

MASS SPECTROMETRIC ANALYSIS OF PEGS AND PEGYLATED PROTEINS BY A COMBINED APPROACH OF ION-MOBILITY SEPARATION AND CHARGE REDUCTION

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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 and PEGylated biotherapeutics 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, PEGylated proteins and peptides has precedent within the biopharmaceutical industry today¹.
- This presentation describes an improved method to accurately measure the average molecular weight of PEGs, PEGylated peptide and protein products using ion-mobility time-of-flight mass spectrometry coupled with gas-phase ion-molecule reactions. Moreover, a PEGylation site mapping strategy is described.
- The method is developed based on a simple and flexible modification to SYNAPT™ HDMS™ System with off-the-shelf components, thus enabling a gas-phase ion-molecule reaction to be effectively coupled to the high-performance tandem mass spectrometer without sacrificing any high performance attributes of the original instrumentation².

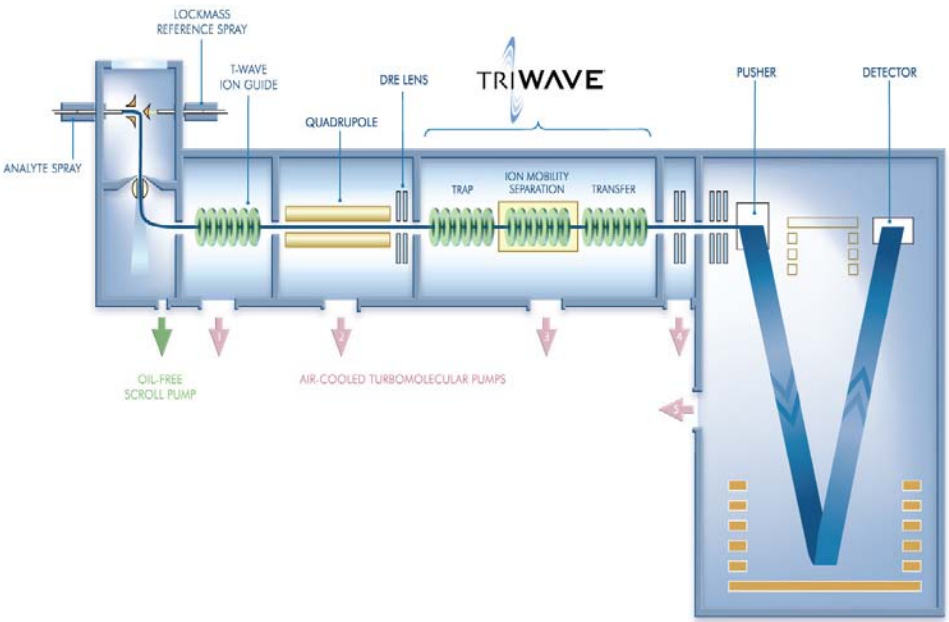


Sample preparation. Samples were prepared at analyte concentrations of 1–2 mg/mL either in a 50/50 (v/v) solution of water and methanol or 10 mM ammonium acetate and methanol. The solutions were directly infused into the ESI source at 10 µL/min by a syringe pump.

MS Conditions
MS System: Waters SYNAPT™ HDMS™
Ionization Mode: ESI Positive
Capillary Voltage: 3.0kV
Cone Voltage: 40V
Source Temp: 100°C
Desolvation temp: 250°C
IMS Cell Pressure: 0.5 mbar (n2)
Wave Pulse Height: 5-14 V
RF Amplitude: 320 V (Peak-to-Peak)
IMS Cell Velocity: 240 m/s

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ADDING ION MOBILITY TO A Q-TOF MS



- The ion-mobility section is comprised by three Traveling Wave-enabled Stacked Ring Ion Guides (SRIG).
- The TRAP ion guide is used to accumulate ions and release them as packets for ion mobility separation.
- The TRANSFER ion guide conveys the mobility separated ions to the oa-TOF for mass analysis.
- Gas phase ion-molecule reactions can take place either in the TRAP cell or in the TRANSFER cell.

SYSTEM CONFIGURATION FOR ION/

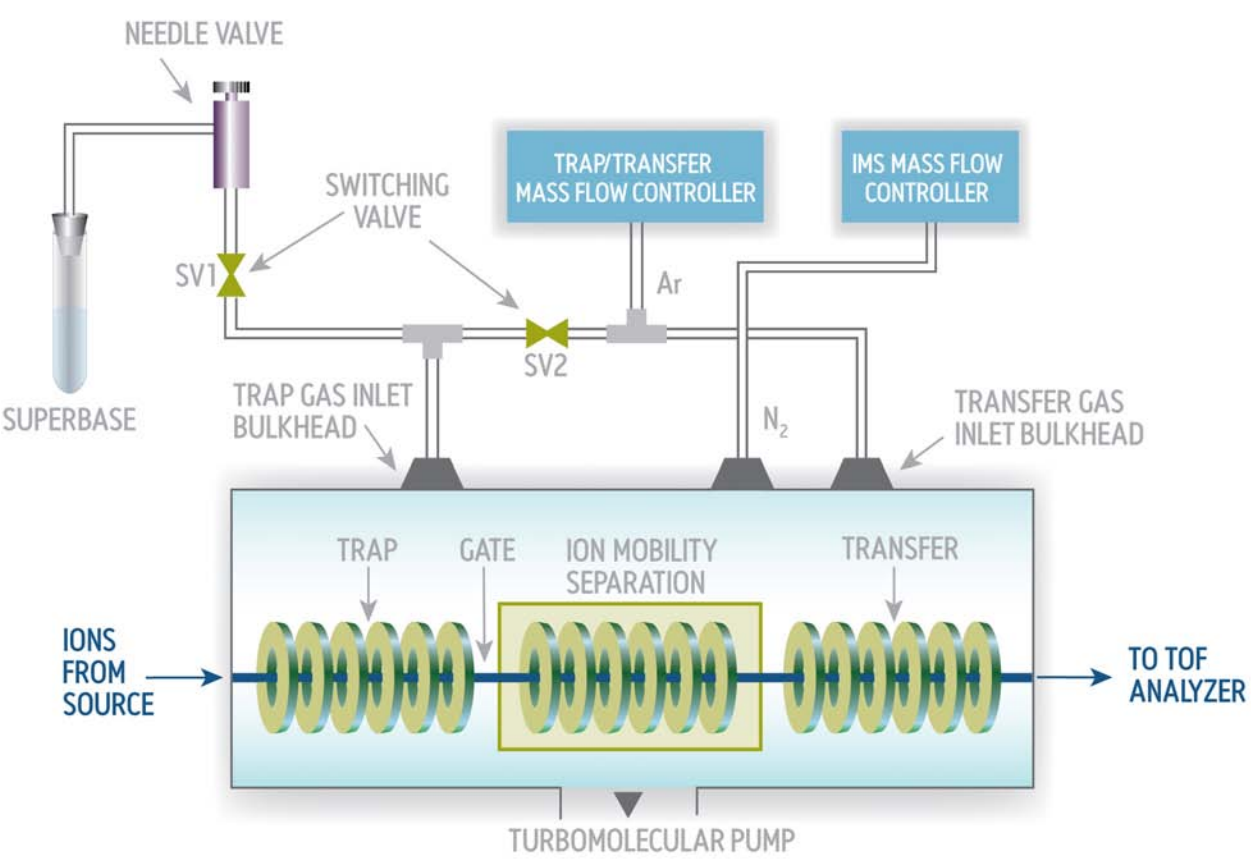


Figure 1. Instrument (SYNAPT HDMS) schematic illustrating the Triwave™ Technology embedded in a high-performance oa-TOF tandem mass spectrometer (A). Schematic diagram showing the modified gas line configuration for performing ion/molecule reaction inside the TRAP cell of SYNAPT HDMS Mass Spectrometer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

ESI-TOF ANALYSIS OF PEG 4450

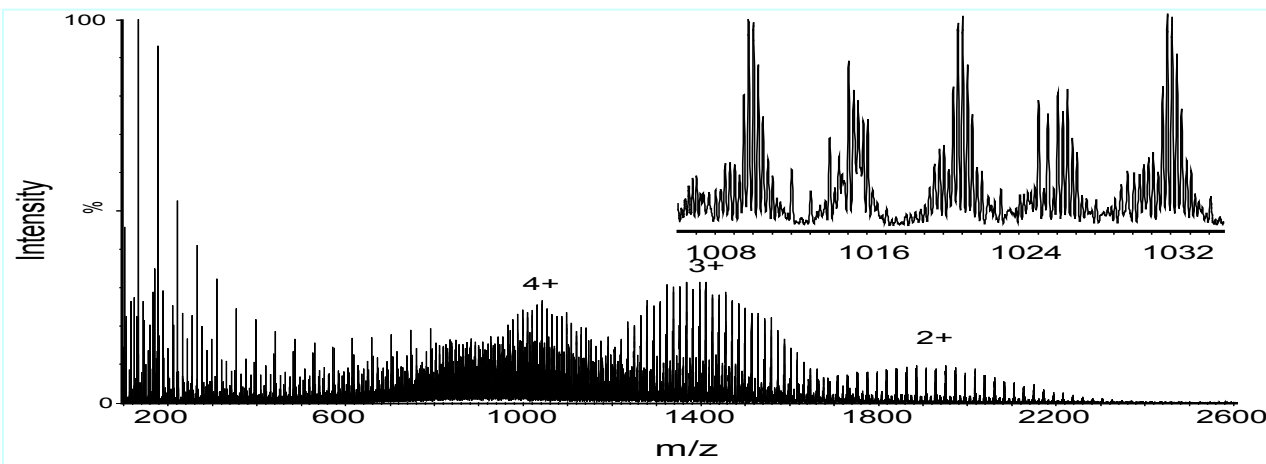


Figure 2. ESI-TOF mass spectrum of PEG 4450 without IMS separation. The isotopic resolution for peaks in the spectrum (inset) showing the sufficient resolving power of the instrument at the collected m/z window. The isotopic resolution permits the determination of the number of charges that each oligomer holds and their charge states distribution in the spectrum. Spectrum contains several charge states ranging from 2+ to 4+ that are due to the addition 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.

ESI-IMS-TOF ANALYSIS OF PEG 4450

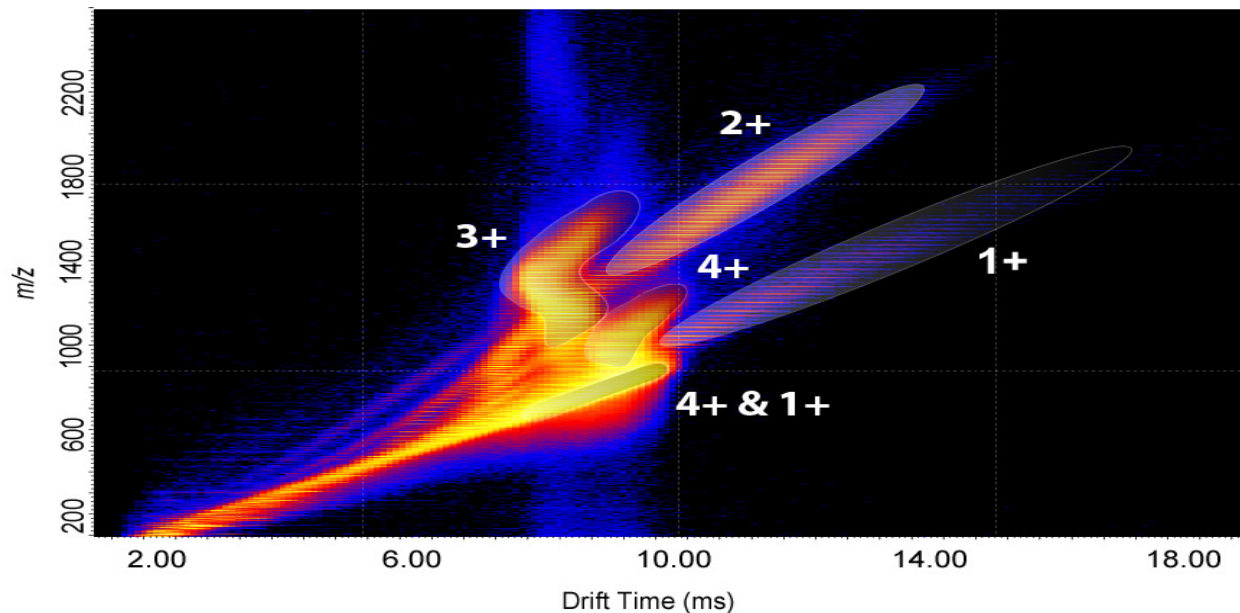


Figure 3. ESI IMS TOF analysis of PEG 4450 using SYNAPT HDMS. Driftscope shows the gas-phase separation power of Synapt in the analysis of PEG 4450. Components with different charge states (1+ to 4+) are separated via ion-mobility, thus enabling the examinations of different (minor) components in the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

Charge Stripping Analysis of PEG 4450

