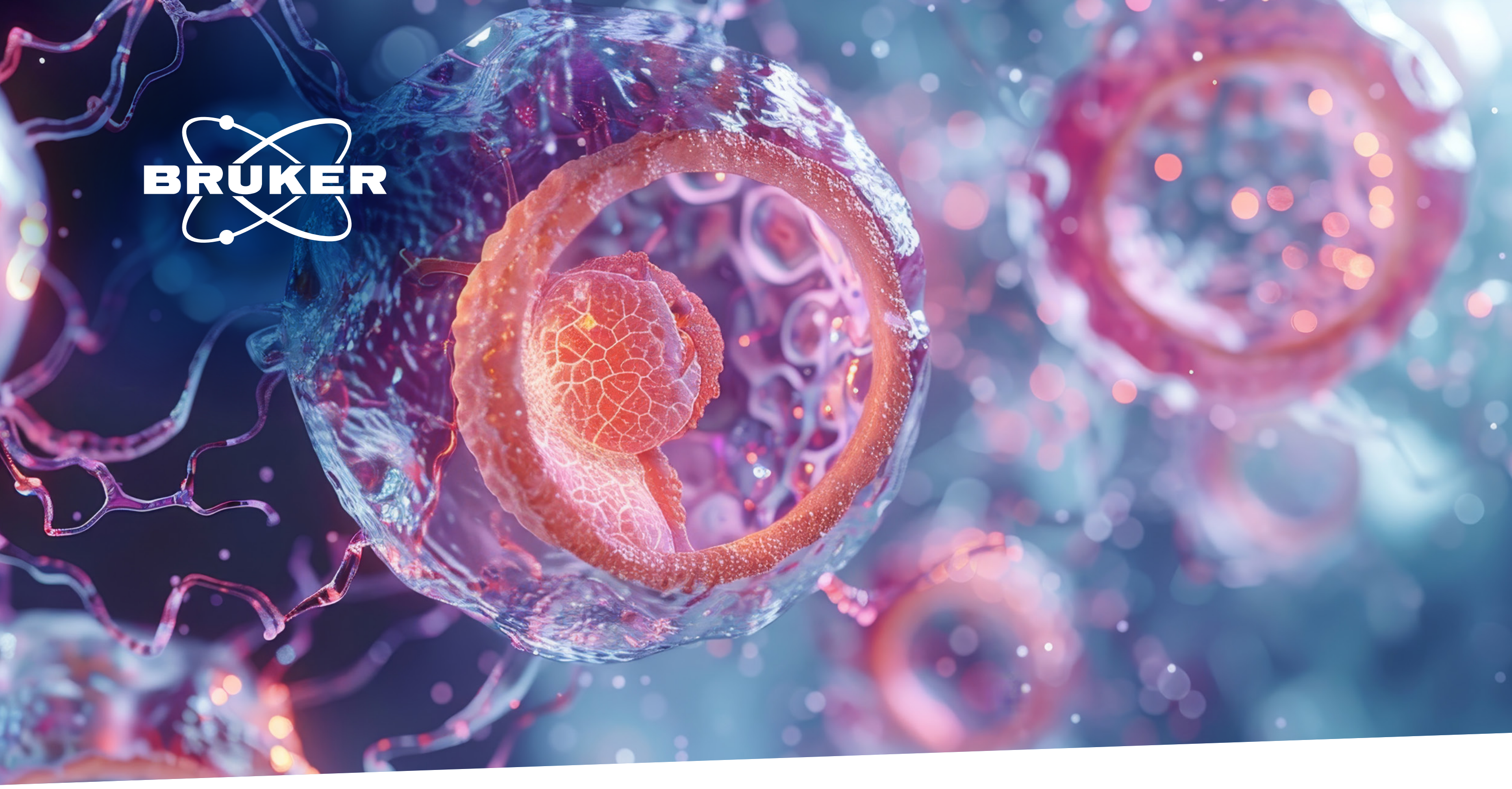

Rapid analysis of lipid nanoparticle components using the neofleX MALDI-TOF/TOF platform

The novel neofleX™ MALDI-TOF/TOF platform was used for the rapid analysis of all four typical components in a lipid nanoparticle (LNP) formulation. Characterization of the PEGylated lipid component uncovered four minor PEG distributions in addition to the main one containing more than 40 oligomers.

Introduction

LNPs have demonstrated significant clinical efficacy as drug delivery systems for mRNA vaccine payloads. As the premier non-viral delivery vehicles for gene therapy, LNPs are composed of four main components: PEGylated lipid, ionizable lipid, helper phospholipid, and cholesterol. These components work synergistically to protect and encapsulate mRNA molecules. In particular, PEGylated lipids enhance the colloidal stability of LNPs in biological fluids and play a critical role in modulating LNP uptake by filter organs [1]. Previously MALDI and ESI trapped ion mobility spectrometry (MALDI-TIMS and ESI-TIMS) were shown to be effective workflows for thorough LNP component analysis [2, 3]. This work focuses on rapid characterization of the LNP components using the neofleX benchtop axial MALDI platform.

Keywords:
Lipid nanoparticles,
LNP components,
PEGylated lipids,
MALDI, neofleX

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Methods

The following LNP components were used in this study: DMG-PEG 2000 (a PEGylated lipid), DOTAP (an ionizable lipid), DSPC (a helper phospholipid), and cholesterol (Figure 1). DSPC, DOTAP, and DMG-PEG 2000 were purchased from Avanti Polar Lipids (Alabaster, AL) and cholesterol from Sigma (St. Louis, MO). DSPC was dissolved in 50% methanol/50% THF, and other LNP components were dissolved in THF. An LNP component mixture was prepared with a 20 mg/mL solution of DHB in THF as a matrix. The mixture was generated by mixing DMG-PEG 2000, cholesterol, DOTAP, and DSPC solutions in 100:40:1:2 molar ratio. DMG-PEG 2000 was prepared with a 20 mg/mL solution of CHCA in THF as a matrix and a 2 mg/mL solution of sodium trifluoroacetate as a cationizing additive. The measurements were performed using a neofleX MALDI-TOF/TOF benchtop mass spectrometer (Bruker Scientiifc, Billerica, MA) equipped with a a smartbeam 3D 10 kHz laser. The spectra were acquired in reflector mode, and 4000 laser shots per spectrum were collected. PEGylated lipid spectra were post-processed in PolyTools software.

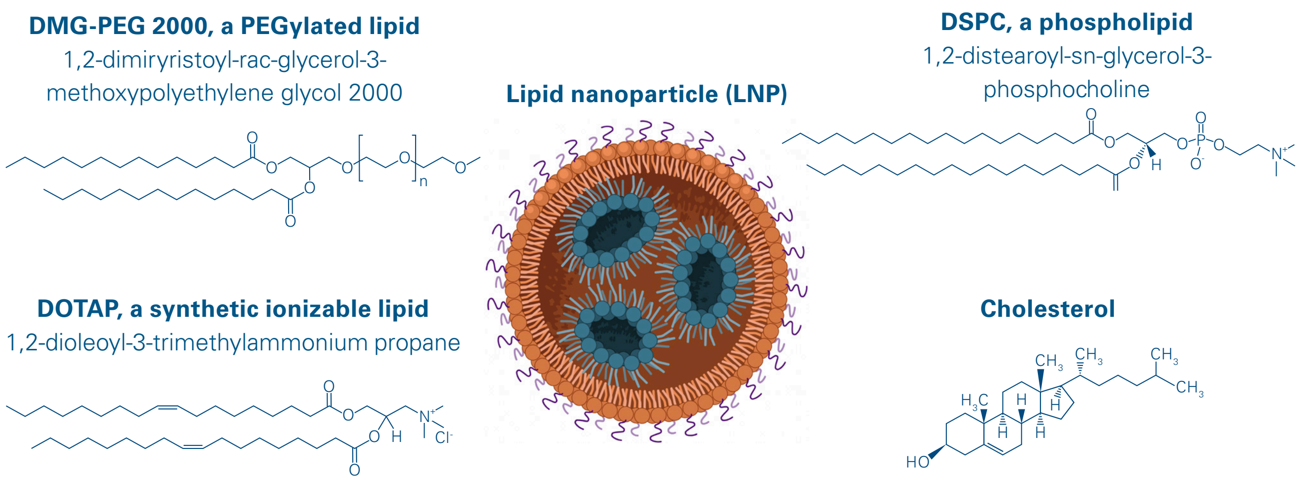


Figure 1
Chemical structures of lipid nanoparticle components

Results

Accurate mass measurements of the LNP components in the mixture resulted in mass errors less than 2 ppm for the three components. Cholesterol was measured as $[M\text{-}H_2O+H]^+$ ion, DOTAP as $[M]^+$ ion, and DSPC as $[M+H]^+$ ion (Figure 2).

Detailed analysis of DMG-PEG 2000 showed several minor PEG distributions in addition to the major one (Figure 3). The subsequent post-processing in PolyTools software revealed the main distribution in red with Mn 2519 Da containing more than 40 oligomers and four minor distributions containing more than 10 oligomers each (Figure 4A, 4C). The four minor distributions were easily recognizable in the remainders of Kendrick mass (RKM) plot and had Mn values in the range of 2302-2497 Da (Figure 4B, 4C).

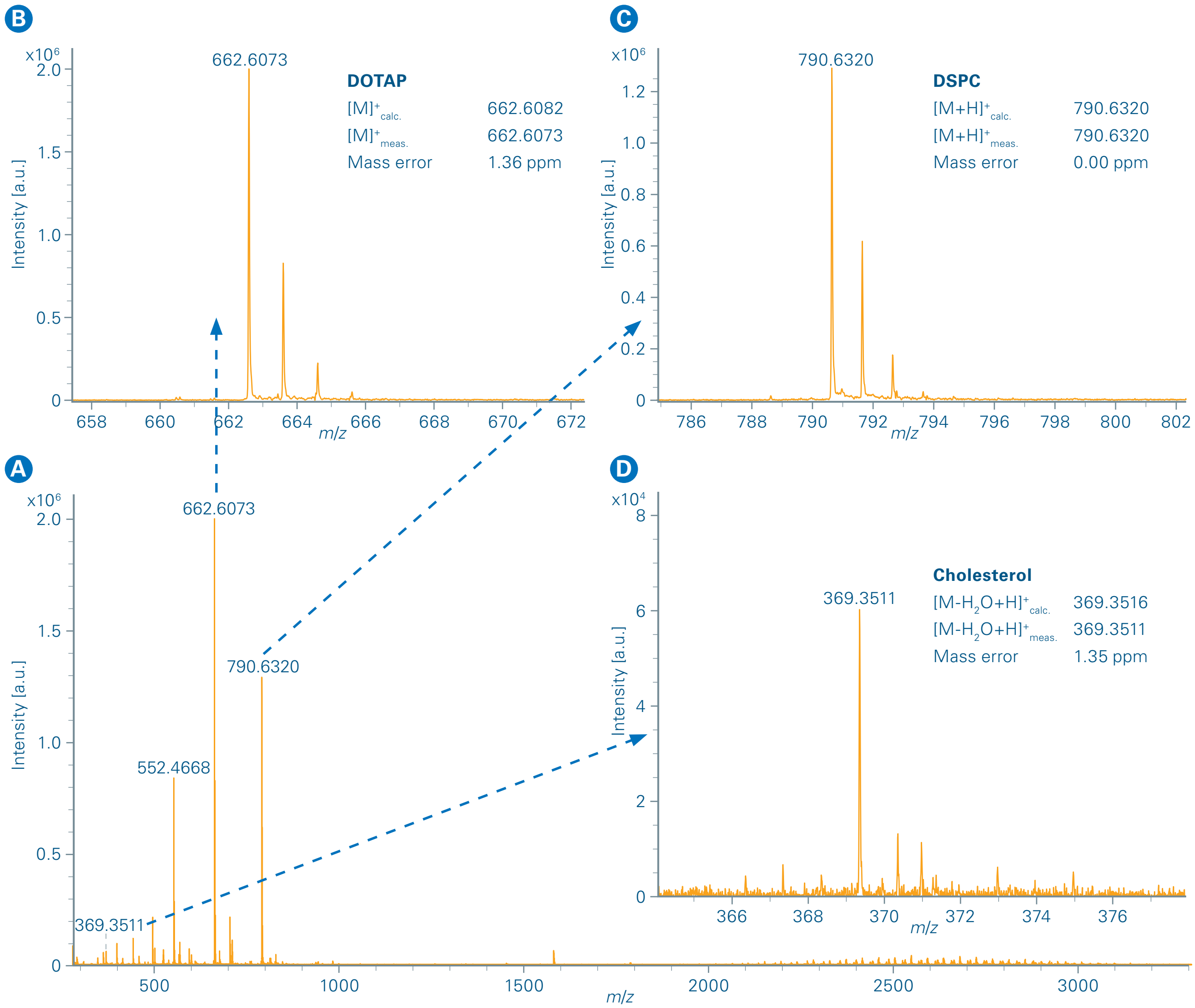


Figure 2

MALDI-TOF spectrum of the LNP component mixture (A) demonstrating accurate mass measurements of DOTAP at *m/z* 662.6082 (B), DSPC at *m/z* 790.6320 (C), and cholesterol at *m/z* 369.3516 (D).

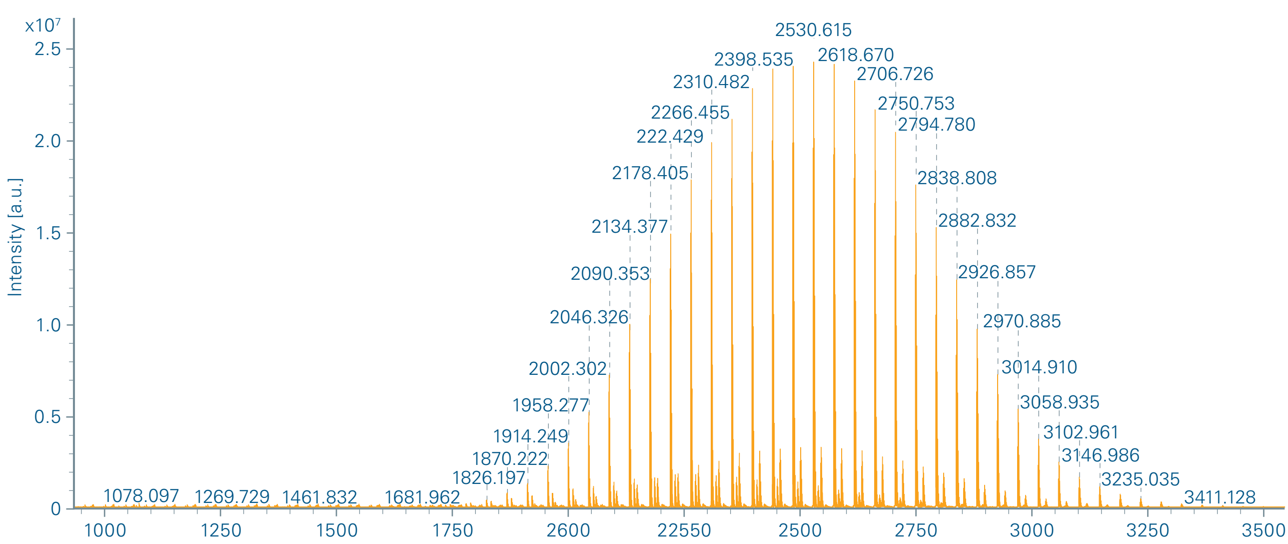
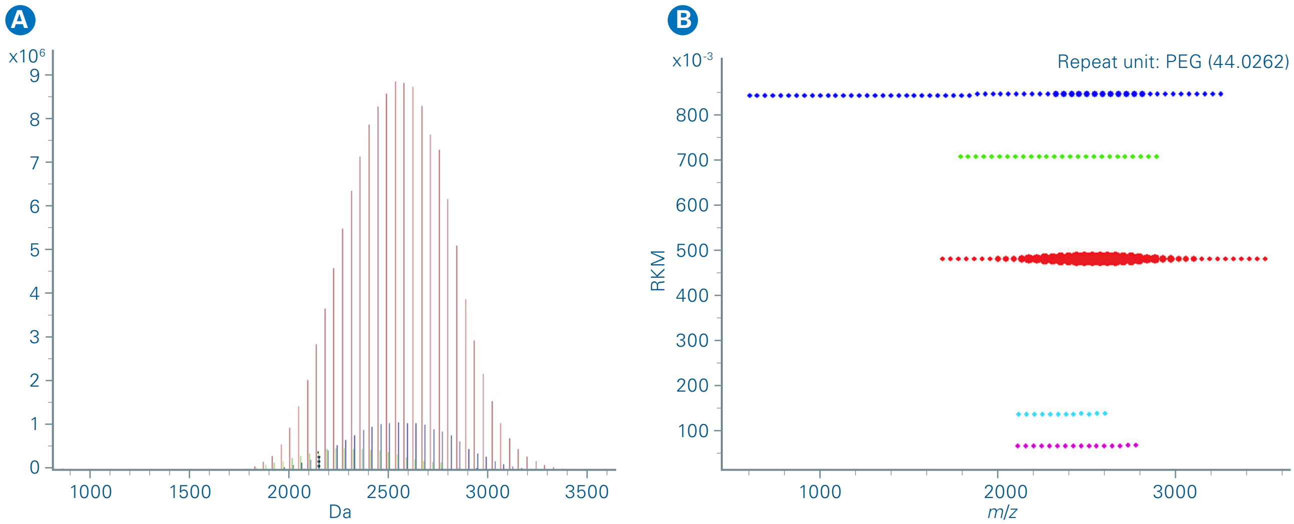


Figure 3

MALDI-TOF spectrum of DMG-PEG 2000



C

n		ser.	rep.unit	orig. resid.	resid.	end1	end2	cation	Mn	Mw	pd	DP	% I.	cnt	Kendrick
☐	1	1	PEG	21.1215	-0.01344	C31H59O5	CH3	Na	2519.45	2545.13	1.01019	57.2261	80.7	42	☑
☐	2	2	PEG	31.0571	9.92222	C31H59O5	CH3	Na	2302.22	2327.74	1.01108	52.2921	4.7	26	☑
☐	3	3	PEG	37.0489	15.9140	C31H59O5	CH3	Na	2496.95	2542.10	1.01808	56.7151	10.7	61	☑
☐	4	4	PEG	3.00942	25.9007	C31H59O5	CH3	Na	2408.89	2424.91	1.00665	54.7149	0.4	16	☑
☐	5	5	PEG	6.09511	28.9864	C31H59O5	CH3	Na	2326.69	2335.86	1.00394	52.8479	0.3	12	☑

Figure 4

Postprocessing of the DMG-PEG 2000 spectrum in PolyTools software established five PEG distributions (A) visualized in the remainders of Kendrick mass plot (B) and characterized with Mn, Mw, polydispersity, and degree of polymerization (C).

Conclusions

- The MALDI-TOF workflow is a rapid approach to characterize all four LNP components ranging from cholesterol to PEGylated lipids.
- Using MALDI to analyze PEGylated lipids enables precise determination of Mn, Mw, and polydispersity.
- Characterizing PEGylated lipids with MALDI aids in detecting minor polymer distributions, which is crucial for quick assessment of the material's purity.

References

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[4] The LNP image was created with BioRender.com.

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