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Analysis of Lipid Nanoparticle Components by MALDI Trapped Ion Mobility Spectrometry

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Introduction

Lipid nanoparticles (LNPs) were proven to be clinically effective drug delivery systems for mRNA vaccine payload. They are the most important non-viral delivery vehicles for gene therapy. LNPs usually include four components: a PEGylated lipid, an ionizable lipid, a helper phospholipid, and cholesterol. These components together protect and encapsulate mRNA molecules. PEGylated lipids play an important role in improving the colloidal stability of LNPs in biological fluids and regulating the LNP uptake by filter organs. Previously ESI trapped ion mobility spectrometry (ESI-TIMS) was demonstrated to be a very effective platform for LNP component characterization. This study focuses on thorough characterization of LNP components by MALDI-TIMS that provides complementary data to the ESI-based approach.

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). An LNP component mixture and three individual small molecule LNP components were prepared with a solution of DHB in THF as a matrix. The mixture was generated by mixing DMG-PEG 2000, cholesterol, DOTAP, and DSPC solutions in 100:20:1:1 molar ratio. DMG-PEG 2000 was prepared with a solution of CHCA in THF as a matrix and sodium trifluoroacetate as a cationizing additive. The measurements were performed with TIMS OFF and ON using a timsTOF fleX mass spectrometer equipped with a dual ionization ESI/MALDI source, a TIMS cartridge, and a smartbeam 3D 10 kHz laser. MALDI spectra were acquired using 2000 laser shots in TIMS ON mode and 4000 laser shots in TIMS OFF mode. PEGylated lipid spectra were post-processed in PolyTools software.

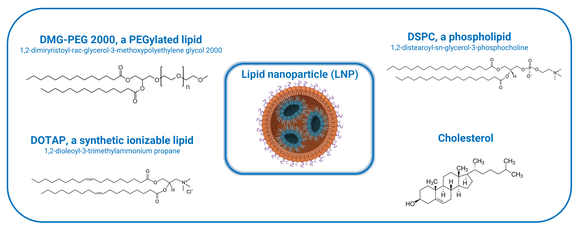

Figure 1. Lipid nanoparticle components

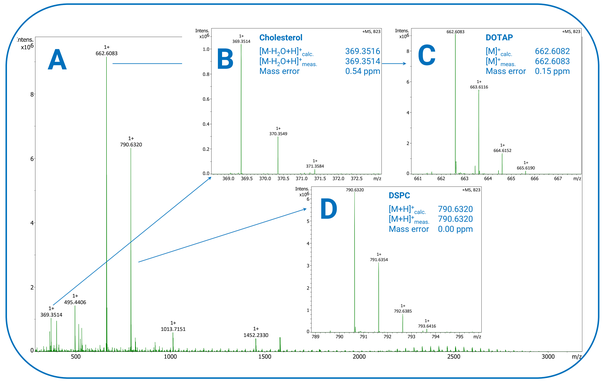

Figure 2. MALDI spectrum of the LNP component mixture (A) showing accurate mass measurements of cholesterol at m/z 369.3516 (B), DOTAP at m/z 662.6082 (C), and DSPC at m/z 790.6320

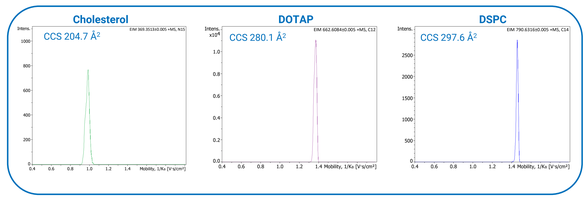

Figure 3. Extracted ion mobilograms from MALDI-TIMS spectra of LNP components showing measured CCS values

Results

- Cholesterol was measured as $[M\text{-}H2O+H]^+$ ion, DOTAP as $[M]^+$ ion, and DSPC as $[M+H]^+$ ion in the LNP component mixture. High resolution accurate mass measurements resulted in mass errors less than 1 ppm for these three components (Figure 2).
- The measurements of the individual LNP components and the mixture were repeated in TIMS ON mode to obtain collisional cross section (CCS) values. The mobilograms of the LNP components allowed determining the CCS values for cholesterol as 204.7 Å², for DOTAP 280.1 Å², and for DSPC 297.6 Å² (Figure 3).
- Ultrahigh resolution MALDI spectrum of DMG-PEG 2000 showed multiple minor polymer distributions in addition to the main one (Figure 4A). The resolution of many oligomers exceeded 90,000. For example, the oligomer at m/z 2574.6468 had resolution 94,214 (Figure 4B).

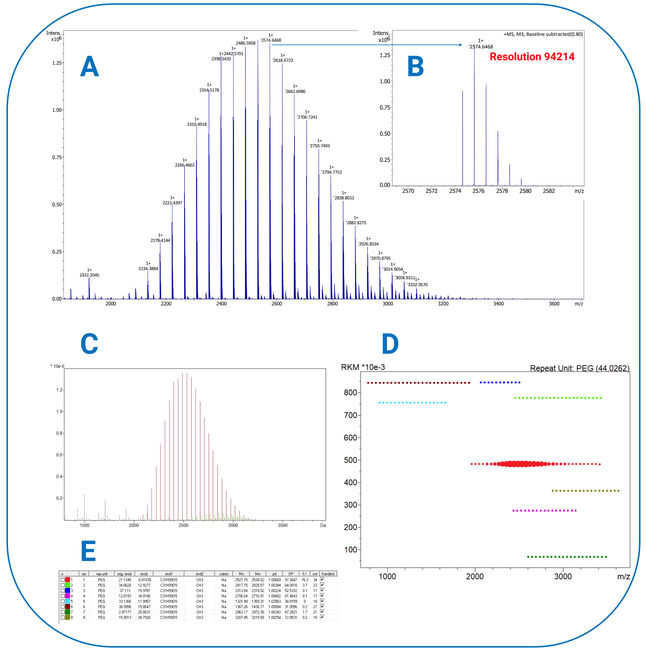

Figure 4. MALDI spectrum of DMG-PEG 2000 (A) showing ultrahigh resolution of a selected oligomer at m/z 2574.6468 (B), PEG distributions calculated in PolyTools software (C), residual Kendrick mass plot of 8 PEG distributions (D), and the table with calculated Mn, Mw, and polydispersity values (E)

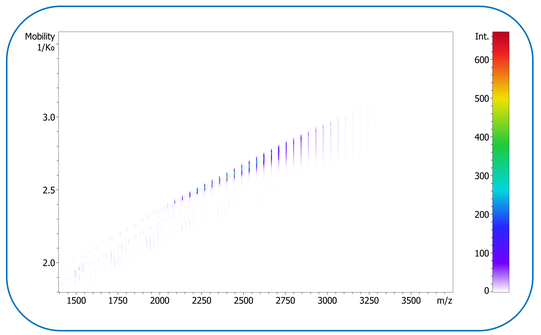

Figure 5. A heat map view of the MALDI-TIMS spectrum of DMG-PEG 2000

- More detailed post-processing in PolyTools revealed a major distribution with Mn 2521 and more than 30 oligomers. In addition, the spectrum contained 7 other minor polymer distributions containing more than 10 oligomers that were easily discernible in the residual Kendrick mass plot (Figure 4C-4E). Four of those minor distributions had Mn values higher that the major one ranging from 2706 to 3207 (Figure 4E).
- The MALDI-TIMS spectra of DMG-PEG 2000 were acquired after lowering TIMS IN cartridge pressure to 2.0 mbar. The spectrum in Figure 5 is shown as a heat map. The CCS value for the oligomer at the center of the polymer distribution ($[M+Na]^+$ ion at m/z 2530.6262) was determined to be 536.7 Å².

Summary

- All four LNP components including DMG-PEG 2000, a PEGylated lipid, DSPC, a phospholipid, DOTAP, an ionizable lipid, and cholesterol were analyzed using MALDI-TIMS workflow to determine the accurate m/z and CCS values.
- MALDI analysis of the PEGylated lipid revealed 7 minor polymer distributions in addition to the major one. Four of those minor distributions had Mn values higher than the major one.

Conclusions

- MALDI-TIMS workflow is an effective approach to characterize all LNP components ranging from cholesterol to PEGylated lipids.
- Characterization of PEGylated lipids by MALDI allows accurate determination of Mn, Mw, and polydispersity.
- MALDI analysis of PEGylated lipids allows straightforward detection of minor polymer distributions/impurities and their accurate characterization.
- MALDI-TIMS workflow applied to LNP components provides complementary data to ESI-TIMS workflow.

MALDI Pharma/Biopharma

The author declares no competing financial interest.
The LNP image was created with BioRender.com.