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アプリケーションノート

Characterizing Polyethylene Glycol (PEG) by Synapt High Definition Mass Spectrometry (HDMS)

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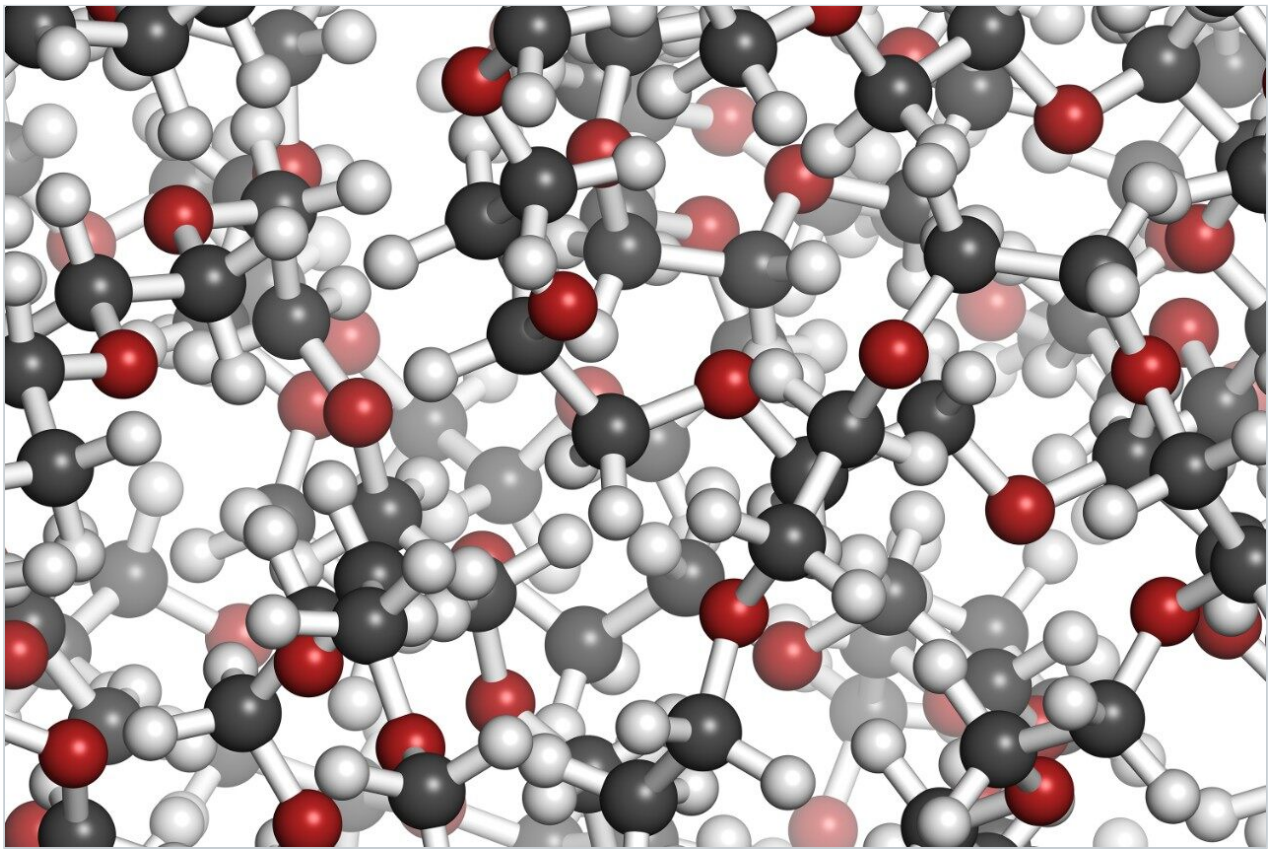

Abstract

Because of the complexity associated with PEG materials, PEG characterization by conventional methods has been extremely challenging. In this application note, we present a method to characterize PEG using the Waters Synapt High Definition MS (HDMS) system, a novel instrument that combines high-efficiency ion mobility (IMS) based measurements and separations with high performance tandem mass spectrometry.

Introduction

Polyethylene glycol (PEG) is a polymer composed of repeating subunits of ethylene oxide. PEG and its functionalized derivatives can be formed in linear or branched shapes with different molecular masses, resulting in significant material complexity and diversity.

Due to the many unique properties of PEG materials – highly water soluble, non-toxic – PEG is often attached to biopharmaceuticals (i.e. PEGylation) to improve pharmacological properties. It is critically important to determine the quality of a batch of PEG prior to attaching it to a biopharmaceutical. Attaching a low-quality batch of PEG to a biopharmaceutical leads to poor end product performance, and increases costs because the final product does not meet specifications.

Because of the complexity associated with PEG materials, PEG characterization by conventional methods has been extremely challenging. In this application note, we present a method to characterize PEG using the Waters Synapt High Definition MS (HDMS) system, a novel instrument that combines high-efficiency ion mobility (IMS) based measurements and separations with high performance tandem mass spectrometry. The additional ion mobility based gas-phase separation of the system provides a unique method to examine – in great detail – the composition of PEG materials. This better enables analysts to identify potential contaminants contained in the material and thus assess the quality of the material, providing for more confidence in the release of a PEGylated biopharmaceutical product.

Experimental

PEG 4450 was obtained from a Waters GPC molecular weight standard kit (part number WAT035711). PEG 20000 was purchased from Sigma [20% (w/v)]. The polymers were prepared at a concentration of 0.5% (w/v) in 50:50 acetonitrile/H_20 for mass spectrometric analysis. Samples were introduced to MS directly by infusion, using a syringe pump (Harvard Apparatus,

Holliston, MA) at a flow rate of 5 µL/min.

MS conditions

MS system:	Waters Synapt HDMS System
IMS gas:	N_2 gas
IMS gas pressure:	0.8 mbar
Pulse height:	Variable, 7 to 15 V
Ionization mode:	ESI positive
Capillary voltage:	3200 V
Cone voltage:	40 V
Desolvation temp.:	400 °C
Desolvation gas:	800 L/Hr
Source temp.:	150 °C
Acquisition range:	100 to 4000 m/z
Trap collision energies:	8 V

Results and Discussion

An electrospray ionization time-of-flight (ESI-TOF) mass spectrum of PEG 4450 [0.5% (w/v) in 50:50 H_2O/ACN solution] results in a distribution of several charge envelopes (Figure 1, left panel). Each charge envelope contains multiple peaks representing a molecular weight distribution of the material. The overlap between each of the charge states, the polydiperse nature of the material, and the presences of low molecular weight PEGs/contaminants all make the complete characterizations of the material via conventional ESI-TOF a formidable task to

undertake, even for a medium-size PEG.

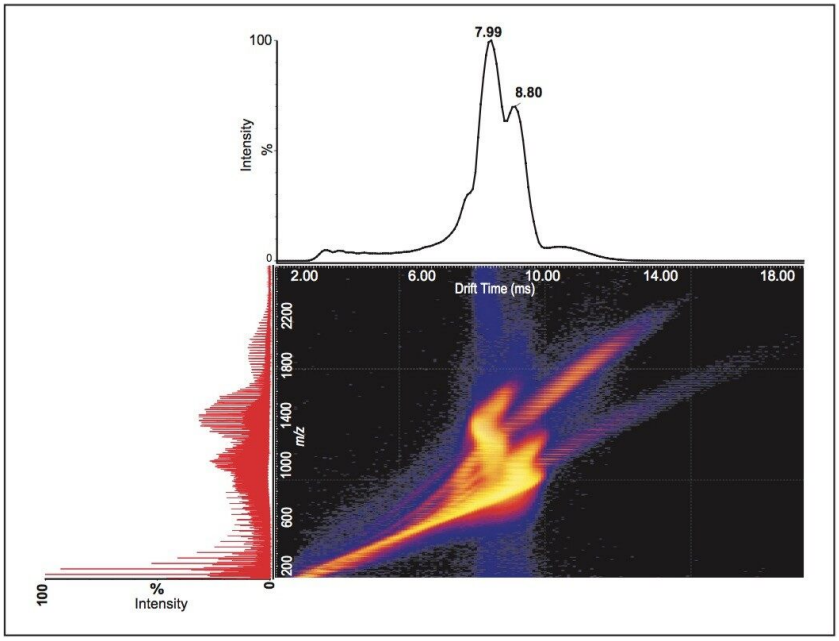

Figure 1. Data for analysis of PEG 4450 using the Synapt HDMS System, displayed in DriftScope Software. Each pixel represents an ion, with color representing its intensity (blue-low, to orange-high). To the left of the plot is the ESI-TOF spectrum without IMS separation. On the top of the DriftScope plot is the composite IMS spectrum from the projection of DriftScope on the drift time axis.

By analyzing the sample in HDMS mode (IMS-MS), Triwave Technology can be used to rapidly separate components in complex mixtures in tens of milliseconds. Here, we have taken advantage of this capability to separate complex PEG ions formed during the ionization process. In these experiments, the time required for IMS separations is <20 ms. Ions with different charge states, or different conformers of the same *m/z* ions, were readily resolved by IMS (Figure 2). The separation greatly simplifies the complexity of the spectrum such that some of the minor components in the samples that cannot be observed otherwise can be easily identified from the sample (Figure 3).

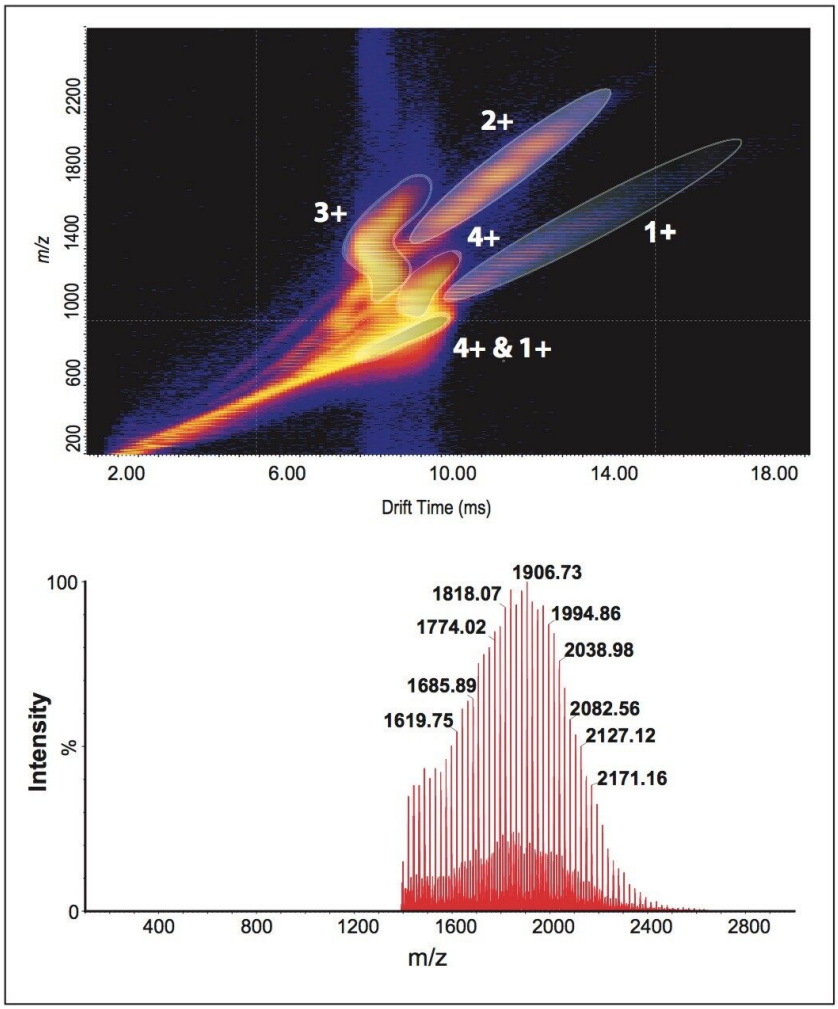

Figure 2. Analysis of PEG 4450 using HDMS. Top panel: HDMS data show the gas-phase separation power of the Synapt HDMS System in the analysis of PEG 4450. Components with different charge states are separated via ion mobility, thus enabling the examinations of different (minor) components in the PEG materials. (Bottom panel: Mass spectrum showing the ions with +2 charge state.)

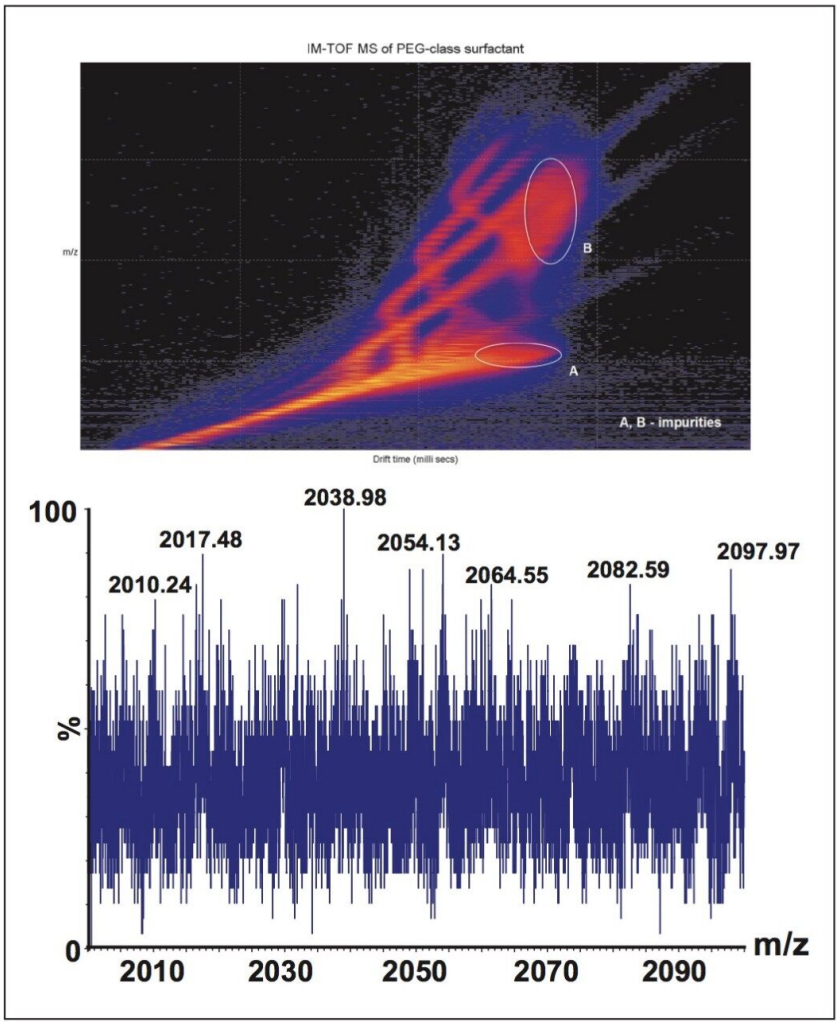

Figure 3. Analysis of PEG 20,000 using HDMS. Top panel: Using DriftScope Software, the HDMS data exhibit the separation of potential impurities (labeled as circle A and B) contained in the PEG materials from the rest of PEG components. These impurities would not be readily discovered without the gas-phase separations. Bottom panel: Zoomed mass spectrum showing the ions in circled region B. The mass difference between neighboring peaks indicated that they are not pure PEG material.

Conclusion

By employing IMS separations in HDMS mode with the Synapt HDMS System, the general molecular weight distribution of PEG material used in biopharmaceuticals can be rapidly

assessed and potential contaminants in the materials can be quickly identified. Fast, more detailed characterizations of PEG are readily achieved. With the level of analytical detail provided by the Synapt HDMS System, analysts can be more confident that their PEGylated biopharmaceutical product will pass quality control tests towards product release.

The consequence of attaching a low-quality batch of PEG to a therapeutic protein is failure of the bioactivity test and the need to scrap a batch of very expensive product.

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720002384, September 2007