

Recent Advances in Quantitative Synthetic-Polymer Mass Spectrometry at NIST

William E. Wallace and Charles M. Guttman

Polymers Division, National Institute of Standards and Technology
Gaithersburg, MD 20899-8541 USA
www.nist.gov/maldi

Commensurate with National Institute of Standards and Technology's mission to advance the state of quantitative measurement science, we have worked to develop a method to determine absolute molecular mass distribution of synthetic polymers by mass spectrometry. Work has centered on using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) on low-mass, narrow-polydispersity polystyrene. Previous work has demonstrated that MALDI-TOF-MS is amenable to the "soft" quantitation of these materials [C.M. Guttman, *et al.*, *Analytical Chemistry* **73** (2001) 1252]. By soft quantitation it is meant that the technique is reproducible (by the same operator on the same instrument on the same day up to different operators on substantially different instruments, and all variations in between), that it is self-consistent, and that it agrees with other methods (within experimental uncertainty). Currently we are exploring the conditions under which MALDI-TOF-MS will produce "hard" quantitation of molecular mass distribution for low-mass, narrow-polydispersity polystyrene. By hard quantitation it is meant that the method requires no physical standards for calibration and that it can be reduced to fundamental physical measures. In practice this entails developing an "uncertainty budget" for the measurement. An uncertainty budget is a determination of the limits of both the Type A ("random") and Type B ("systematic") uncertainties. Our work has centered on developing a set of methods to create this budget.

For a mass spectrum the uncertainties are considered separately as those of the mass axis and those of the intensity axis. For Type A the mass axis shows a relatively small uncertainty, while the intensity axis shows a more substantial uncertainty. The intensity axis uncertainty is mitigated somewhat by the need to know ion intensities relative to one another and not in absolute terms. Furthermore, for Type A uncertainty these values can be reduced by repeated measurement. For Type B uncertainty repeat measurements cannot reduce the uncertainty. For the mass axis three types of calibration are available: (1) biopolymers (as is typically used), (2) calibration on the knowledge that the polymer has a constant-mass repeat unit, and (3) absolute calibration using the extraction voltage, flight path length, and time of flight. As you move from method (1) to (3) you reduce the reliance of physical standards to calibrate the mass axis but you also decrease the accuracy of the calibration. The Type B uncertainty for the signal intensity axis is complicated greatly by the polydispersity of the material. This is the leading reason that narrow-polydispersity polystyrene is used. In the limit of a polydispersity of unity (i.e., a single oligomer) the systematic uncertainties in the signal axis vanish. However, the polydispersity of a real polymer affects the relative intensities of the peaks on the signal axis via differences in behavior of the low-mass species compared to the high-mass species. This occurs in a variety of ways: (1) through differences in laser desorption probability, (2) through differences in cationization probability, (3) through detection efficiency (because the high-mass ions move more slowly in time-of-flight mass separation which can affect detector sensitivity), and (4) through sample preparation (because of solubility/phase separation differences based on mass).

There are two requirements for an oligomer-resolved mass spectrum of a polydisperse polymer to be useful in obtaining a quantitative measure of the molecular mass distribution. First, the total signal intensity for each n-mer should be proportional to the concentration of that n-mer in the MALDI target. So for the *i*th n-mer:

$$S_i = k_i n_i \quad \text{Equation (1)}$$

where S_i is the signal intensity (i.e., integrated peak area), n_i is the concentration of n-mers in the MALDI target, and k_i is a constant of proportionality (that may be different for each n-mer). The second

requirement is that k_i is largely independent of i and follows a functional form that can be measured. Determining the functional form and value for each k_i is the central objective of our research.

For the first condition, we have shown that there is a set of experimental conditions where the total polymer signal, ΣS_i , is constant for a polystyrene with a narrow polydispersity as shown in Figure 1. That is

$$\Sigma S_i = \langle k_i \rangle n_T \quad \text{Equation (2)}$$

where $\langle k_i \rangle$ is the average k over all n -mers and is independent of n_T , where n_T is the total amount of polymer of all molecular mass in the MALDI sample. In addition, the second criterion was shown by Li *et al.* [H. Zhu, T. Yalcin, L. Li, *J. Am. Soc. Mass Spectrom.* **9** (1998) 275] to be true. They showed the molecular mass distribution to be proportional to analyte concentration when two narrow-polydispersity polystyrenes of almost the same average molecular mass were mixed in different ratios. That is

$$\Sigma S_i = S_T = R n_T \quad \text{Equation (3)}$$

where R is not just the average of all k_i but is independent of i altogether. What then remains is to determine a method to find the sample preparation and instrument parameters that satisfy equations (2) and (3). Remember that these conditions will vary depending on the species of polymer, its molecular mass, the brand of instrument used, and many other variables. We are developing a Taylor's expansion approach that may be used to guide the experimentalist in finding the correct measurement conditions to assure quantitation. Along with this we are developing an uncertainty budget (for both Type A and Type B uncertainties) so the experimenter can ascertain the level of confidence in the final quantitative measure of molecular mass distribution.

Figure 1 MALDI-TOF mass spectrum of Standard Reference Material 2888 (a low mass polystyrene), taken using retinoic acid as a matrix and silver trifluoroacetate as the cationizing agent. See the reference of Guttman *et al.* for measurement details.

