79%
0.044 mg/mL	0.044 mg/mL	–0.68%	0.056 mg/mL	0.057 mg/mL	+1.90%
0.022 mg/mL	0.022 mg/mL	+0.23%	0.028 mg/mL	0.030 mg/mL	+5.95%
0.011 mg/mL	0.011 mg/mL	–3.64%	0.015 mg/mL	0.015 mg/mL	+0.60%
0.005 mg/mL	0.005 mg/mL	–3.77%	***	***	
R^2 = 0.9991			R^2 = 0.9998		

mRNA Concentration, Expected (Aliquot 3)	mRNA Concentration, Measured	Deviation	mRNA Concentration, Expected (Aliquot 4)	mRNA Concentration, Measured	Deviation
0.416 mg/mL	0.416 mg/mL	0.00%	0.419 mg/mL	0.419 mg/mL	0.00%
0.208 mg/mL	0.204 mg/mL	–1.84%	0.209 mg/mL	0.202 mg/mL	–3.60%
0.102 mg/mL	0.104 mg/mL	+1.32%	0.101 mg/mL	0.102 mg/mL	+1.03%
0.052 mg/mL	0.050 mg/mL	–3.98%	0.051 mg/mL	0.050 mg/mL	–1.29%
0.025 mg/mL	0.025 mg/mL	–0.34%	0.025 mg/mL	0.024 mg/mL	–3.48%
0.416 mg/mL	0.416 mg/mL	0.00%	0.012 mg/mL	0.012 mg/mL	+2.31%
***	***		***	***	
R^2 = 0.9999			R^2 = 0.9996		

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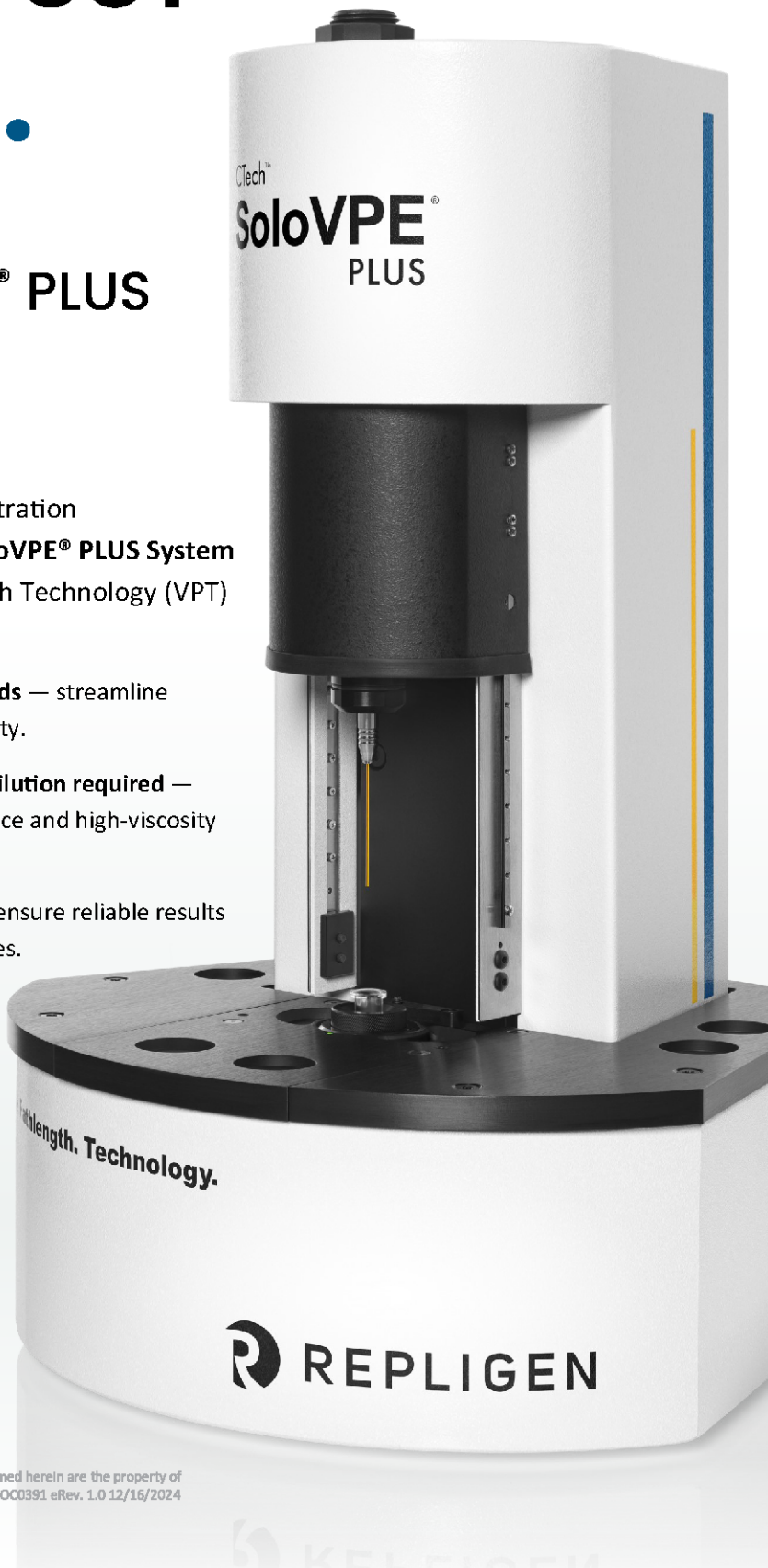

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