

DETERMINATION OF THE MOLECULAR MASS DISTRIBUTION OF A NARROW POLYDISPERSITY POLYSTYRENE BY MOLEDI-TOF-MS: INTERLABORATORY COMPARISON

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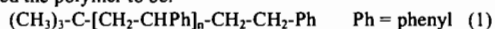
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Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a new and important technique in synthetic polymer characterization. Yet much is still unknown about the molecular mass distribution (MMD) that is obtained through the MALDI process. 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 18 institutions participated.

Preparation

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:



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 [1].

Light Scattering Methods

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 °C in all light scattering experiments. Light scattering data from polymer solutions of concentration c and scattering angle were fit following normal Zimm Analysis [2]. Normally for homopolymers the refractive index increment, (dn/dc) , is independent of molecular mass. At lower molecular masses, however, because the refractive index increments from the end groups are different than that of the repeat units, we expect the (dn/dc) to have a molecular mass dependence. The measured (dn/dc) for this PS was found to be (0.1030 ± 0.0010) mL/g. Such (dn/dc) corrections are also important in SEC at low molecular masses of the polymer. With this (dn/dc) we estimate $M_w = (7300 \pm 600)$ g/mole. The methods of data analysis and uncertainty analysis used here are described in more detail in reference [3]. (\pm refer to standard uncertainty throughout.)

NMR Methods

Proton NMR spectra at 400 MHz were recorded on the PS at ambient temperature on a WM-400 spectrometer (Bruker Instruments, Inc, Billerica, 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. Spectra were taken with single pulse excitation. Pulse nutation (tip) angle was 30 ° and the delay between acquisitions was 20 s. This combination of conditions was confirmed to give quantitative results for all protons. Signal accumulations after 64 scans had adequate signal-to-noise in the Fourier Transform spectra for evaluating the integrals of interest. The Fourier transforms were made large enough, by zero-filling, so that the relative integrals, for even the narrowest lines, were reliable. 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 $M_n = (7050 \pm 400)$ g/mole.

NIST MALDI study of Interlaboratory Polystyrene

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 1 & 2. 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; see figure 2. We were concerned that some of these intermediate peaks indicated end groups not seen in the FTIR. As is discussed in the next paragraph, 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.

The positions of the secondary series ions in relation to the main series ions depend on the choice of matrix and also on the salt added to effect cation attachment to PS. To see these effects, three matrices, retinoic acid (RA), trans-3-indoleacrylic acid (IAA), and dithranol, were used; the salts used were silver trifluoroacetate (AgTFA) and copper trifluoroacetate (Cu(II)TFA). Post-source decay (PSD) analysis of the interlaboratory PS sample and also of another PS of M_n of about 3900 u indicated that the secondary series are primarily due to the formation and decay of adducts of PS with matrix molecules and metal cations. Some fragmentation of the PS polymers alone may also occur with the loss of between one to four repeat units[4]. These results are in contrast to our fragmentation studies earlier on PEO where fragments of all chain lengths less the molecular mass of the polymer were found [5].

Interlaboratory Protocol

Each participating laboratory was asked to perform MALDI mass spectrometry using two distinct protocols. The different protocols involved different sample preparations. One of the protocols was specified. It is described in Appendix A. The other protocol allowed the laboratory use its own favorite sample preparation for PS. 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.

Analysis of Interlaboratory Data

Here we describe results from the eighteen 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 [6]. Three other moments were also considered, $M_{1/n}$, $M_{1/w}$, $M_{1/z}$ defined as;

$$M_{1/n} = \frac{\sum N_i}{\sum N_i M_i^{-1}} \quad M_{1/w} = \frac{\sum N_i M_i^{-1}}{\sum N_i M_i^{-2}} \quad M_{1/z} = \frac{\sum N_i M_i^{-2}}{\sum N_i M_i^{-3}}$$

where N_i = the number of molecules with a molecular mass of M_i . These moments take into consideration the lower molecular mass end of the distribution. The six moments were then compared using statistical techniques.

For such a narrow MMD, these six moments do not represent the entire molecular mass distribution, only the center of the distribution (see figure 1). 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 6614 u and an M_w of 6743 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 matrices. 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.

Conclusions

NIST Polymers Division interlaboratory comparison of TOF MALDI MS using a polystyrene of about 7000 g/mol showed good agreement between all participating laboratories, in general. Some differences were found but differences were in the range of hundreds of mass units for moments, not thousands of mass units. By compiling all the returned data using both protocols into one preliminary analysis it was found that MALDI mass spectrometry returned an M_n of (6600 ± 100) u and an M_w of (6700 ± 90) u. These numbers were below those of the classical methods, but still within the overlapping uncertainty ranges.

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) for a description of this process for an Standard Reference Material, see for example Guttman, C.M.; Blair, W.R.; Maurey, J.R., "Recertification of SRM 706a, a Polystyrene", Nat. Inst. Stds. and Tech. (U.S.) *NISTIR 6091*; 1998 March. 20 p.

(2) M. B. Huglin, editor, "Light Scattering from Polymer Solutions," Academic Press, N.Y., (1972).

(3) Blair, W. R.; Fanconi, B. M.; Goldschmidt, R. I.; Guttman, C. M.; Wallace, W. E.; Wetzel, S. J.; VanderHart, D. L. *Proceedings of 47th ASMS Conference on Mass Spectrometry and Allied Topics*, 1999.

(4) Goldschmidt, R. J.; Wetzel, S. J.; Blair, W. R.; Guttman, C. M. *Proceedings of 47th ASMS Conference on Mass Spectrometry and Allied Topics*, 1999.

(5) Kowalski, P.; Guttman, C. M.; Wallace, *Proceedings of 46th ASMS Conference on Mass Spectrometry and Allied Topics*, 1998, 1060.

(6) Wetzel, S. J.; Guttman, C. M.; Girard, J. E. *Proceedings of 47th ASMS Conference on Mass Spectrometry and Allied Topics*, 1999.

Appendix A: Required Protocol

MALDI participants used the following required recipe: 5 mg/mL of PS in THF: 75 mg/mL retinoic acid in THF :5 mg/mL AgTFA in THF :Mix solutions of PS:retinoic acid:AgTFA by volume 1:10:1. Make three different sample spots, if you can, and take each spectrum from a different spot. If you can make only one spot, please take each spectrum from different area on that spot. (It is required that only one solution of solvent, polymer, matrix and salt be prepared with several sample spots made from this solution.) An individual spectrum should be at least 100 laser pulses. Do not change laser or machine settings during the time you make all 3 spectra (You may want to make 4 spots so you can get the best machine setting to get the best spectra before you begin).

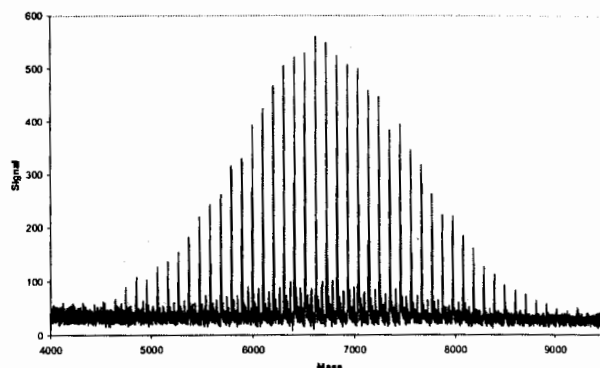


Figure 1. Typical MALDI TOF MS of PS

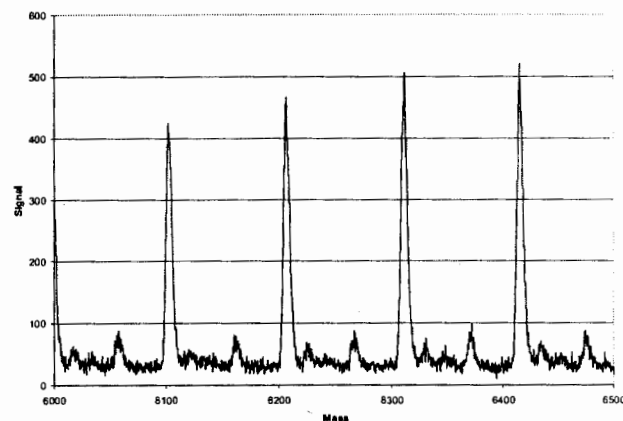


Figure 2. Typical local region in MALDI TOF MS of PS

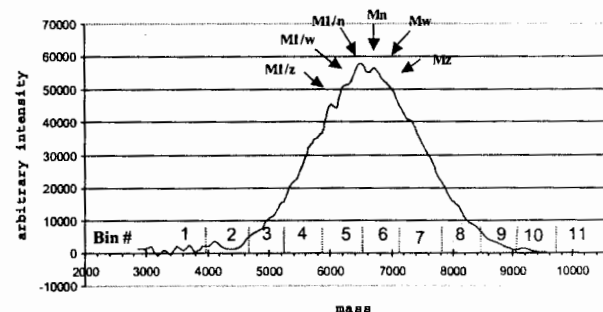


Figure 3. The moments and bins for a typical PS MMD

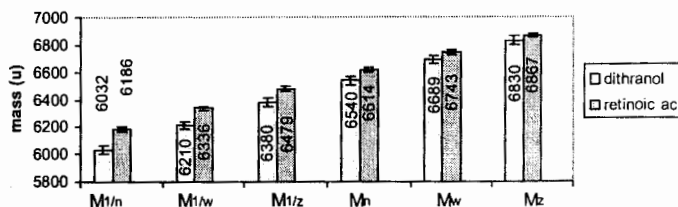


Figure 4. A comparison of the mean moments from two different matrices, retinoic acid and dithranol, used in the sample preparation. The error bars estimate the standard error of the mean.