Waters Alliance™ Systems for LC/MS
ESI/APCI Applications

Part 2: Factors that Affect the Analysis of Proteins by Electrospray LC/MS

Highlights: Choices that are made during set up and sample acquisition using a benchtop LC/MS system greatly affect the resulting data. For Parts 1 & 2 of this experiment, three elements utilized during the analysis of a horse heart myoglobin sample were investigated. Part 1 examined the effect of Cone Voltage ramping and post-acquisition software processing of the mass spectral data. Part 2 looks at the effect of calibrant used for mass axis calibration when comparing the theoretical vs. calculated molecular weight of a multiply-charged protein molecule using both the raw data and the deconvolution software-derived data.

Experimental: The system used was a Waters Alliance™ LC/MS System Featuring the Platform LCZ Detector. The system was controlled using the MassLynx™ NT operating system. A myoglobin solution (approximately 200 ng/uL) was infused into the mass spectrometer source and analyzed under positive electrospray conditions. Various factors affecting the raw and derived data were examined. The two post-run software processing options that were studied were Transform and Maximum Entropy (MaxEnt). Transform identifies any components present in a multiple charge envelope for a large molecule, and then identifies the charge state and/or components for each ion. Lastly, the Transform algorithm calculates the true molecular weight of the uncharged molecule. MaxEnt further aids in the enhancement of complex spectra. MaxEnt deconvolutes overlapping multiply charged spectra and yields enhanced resolution of m/z data within the mass range of the mass spectrometer (4000 Da) to give unambiguous assignment of the charge state of multiply charged peaks.

Sodium Iodide vs. Myoglobin Calibration:

When analyzing large, multiply-charged biomolecules such as proteins, the use of a protein to calibrate the mass axis is recommended. Figures 1 & 2 compare the raw data and MaxEnt output of Myoglobin for both sodium iodide (Figure 1) and Myoglobin-derived (Figure 2) calibration. While the raw data in both cases looks very similar, the deconvoluted molecular ion (inset in both traces) is closer to the theoretical value of 16951.5 when a protein (myoglobin, in this case) is used as a calibrant.

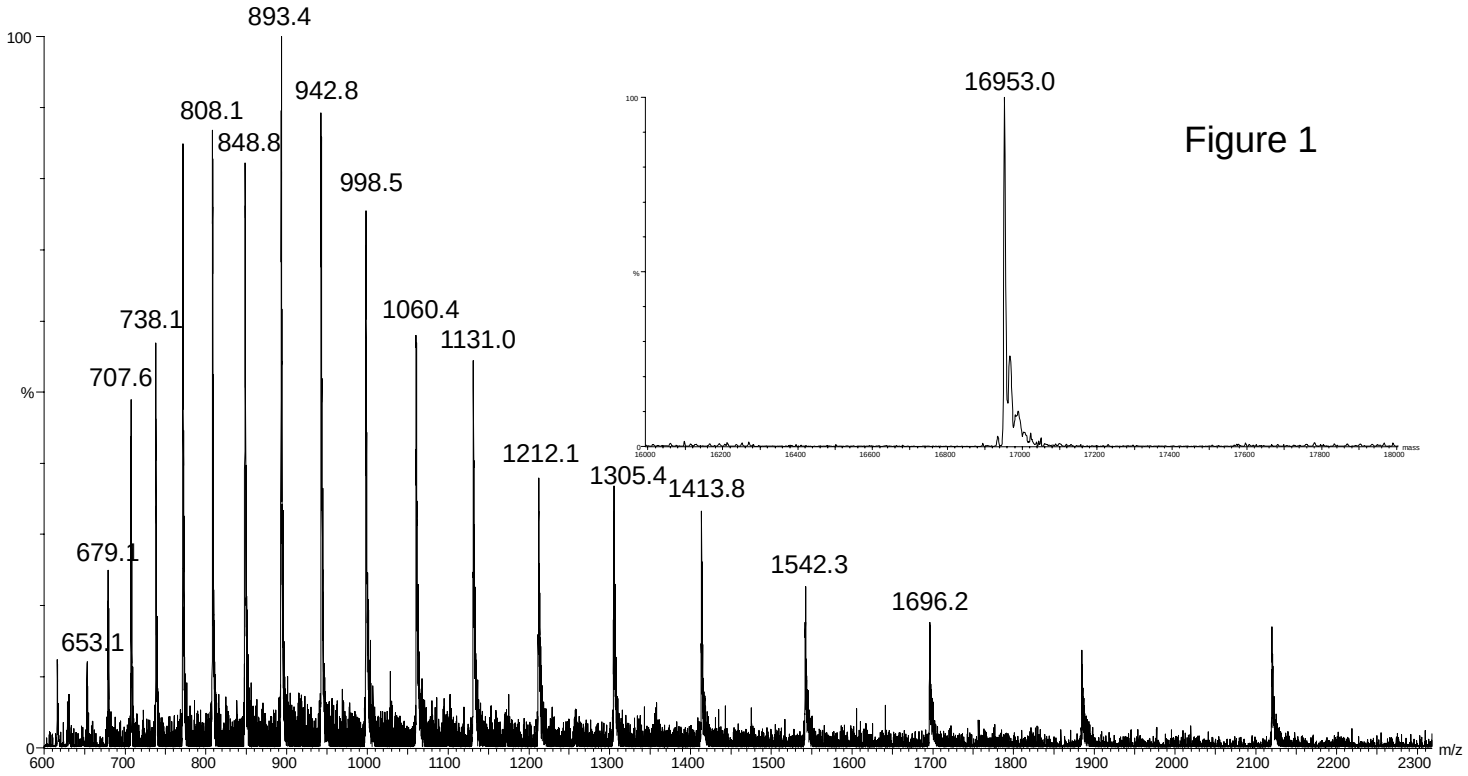

Figure 1

Figure 1. MaxEnt output from Myoglobin infusion calibrated with Sodium Iodide.

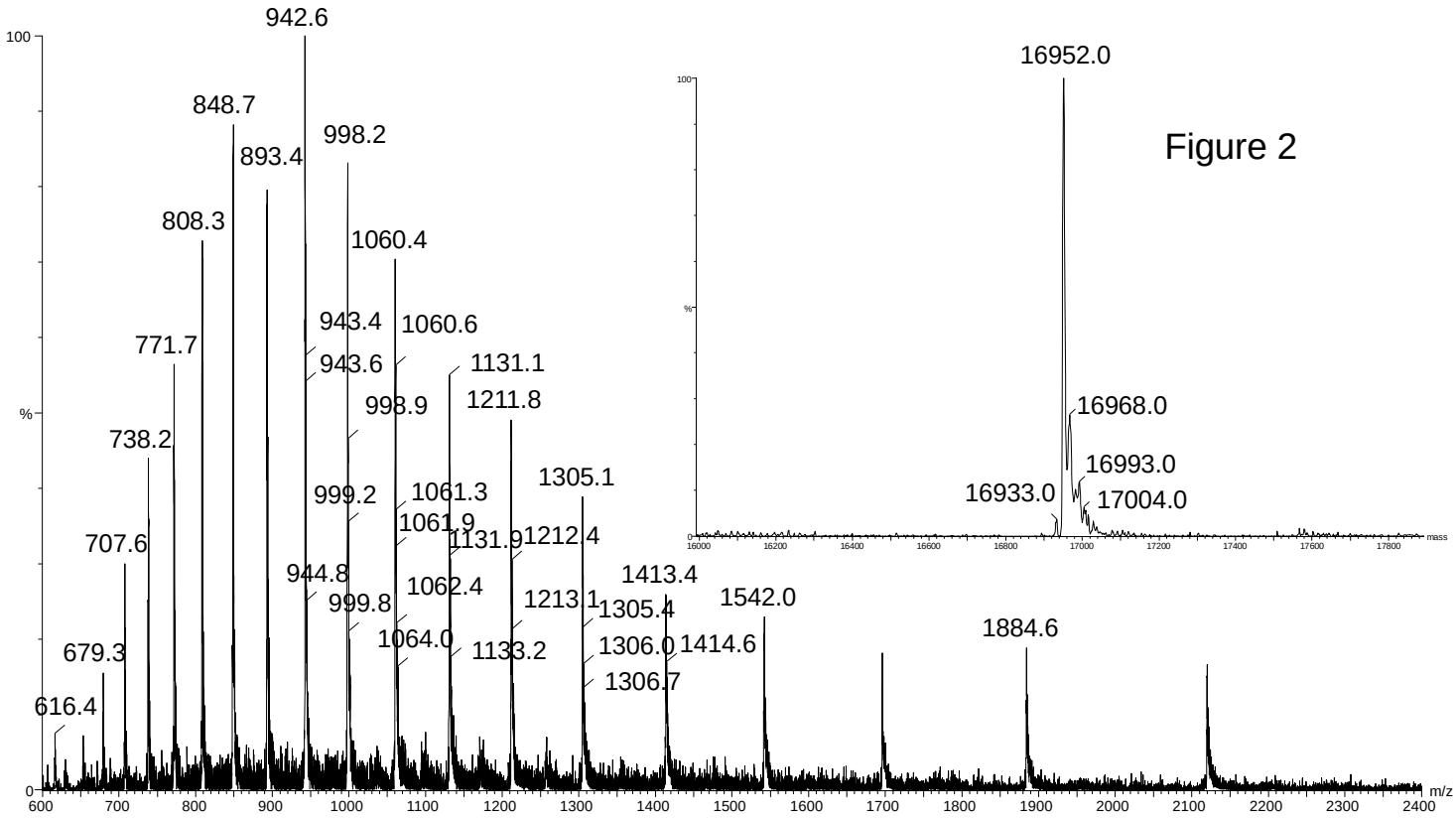

Figure 2. MaxEnt output from Myoglobin infusion calibrated with Myoglobin.

MaxEnt vs. Transform:

The Transform program consists of two separate functions. The first identifies any components present, then identifies the charge state/component for each ion, and calculates the true molecular weight of the uncharged molecule. Once the components and charge states have been identified, Transform next creates a deconvoluted mass spectrum of the uncharged compound(s) displayed on a true molecular weight scale. See Part 1 of this study to view the results of Transform compared to MaxEnt reduced data for myoglobin.

The MaxEnt program treats multiply-charged spectra somewhat differently. It uses a sophisticated statistical analysis of such factors as peak shape and baseline noise in order to derive the molecular weight of the uncharged protein. The only required user input is a mass range which brackets the expected molecular weight of the sample.

Together, MaxEnt and Transform are powerful software options that serve to deconvolute complex, multiply-charged spectra. They are most often, but not exclusively, used during the analysis of large biomolecules. Through the use of these software algorithms, the functional mass range of the instrument is theoretically extended to accommodate the analysis of such large biomolecules. These are just two of the optional software tools available for MassLynx NT software.