OVERVIEW

- Poly (ethylene glycols) (PEGs) and its derivatives are widely used in the biopharmaceutical industry for the delivery of therapeutic drugs.
- Mass spectrometric analysis of high molecular weight PEGs is very challenging due to the great complexity and diversity of the materials.
- The use of gas phase ion-molecule reactions for the analysis of PEG and PEGylated proteins has precedent within the biopharmaceutical industry today.
- This presentation describes an improved method to accurately measure the average molecular weight of PEGs using ion-mobility time-of-flight mass spectrometry coupled with gas-phase ion-molecule reactions.
- The method is developed based on a simple and flexible modification to Synapt™ HDMS™ instrument with the added ability to use gas-phase ion-molecule reaction to effectively couple the high-performance tandem mass spectrometer without sacrificing any high performance attributes of the original instrument.

SYSTEM CONFIGURATION FOR ION/MOLECULAR REACTIONS

Sample preparation. Samples were prepared at analyte concentrations of 200 ng/μL in a 500 μL aliquot of water. The analytes were directly infused into the ESI source at 10 μL/min by a syringe pump.

MS Conditions
- MS Source: Waters Synapt™ HDMS™
- Capillary Voltage: 3.0 kV
- Capillary Temperature: 300°C
- Source Temp: 100°C
- Desolvation Temp: 350°C
- Desolvation Gas: N2 (1.5 L/min)
- Scan Range: m/z 20-2000
- Mass Range: -50 m/z to +50 m/z
- IMS Cell Voltage: 240 mV

Figure 1. Instrument (Synapt HDMS™) schematic illustrating the Triwave technology embedded in a high-performance Q-ToF tandem mass spectrometer (A). Schematic diagram showing the modified gas line configuration for performing ion/molecule reaction inside the TRAP cell of Synapt HDMS mass spectrometer (B).

ChiNG STRIPPING ANALYSIS OF PEG 4450

Figure 2. ESI-TOF mass spectrum of PEG 4450 without IMS separation. The isotopic resolution for peaks in the spectrum (inset) demonstrating the sufficient resolving power of the instrument at the collected m/z window. The isotopic resolution permits the determination of the number of charges that each oligomer holds and their charge states distribution in the spectrum. As depicted in the Figure above, spectrum contain several charge states ranging from 2+ to 4+ that are due to the attachment of multiple cations (e.g., Na+, K+, and H+) to each PEG oligomer, generating many different ion series in the spectrum. Consequently, the average molecular weight cannot be readily determined.

Figure 3. ESI IMS TOF analysis of PEG 4450 using Synapt HDMS. Driftscope shows the gas-phase separation power of Synapt in the analysis of PEG 4450. Components with different charge states (1+ to 4+) are separated via ion-mobility, thus enabling the examination of different (minor) components in the PEG materials.

Figure 4. ESI-TOF mass spectrum of PEG 4450 after reaction of all charge states in the trap cell of the instrument with super base, 1,8-diazabicyclo[5.4.0]undec-7-en (DBU). The inset shows the expanded view of the best fit to a Gaussian distribution.

Figure 5. ESI-TOF mass spectrum of PEG-aldehyde 20 kDa before (A) and after (B) reaction with DBU in the TRAP cell of the Triwave. The deconvoluted zero-charge mass spectrum obtained by adding together the distributions from the 4+ and 5+ envelopes. The Gaussian fit of the distribution is shown as a dashed line. The zero-charge distribution provides an average MW of 21,352 Da and a MW of 1,228 Da. This MW value is in close agreement with the literature value measured by similar MS approach as well as an NMR method (21,520 Da).

CONCLUSIONS

- We have demonstrated a method to accurately measure the average MWS and MW distributions of large PEG using ion-molecule reaction inside a Waters Synapt™ HDMS™ mass spectrometer.
- With many unique capabilities of the instrument, such as ion-mobility separation, the ability to perform ion-molecule reaction inside Synapt has truly expanded the applicability of the instrument. It is conceivable that this configuration can be readily applied to many challenging analytical tasks in pharmaceutical industry.

References:

Figure 3 in Anal. Chem. 80, 2050-2059 (2008).