Determining the molecular weight of PEG standards by MALDI-TOF MS

Introduction

Poly(ethylene glycol) (PEG) is a water-soluble, non-toxic polymer used in a range of therapeutics, cosmetics, personal care products and many others. An important property of PEG is the molecular weight, which ultimately determines the properties of the material. An accurate method to determine molecular weight and characterise the structural properties of PEG is therefore essential.

Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) is such a technique as it allows for an absolute measurement of the molecular weight of each individual polymer chain in a sample, allowing for the polymer repeat unit and chain end groups to be determined. However, optimising the MALDI-TOF-MS sample prep method is critical to maximising the ionisation and MS detection. This application note outlines an optimised sample prep recipe for the MALDI-TOF-MS of PEG standards over a range of molecular weights , allowing for the quality of the PEG standards to be assessed.

Experimental details

- Instrument: *Bruker* Autoflex Speed
- The matrix was prepared by dissolving 15.8 mg of α-cyano-4-hydroxycinnamic acid (CHCA) in 1mL ethanol using an ultrasonic bath. The cationising agent was prepared by dissolving 5.9 mg of sodium trifluoroacetate (NaTFA) in 1mL ethanol using an ultrasonic bath. The PEG standard samples were supplied in aqueous solution at ~2 mg/mL. The three components were pipette mixed in an Eppendorf in a 5:1:1 (v/v/v) ratio, and 0.5µL was spotted onto a ground steel target plate and allowed to dry before being inserted into the instrument for analysis.

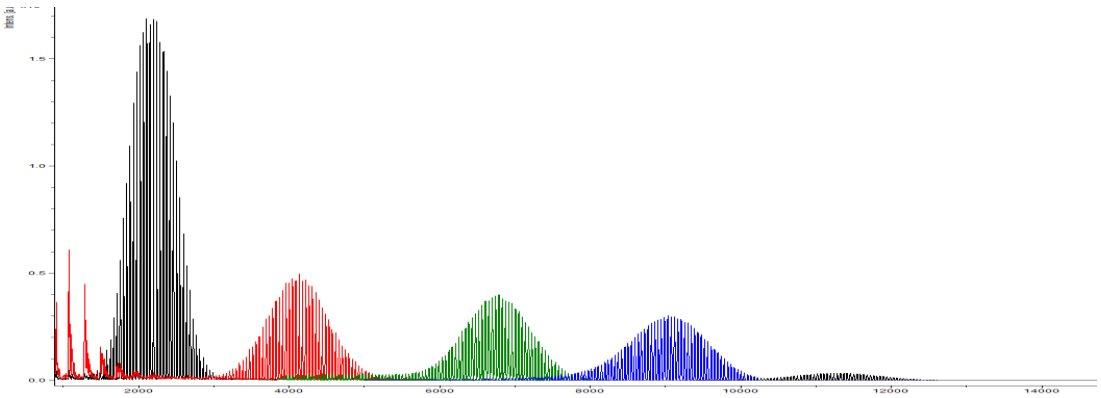

***Figure 1**: Overlaid MALDI-TOF-MS spectra of six PEG standards, ranging from 2,000 Da to 10,000 Da, clearly showing the distribution of chain lengths in each standard solution.*

Results

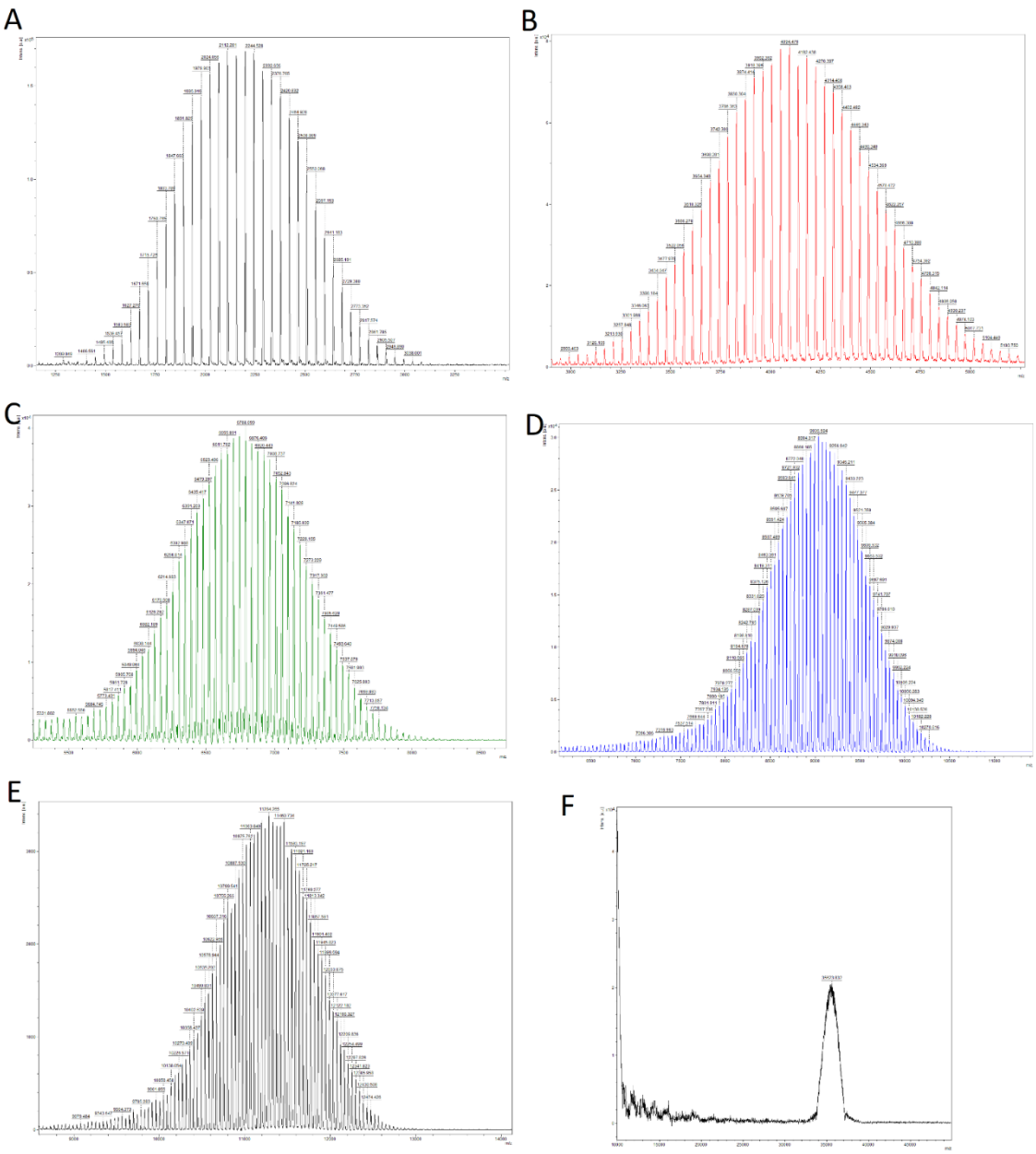

Figure 2: *MALDI-TOF-MS spectra of (A) PEG 2000 (B) PEG 4000 (C) PEG 6000 (D) PEG 8000 (E) PEG 10000 and (F) PEG 35000*

Code	Mp / Da (from manufacturer)	Mn / g mol-1 (as detected by MALDI-TOF-MS)
A	2,000	2,220
B	4,000	3,990
C	6,000	6,740
D	8,000	9,010
E	10,000	11,000
F	35,000	35,000

Table 1: *Comparison between manufacturer supplied MW for PEG standards, and experimentally determined Mn*

Conclusion

- Sample preparation protocols for the MALDI-TOF-MS analysis of PEG have been optimised such that molecules with molecular weights <35,000 Da can be readily detected by the technique.
- Resolution of individual chain length related ions is obtained up to 10,000 Da, allowing for the determination of repeat unit.
- Experimentally determined average molecular weights by MALDI-TOF-MS are largely in agreement with the supplier labels on the bottle, indicating that the PEG standards have not degraded, and providing more accurate information of the actual molecular weight of the polymer sample.
- The peak spacing is 44 Da in all cases, which correlates with the PEG monomer unit, and confirms the sample purity.

- [1] Li, Y.J.; Hoskins, J.N.; Sreerama, S.G.; Grayson, S.M., **Macromolecules**, 43, 14, 2010, 6225-6228
- [2] https://maldi.nist.gov/ accessed 24th April 2019.