Characterization of LNPs with SEC-MALS: Encapsulation Efficiency, Concentration, Size and Molecular Weight

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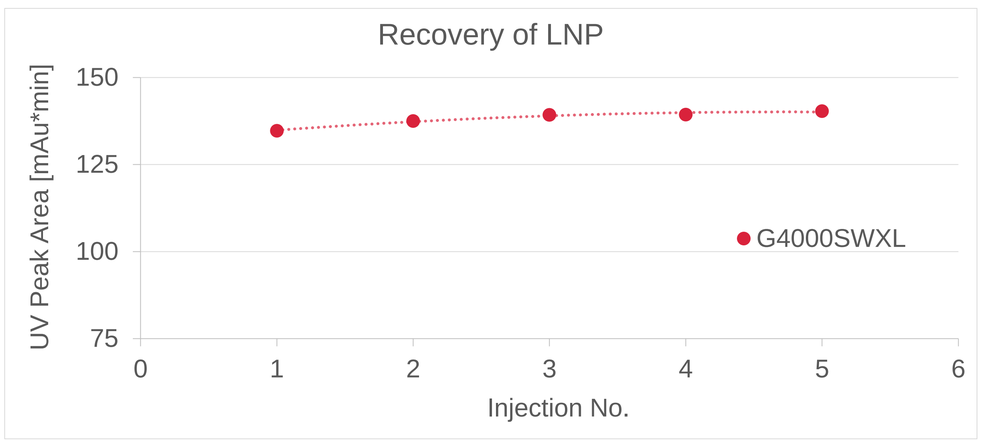
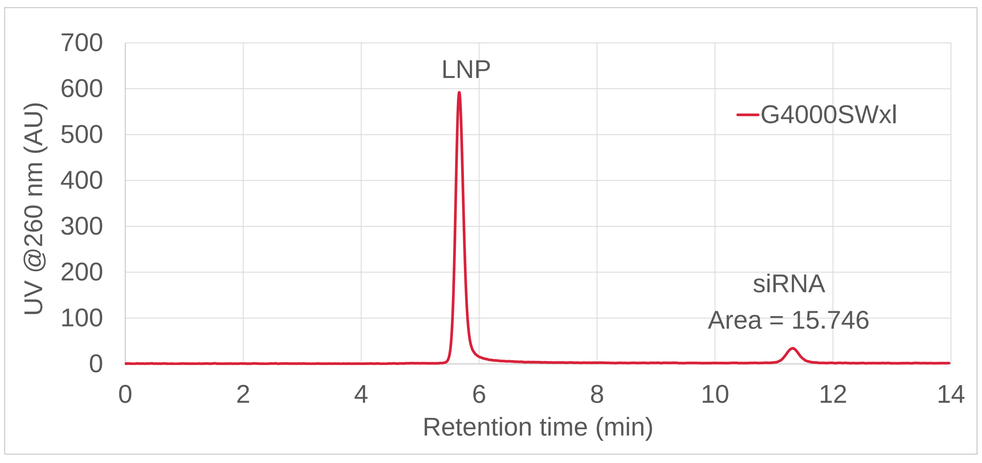
Lipid nanoparticles (LNPs) are popular delivery particles for siRNA therapeutics and vaccines based on mRNA or DNA. They are composed of lipids that build the core structure, the nucleic acid payload and further lipids and PEG to stabilize the particle and determine particle size. Important characteristics that impact the effectiveness and stability of lipid nanoparticle drugs are the encapsulation efficiency determining the dose that is delivered, as well as particle size that affects particle stability and cellular uptake pathways. Current methodologies only cover one or two of these characteristics per assay, resulting in time- and resource-consuming analytics. Here, we present the development of a method employing size exclusion chromatography (SEC) in combination with UV and multi-angle light scattering (MALS) to determine multiple parameters of an siRNA-encapsulating LNP in a single HPLC run. A silica-based SEC column separated nanoparticle and free payload. This way, particle concentration was determined as well as the concentration of the free payload, which served to calculate the encapsulation efficiency. The addition of the LenS3 MALS detector further determined the size and molecular weight of the particle. The methodology analyzes the LNP characteristics particle size and molecular weight, particle concentration and encapsulation efficiency in a single HPLC run. Therefore, it potentially replaces the need for various analysis methods and speeds up LNP characterization.

CHARACTERIZATION OF AN SEC COLUMN FOR LNP ANALYSIS

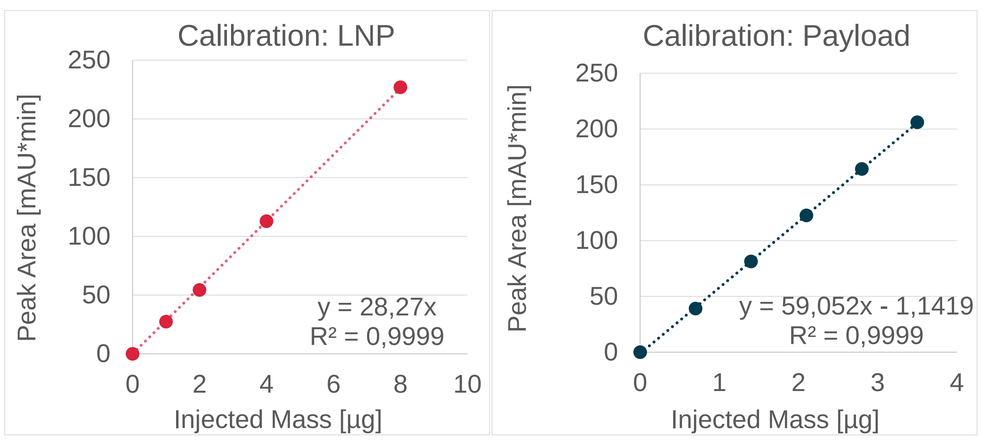
In an initial step, the suitability of the TSKgel G4000SWXL column for separating LNPs from free payload was tested. First it was analyzed, whether the LNP could be reliably recovered from the column. As the peak area was stable for consecutive injections with the nanoparticle, it was assumed, the particle completely elutes from the column with high recovery



Next, the separation between LNP and siRNA payload was tested on the G4000SWXL column. A baseline separation was achieved, which is the prerequisite for the concentration determination of the siRNA payload and encapsulation efficiency calculation.



A further requirement is the linearity between injected sample amount and the peak area derived from the UV signal. For both, LNP and siRNA, good linearity was achieved for the injected mass range. This allows establishing calibration curves and concentration calculation the siRNA of unknown samples. For the LNP sample analyzed in this experiment, the concentration of free payload was 0.0284 g/l.



EXPERIMENTAL CONDITIONS

Sample: Lipid nanoparticle* with siRNA

Column: TSKgel® G4000SWXL, 8 µm, 7.8 mm ID x 30 cm L

Mobile phase: 100 mM sodium phosphate buffer, pH 7.4

Flow Rate: 1.0 mL/min

Instrument: ThermoFisher Scientific Vanquish Flex

Detection: UV absorbance @260 nm, refractive index detector, MALS with LenS3 detector

DETERMINE ENCAPSULATION EFFICIENCY

The encapsulation efficiency can be calculated using the calibration curve generated for the siRNA payload, the area of free payload and the amount of siRNA payload initially employed for LNP production (given by manufacturer). The three calculation steps are outlined below (1) calculation of mass of the free payload (2) calculation of the concentration of free payload (3) calculation of the encapsulation efficiency.

$$m_{RNA_{free}} = \frac{Area - Intercept}{Slope}$$

$$c_{RNA_{free}} = \frac{m_{RNA_{free}}}{V_{inj}}$$

$$Encapsulation\ \% = \left(1 - \frac{c_{RNA_{free}}}{c_{RNA_{init}}}\right) * 100\%$$

$$m_{RNA_{free}} = \frac{15.746\ -1{,}1419}{59{,}052} = 0.2473\ \mu g$$

$$c_{RNA_{free}} = \frac{0.2473\ \mu g}{10\ \mu L} = 0.02473 \frac{g}{L}$$

$$c_{RNA_{init}} = 0.26 \frac{g}{L}$$

$$Encapsulation\ \% = \left(1 - \frac{0.02473 \frac{g}{L}}{0.26 \frac{g}{L}}\right) * 100\% = \mathbf{90.05\ \%}$$

DETERMINE MOLECULAR WEIGHT AND RADIUS OF GYRATION (R_G) OF LNP

By adding MALS as a detection method, more information can be directly obtained from the SEC analysis of LNPs. Using the low-angle light scattering signal (LALS) in combination with concentration detection by refractive index (RI), the average MW of the LNP was calculated to be 110 MDa. Furthermore, the signals from low-, right- and high-angle scattering were employed to calculate the average radius of gyration of the LNP: 34.3 nm. This is close to the size indicated on the LNP data sheet of 82 nm (radius – 41 nm). The SEC-MALS analysis was further employed to determine the particle concentration of $8.726*10^{12}$ /ml.

Av. radius of gyration	Av. molecular weight	Particle concentration
34.3 nm (size ~78.6 nm)	111 MDa	$8.726*10^{12}$ /ml

CONCLUSION

This poster shows the results that can be obtained from a single HPLC analysis of an LNP sample with multi-angle light scattering detection. The LNP was characterized in terms of its encapsulation efficiency, size (gyration radius), MW, and concentration. This is made possible by an SEC column that separates LNP from the free payload and provides high linearity and recovery of both. Conventionally, other analytical methods such as fluorescence assays and DLS are used for such characterization. With the SEC-MALS approach presented, these methods can be omitted, saving time and resources.

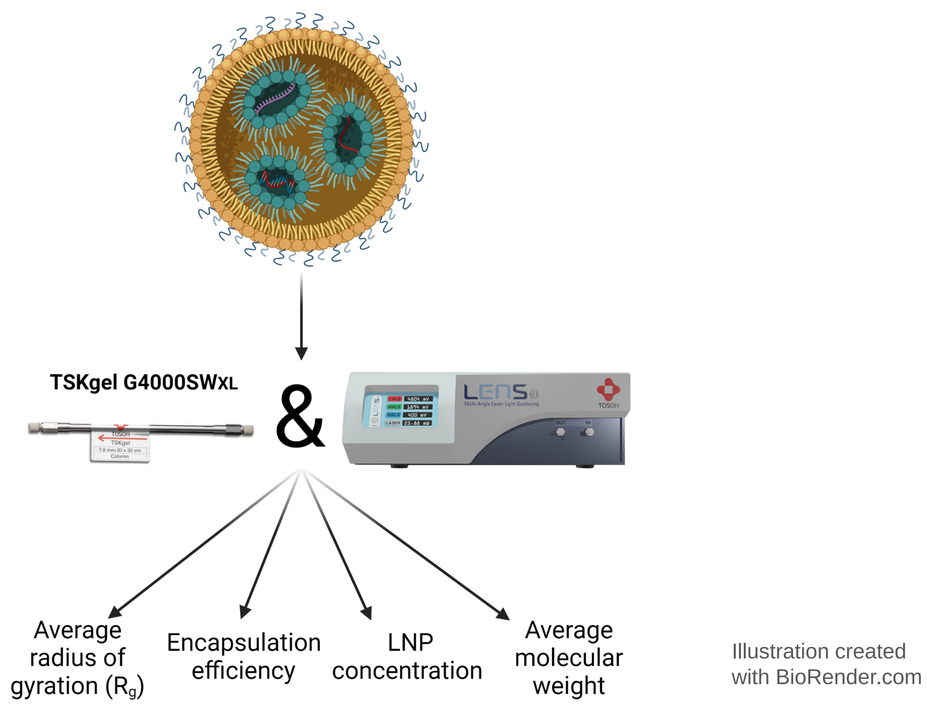


Illustration created with BioRender.com

*Material was kindly provided by axolabs gmbh

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