

Investigation of Two Broad Features often Observed in MALDI Mass Spectra of Synthetic Polymers

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In performing molecular mass (MW) or other quantitative determinations from mass spectra of synthetic polymers obtained by matrix-assisted laser desorption/ionization (MALDI), one is confronted with several issues related to the baseline. Often the apparent baselines are far from flat, and one must make decisions about how to perform a baseline subtraction without distorting the analyte signal. There may not be a clear separation between analyte and matrix or other signal, and the beginning and end points of the polymer distribution may not be easily discerned.

Two broad features observed in many MALDI mass spectra of synthetic polymers contribute to baseline difficulties. One of these is a sloping feature proceeding from the lower mass regions of the spectrum that may extend in an asymptotic fashion to several thousand mass-to-charge ratio (m/z) units. In some cases this feature is quite large and the slope is steep. For low MW samples, the slope may extend into or through the region containing the polymer distribution. This sloped feature is generally assumed to be matrix signal, and it is usually not difficult to correct for it by use of background correction routines available in commercial or in-house software packages. However, though the baseline can be flattened, noise associated with the feature remains and can obscure analyte signal. Also, the noise tends to be larger on the lower mass part of the slope; this can skew the baseline-corrected polymer distribution if the noise level is not accurately zeroed.

The other feature appears under the peaks of the polymer distribution. This is a broad, unresolved feature that we will refer to as a hump. The hump is usually offset to the higher mass side with respect to the envelope of the peak distribution, and it may extend past the last identifiable peak of the distribution. Including the hump as part of the analyte signal can result in a significant difference in calculated MW compared to results obtained when the hump is excluded. A valid decision about whether the hump should be removed during baseline correction can only be made if its source is known. If the hump is due to limitations in resolution such that some of the expected polymer ions have flight times that are distributed about, but do not deviate substantially from, those expected from mass calibration, then the best course is to include the hump as part of the analyte signal. Excluding the hump in that case would lead to errors in calculated MW. However, if the hump is due to species other than the expected polymer ions, e.g. polymer-matrix adducts or fragmentation products, then including the hump would lead to errors in calculated MW. In this work we examine the sources and behavior of the hump and the other broad feature discussed above.

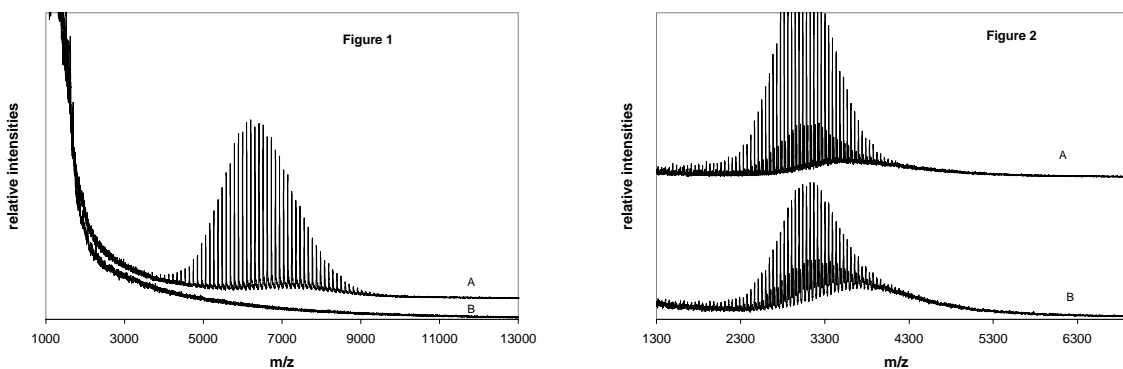
Mass spectra were acquired on a Bruker Reflex II time-of-flight mass spectrometer equipped with dual microchannel plate detectors for both linear and reflectron modes and with a 3 ns pulsewidth nitrogen laser [1]. Samples examined include polystyrene (PS), poly(ethylene glycol) (PEG), and poly(methyl methacrylate) (PMMA). Matrices used include 2,5-dihydroxybenzoic acid (DHB), indoleacrylic acid (IAA), and α -cyano-4-hydroxycinnamic acid (CCA), and added salts include sodium trifluoroacetate (NaTFA) and silver trifluoroacetate (AgTFA).

Figure 1A shows a reflectron mode MALDI mass spectrum of PS7900 obtained using the matrix IAA and with AgTFA added for silver cationization. A corresponding mass spectrum of a sample containing IAA and AgTFA, but no PS, is shown in Fig. 1B. It is apparent that signal related to PS7900 contributes little or nothing to the large sloped feature proceeding from the lower mass portion of the spectrum. In linear mode the sloped feature is larger and extends to higher mass than in reflectron mode. The intensity of the sloped feature is also found to depend on delay time before ion extraction from the source. Post-source decay (PSD) experiments indicate that mass selection of portions of the sloped feature yields broad decay signal, even when, as is the case above a certain mass, little or no signal appears in the selection window. The lower mass broad feature thus appears to be due to a variety of matrix clusters that decay continuously upon desorption. The poor resolution of the feature is likely due to the

existence of a wide variety of cluster species and decay products, and may be exacerbated by decay that occurs during acceleration.

The hump under the peaks of the PS7900 distribution in Fig. 1 is relatively small, but still noticeably affects calculated MW. In contrast, the hump obtained for PEG2890/DHB/NaTFA, shown in Fig. 2, is larger, and the lower mass matrix feature is small. The hump is larger in linear mode (Fig. 2B) than in reflectron mode (Fig. 2A). The effects of the hump on calculated MW were determined for PS7900/IAA, PEG2890/DHB, and PMMA6400/DHB. Repeat measurements under conditions we typically use for each polymer gave increases (with 95 % confidence limits) ranging from 125 (\pm 45) g/mol for M_w of PS7900 in reflectron mode to 880 (\pm 220) g/mol for M_w of PEG2890 in linear mode. The signal strength and extent of the hump decrease with increasing delay before ion extraction, and the hump is observed to behave differently with respect to changes in reflector voltage compared to the polymer peaks. In PSD experiments in which the mass window is moved over the range of the hump and peak distributions, we observe for PEG2890/DHB broad decay signal with intensity that tracks with the reflectron mode hump intensity. The likely source of the hump is polymer-matrix adducts and their decay products. As with the matrix cluster feature, the poor resolution of the hump can be attributed to a wide variety of adduct species and decay products, with some decay during acceleration probable. Also, overlap is bound to occur if each oligomer in the distribution forms a range of adducts. In addition to the broad decay signal, there is some more peak-like PSD signal. This may also be due to polymer-matrix adduct decay [2], but could have contributions from PSD of (PEG + Na)⁺ ions [3]. Other tested systems give less of the broad decay signal, but superposition of PSD signal from adjoining regions suggests that overlap leads to poor resolution and that this signal accounts for the hump. In linear mode for all tested systems, mass selection experiments indicate that part of the hump is due to neutral species.

The hump tends to increase, relative to the size of the polymer peaks, with increasing MW for polymers of the same type and of similar polydispersity. For example, with DHB matrix, PEG1000 exhibits no hump even at fairly high laser fluence. PEG6770 exhibits a relatively larger hump than observed for PEG2890. Choice of matrix can also effect the hump intensity. For example, when using CCA or IAA matrices with PEG 2890, we observed only very small humps in reflectron mode, though in linear mode the humps were still significant.



References

1. Certain commercial equipment is identified in this article in order to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the items identified are necessarily the best available for the purpose.
2. Goldschmidt R. J.; Wetzel S. J.; Blair W. R.; Guttman C. M. *J.Am.Soc.Mass Spectrom.* **2000**, *11*, 1095-1106.
3. Kowalski, P.; Guttman, C. M.; Wallace, W. E. *Proc. of the 46th ASMS Conf. on Mass Spectrom.* Orlando, FL, May 31- June 4, 1998, p. 1060.