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

Part 1: Factors that Affect the Analysis of Proteins by Electrospray LC/MS

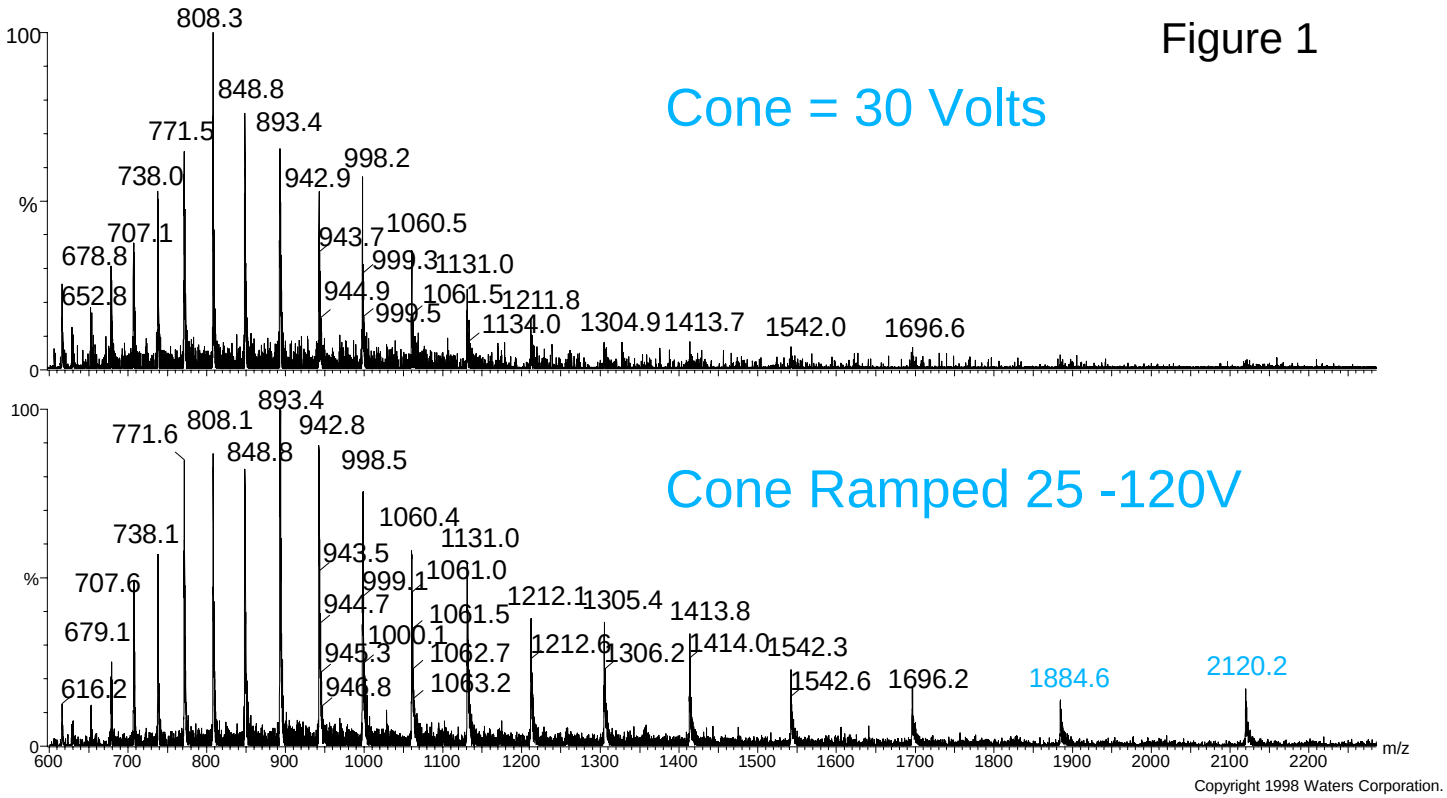
Highlights: Choices that are made during set up and sample acquisition using a benchtop LC/MS system greatly affect the resulting data. For parts 1 & 2 of this experiment, three elements used during the analysis of a Myoglobin sample were investigated. Part 1 looks at two of these elements studied during the analysis of a myoglobin sample. The appearance of mass spectra when static vs. ramped Cone Voltage was utilized was investigated, and the effect on data integrity of two post-run software processing options were examined in Part 1. The choice of calibrant (See Part 2) was considered 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.

Experiment A. Effect of Static vs. Ramped Cone Voltage:

Figure 1 shows the comparison of the averaged mass spectrum of horse heart myoglobin acquired with a static 30 Volt cone voltage (top) and a spectrum illustrating ramped Cone Voltage from 25 to 120 Volts (Bottom). As may be seen in the upper spectrum, the higher charge states are favored by a low Cone Voltage. When a higher static Cone Voltage is used, the lower charge states are enhanced, but the noise level at the low end of the spectrum is greatly increased. By ramping the Cone Voltage, peak height is increased over a wider envelope of charge states without significant increase in baseline noise. This is of great benefit to the overall integrity of the resulting data since the larger number of charge states with good signal-to-noise increases the quality of the subsequent deconvolution.

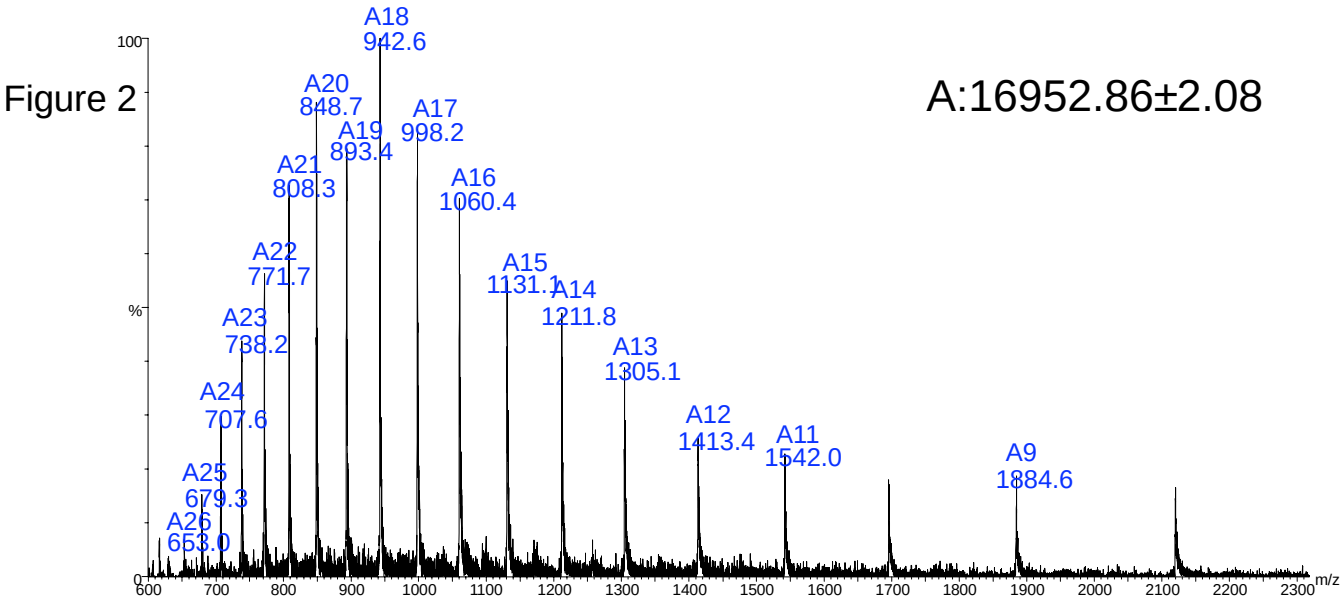
Figure 1



Experiment B. MaxEnt vs. Transform:

The mass spectrum obtained in Experiment A was processed with two deconvolution programs: Transform and Maximum Entropy (MaxEnt). The Transform program consists of two separate functions. The first identifies any components present, identifies the charge state/component for each ion, and calculates the true molecular weight of the uncharged molecule. Figure 2 shows the results of automatic component identification. As may be seen by the number in the upper right-hand corner of this figure, a single component was found, with a calculated mass of 16952.9 Daltons (versus the theoretical weight of 16951.5 for horse heart myoglobin). 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, as is shown in Figure 3.

The MaxEnt program treats multiply-charged spectra somewhat differently, using 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. In this case, a range of 10,000 to 20,000 Daltons was entered. Figure 4 shows the output of the MaxEnt program obtained from the raw data shown in Figure 1 (bottom).



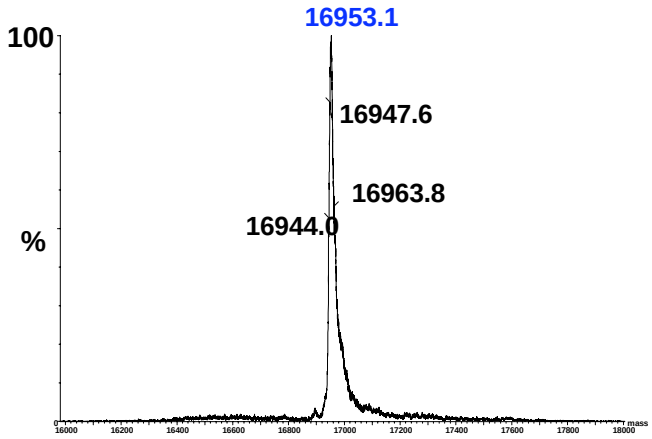


Figure 3. Deconvoluted mass spectrum of Myoglobin obtained from the Transform program

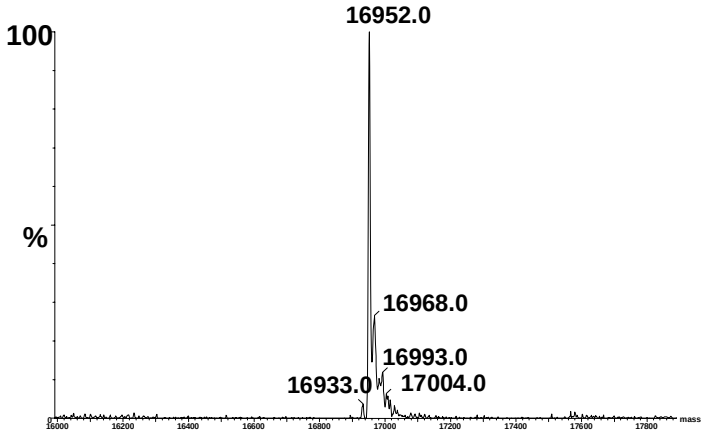


Figure 4. MaxEnt output of the raw data shown in Figure 1 (bottom).