MAKING MALDI-TOF-MS AN ABSOLUTE METHOD FOR THE DETERMINATION OF THE MMD OF POLYMERS

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Introduction

In 1963, Donald McIntyre of the NBS Polymers Division produced the first polymer molecular mass standards to be issued by NBS as Standard Reference Materials (SRM), SRM 705 [1]. The appeareance of these SRM's marked the beginning of a long and continuing tradition of production of polymer molecular mass standards characterized by absolute classical measurement methods that include static light scattering, membrane osmometry and ultracentrifugation. In 1997, the NIST Polymer Characterization group initiated an effort to utilize Matrix Assisted Laser Desorption Ionization (MALDI) Time of Flight (TOF) Mass Spectroscopy (MS) to obtain the molecular mass distribution (MMD) of synthetic polymers. This talk will describe current efforts using this method to obtain the absolute MMD of polymers for future SRM products.
Specifically, we shall discuss work done by NIST on the sample preparation methodology to improve the repeatability of the MALDI spectra, and a NIST sponsored interlaboratory comparison that shows the robustness of the method from laboratory to laboratory.

Sample Preparation Methodology

Efforts to modify MALDI sample preparation techniques for synthetic polymers with the goal of increasing sample homogeneity and subsequent peak signal intensity have met with modest success. The usual sample preparation method involves depositing on the sample plate a mixture of the analyte (polymer), a low mas UV absorber (matrix) and a metallic salt (for cationization) from a common solvent. Believing that the relationship of the polymer molecules and matrix crystals in a sample preparation had a significant influence on the evolution of charged polymer species when the target was ablated by UV laser, we investigated the use of non-crystalline, UV absorbing matrix compounds. If the random crystallization of the matrix produces inhomogeneous sample surfaces, eliminating the matrix crystallization event from the sequence of events occurring during sample preparation seem to hold promise for improved sample homogeneity. Using a series of specially synthesized glassy azodyes as matrix material and electro spray sample deposition, we were able to generate replicate MALDI spectra with integrated peak area variations as low as 4.4%. However, due to solubility problems, the applicability of the azodyes was limited to relatively low molecular mass poly(ethylene glycol) polymers. Additionally, the lack of commercial availability of the azodyes limited their use.

During the above investigations, we discovered that the electro spray sample preparation technique could, by itself, provide a significant improvement in the consistency of peak signal integrals. This effect was not realized if the electro sprayed sample surface coating was thin, prepared using only 2 to 4 uL of matrix/polymer solution. Improvement in the statistical variation of the peak intensities did not occur until the electro sprayed coating was made thick. Lacking a technique to measure the thickness of our electro sprayed samples, they were designated as thick or thin by the volume of matrix/polymer solution sprayed onto the target surface. A solution volume of 30 to 40 uL was sprayed onto the target to produce a thick sample.

To gain further understanding of the MALDI sample preparation process, we have examined hand spotted and thick electro sprayed targets prepared with a glassy azodye, dithranol and 2,5-dihydroxybenzoic acid (DHB) by scanning electron microscopy (SEM). An electro spray apparatus was assembled from the following components: A Spellman High Voltage Electronics Corp. (Hauppauge, NY 11788) adjustable high voltage DC power supply, a Harvard Apparatus (Holliston, MA 01745) Model 22 syringe pump and a manually adjusted x-y-z positioning movement (Edmund Scientific, Barrington, NJ 08007) to hold the MALDI target. All these components were mounted on a small (8" x 24") optical bench plate (Edmund Scientific, Barrington, NJ 08007) to provide easy, reproducable alignment of the syringe needle and MALDI target. For SEM examination, samples were given a light gold coating. A JEOL model 5300 electron microscope was used for all SEM microscopy. MALDI analyses were performed on a Bruker Reflex II MALDI-TOF mass spectrometer[1].

The SEM micrographs below both show a dithranol, silver trifluoroacetate, and polystyrene 10,200 MW polymer preparation. The illustration on the left was prepared by hand spotting. The one on the right is a thick electro sprayed sample. The more uniform structure of the electro sprayed sample, apparently composed of hollow spheres of 2 to 3 micrometers diameter, may be a significant factor in the generation of replicate MALDI analyses with small variations in integrated peak area intensities.

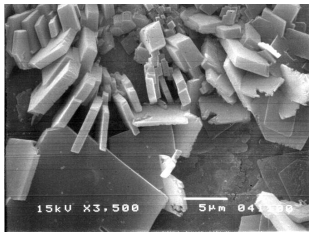

Figure 1. Hand spotted sample

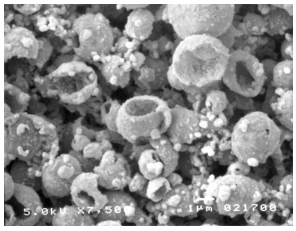

Figure 2.Electrosprayed sample

Interlaboratory Comparison

NIST has sponsored an interlaboratory comparison using well-characterized polystyrene to determine the lab-to-lab reproducibility of MALDI-TOF-MS in determining the MMD and to learn more about the parameters that influence the MMD. Samples of a well-characterized low molecular mass polystyrene were sent to any institution requesting it. A total of 23 institutions participated [2].

The polystyrene (PS) used in this interlaboratory comparison was prepared commercially (Polymer Source, Dorval, Quebec, Canada)[#] for the NIST Polymers Division. The polymer was specially prepared by anionic polymerization with well-defined end groups. From the preparation chemistry, we expected the polymer to be:

$$(CH_3)_3\text{-C-}[CH_2\text{-CHPh}]_n\text{-}CH_2\text{-}CH_2\text{-Ph} \qquad \text{Ph = phenyl} \quad (1)$$

FTIR studies performed at NIST on the polymer confirmed that the end groups were as expected. The polymer was bottled and homogeneity testing was done by size exclusion chromatography (SEC) with samples selected by stratified random sampling.

Light scattering measurements on toluene solutions of the PS were made on a Brookhaven Instrument Model BI-200 (Brookhaven Instrument Corp., Ronkonkoma, NY) light scattering apparatus with a 10 mW He-Ne laser light source. The temperature was controlled at 25.0 ºC in all light scattering experiments. Light scattering data from polymer solutions of concentration c and scattering angle were fit following normal Zimm Analysis [2]. We estimate Mw = (7300 ± 600) g/mole. The methods of data analysis and uncertainty analysis used here are described in more detail in reference [3]. (± refer to standard uncertainty throughout.)

Proton NMR spectra at 400 MHz were recorded on the PS at ambient temperature on a WM-400 spectrometer (Bruker Instruments, Inc, Billirica, MA). Resolution was found to be adequate for evaluating the integrals of interest, for both the 50 g/L and the 130 g/L solutions, under the following conditions. Based on the assumed structure for the PS polymer shown above, the integrals (of both aromatic and aliphatic PS protons plus the end-group methyl protons) measured from the NMR, yield an M_n of 7100 g/mole based on measurements from the 50 g/L solution and M_n of 7000 g/mole, based on the measurements of 130 g/L solution. The 130 g/L solution had poorer resolution, as expected from a more concentrated solution, but smaller corrections had to be made to the measured integrals than for the 50 g/L solution. Thus the confidence level from each solution was similar. We estimate from NMR the Mn = (7050 ± 400) g/mole

The polystyrene (PS) sample which was used in the interlaboratory comparisons was expected from the preparation chemistry to consist of

oligomers of the form shown in eq. 1. The spectral main peaks from a calibrated instrument agreed well with the structure in eq. 1. See figures 3 However, matrix-assisted laser desorption/ionization (MALDI) mass spectra of the sample revealed an unexpected secondary series with 104 u mass separation in addition to the expected main series ions. We were concerned that some of these intermediate peaks indicated end groups not seen in the FTIR. As is discussed in ref [3],we were able to show the secondary peaks were shown arose from two sources: either adducts of the matrix and/or cations with the polymer or possibly fragmentation of the polymer along the main chain. None of the secondary peaks were attributable to additional end groups.

Each participating laboratory was asked to perform MALDI mass spectrometry using two distinct protocols. The different protocols involved different sample preparations. Each laboratory was asked to do three repeats of each protocol to check for intralaboratory variability. Each laboratory was asked to provide M_n and M_w for each repeat as well as the integrated signal for each separate peak..

Here we describe results from the 23 participating laboratories analyzing the polystyrene interlaboratory sample. To analyze such a body of data the first consideration is how to reduce the data into estimators, which can then be compared and interpreted. The moments of the MMD were considered. M_n, M_w, & M_z are the traditional moments considered in polymer molecular mass determination .

For such a narrow MMD, these three moments do not represent the entire molecular mass distribution, only the center of the distribution . The tails of the distribution, which are expected to have the greatest variation, will not be well represented in any analysis using these moments.

Another method of reducing the data for analysis is to divide the MMD range into a few bins. This method is particularly useful for comparing the tail regions of the molecular mass distribution. The distribution was separated into eleven equal mass divisions for comparison.

The first step in the data analysis was to identify any outliers. The outliers were identified by examining the distribution of the moments and determining whether any data points fell outside of three standard deviations. The moments of two repeats conducted by one laboratory fell outside of the normal distribution of the moments. These data points were classified as outliers and excluded in further data analysis.

The reduced data, both the moments and the bins, were compared using ANOVA. ANOVA indicates a difference in population means by comparing the variation among the group means with the variation within the groups. Analysis of all of the moments showed the significant parameters to be the laboratory performing the measurement, and the matrix used in sample preparation. The variation among laboratories was tested for all of the moments and bins by ANOVA. The results indicate that the variation among laboratories is much greater than the variation within laboratories.

When a matrix of all-*trans*-retinoic acid was used in the sample preparation, an M_n of 6610 u and an M_w of 6740 u were obtained. When the polystyrene sample was prepared with a dithranol matrix, an M_n of 6539 u and an M_w of 6689 u were obtained (see figure 4). ANOVA tests were performed on the moments to test variation between matrices. The analysis compared data from laboratories which ran all-*trans*-retinoic acid and dithranol as matricies. This analysis shows that the matrix used in the sample preparation has no significant influence on the molecular mass distribution obtained by MALDI.

Also compared was the influence of linear or reflectron modes of the instrument on the molecular mass distribution. When the moments were compared, no difference in variance was seen between the instrument modes and within the modes. However, the ANOVA of the bin data shows that the linear or reflectron modes affect the tails of the distribution. The null hypothesis, that no difference exists between the linear and the reflectron modes of analysis, is accepted for all of the bins except for those bins representing the tails of the distribution. The data indicate that the reflectron mode is more sensitive to the low mass species, whereas the linear mode is more sensitive to the high mass species.

References

[#] Certain commercial materials and equipment are identified in this paper in order to specify adequately 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 necessarily the best available for the purpose.

1. McIntyre, D., J. Res. Nat. Bur. Stand. (U.S.) , 71A 43 (**1967**)

2. A more detailed description of the analysis of the MALDI TOF MS Interlaboratory Comparison is given in Guttman, C.M., Wetzel, S.J., Blair, W.R., Fanconi, B.M., Girard, J.E., Goldschmidt, R.J., Wallace, W.E., and VanderHart, D.L., submitted for publication to Analytical Chemistry

3. Goldschmidt, R.J., Wetzel, S.J., Blair, W.R., Guttman, C.M., Amer. Soc. for Mass Spectrom..,November **2000**

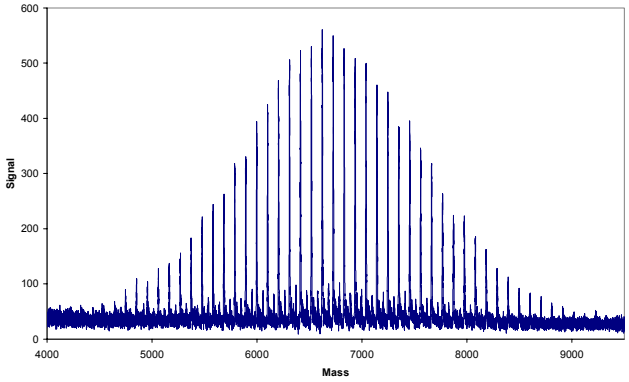

Figure 3. Typical MALDI TOF Spectrum of Interlaboratory PS