

Development of NIST SRM 2881, An Absolute Molecular Mass Distribution Polymer Standard*

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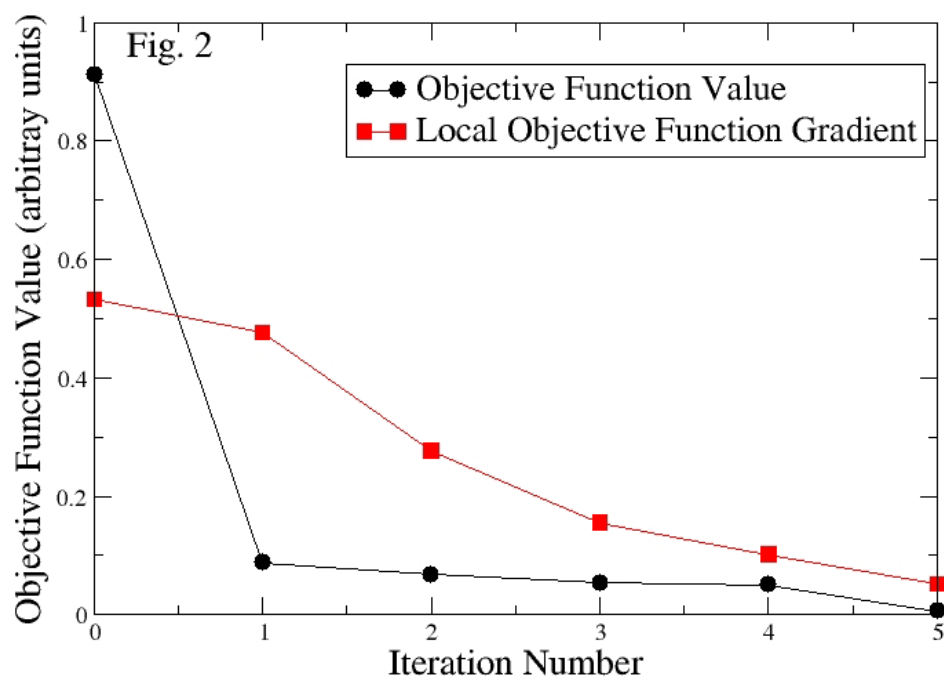
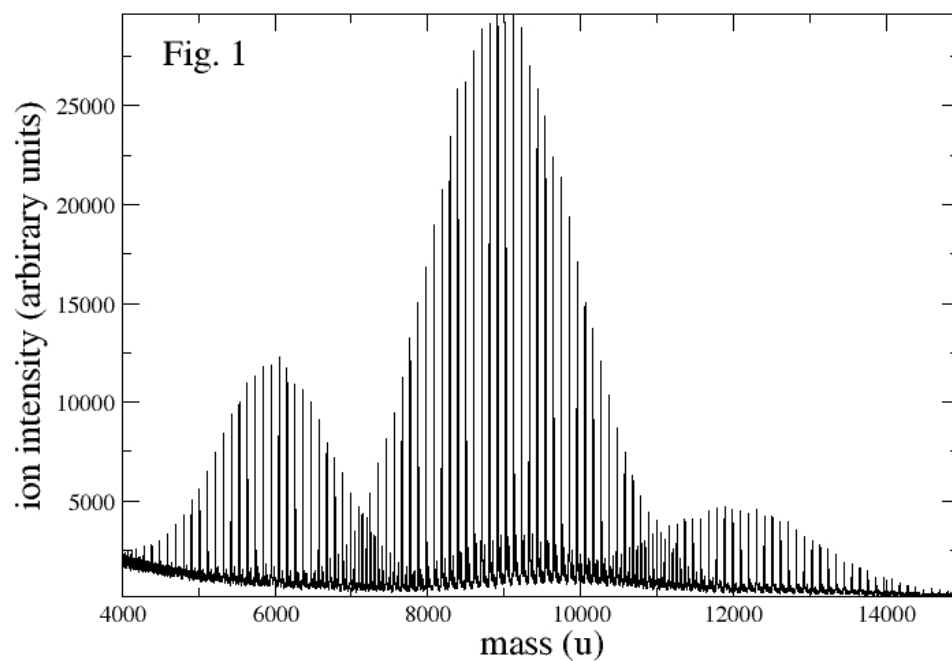
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Introduction Currently, all absolute molecular mass standards provide a single moment (e.g. M_n) of the molecular mass distribution (MMD). (By *absolute* it is meant that the standard is based on direct measurement of a physical property and not on calibration against another polymer.) We present a strategy to create an absolute molecular mass distribution polymer Standard Reference Material using MALDI-TOF MS. To create such a standard our methods must be free of operator bias (intentional or unintentional). Furthermore, the Type A (“random”) and Type B (“systematic”) uncertainties must be determined for each part of the experiment. The required unbiased, repeatable steps are 1) sample preparation, 2) instrument tuning, 3) data collection, and 4) data analysis. These steps ultimately lead to a signal intensity calibration function that relates the true MMD of the analyte to the MMD measured by mass spectrometry.

Methods We use narrow polydispersity polystyrene initiated with an n-octyl group and having an M_p of 9000 u. To this polymer we gravimetrically mix two n-butyl-initiated polystyrenes one having an M_p of 6000 u and the other an M_p of 12 000 u. By controlling the gravimetric mix we can create a signal-intensity calibration curve. We describe the use of electrospray methods to create uniform MALDI targets that are free of sweet spots, that can be reproducibly made, and whose preparation can be described precisely. Removal of the gross uncertainties inherent in typical MALDI sample preparation methods (e.g. “hand spotting”) is critical for quantitation work. With gradient-free numerical optimization methods we locate the instrument parameter settings (extraction, lens, and reflectron voltages; laser intensity; and extraction delay time) that give the least bias in the molecular mass distribution. We do this by comparing the difference between the gravimetric-mixture ratios with the ratios found by mass spectrometry (via an numerical expression called an “objective function”) until an optimal set of instrument settings is found. Data collection is randomized to prevent unknown changes in experiment procedure or instrument performance from creating a systematic bias in the data over periods of hours, days, and weeks. Using our time-series segmentation method, *MassSpectator* [1], we automatically identify and integrate mass peaks. Finally, using standard additions of the three polystyrenes we create a signal intensity calibration curve. Variations in relative ratios of the three polymers let us determine the uncertainty in the calibration curve.

Results Figure 1 shows a typical mass spectrum of the three polymers mixed in a gravimetric ratio of 10:70:20. Retinoic acid was the matrix; silver trifluoroacetate was the cationizing agent. Notice that even though the distributions overlap they can be distinguished from each other because the center distribution is n-octyl initiated and, therefore, its mass is 54 u higher than the corresponding n-mer for the two n-butyl-initiated polymers on either side of it. In this way the total amount of each polymer can be determined from the spectrum and easily compared to the gravimetric ratio. From this the instrument parameters can be tuned until the best match between the gravimetric to the MALDI ratios is found. Figure 2 shows the scaled difference between the gravimetric and the MALDI ratios (the objective function) at the initial parameter setting [2] and for 5 iterations. (The Type A uncertainties at each point are approximately the size of the plotting points.) By the last iterated value the improvement made is small enough to make further iterations unprofitable. The local gradient of the objective function tells us how close to the minimum the current values are. The gradient, that is the derivative, would be zero at the local minimum. Its monotonic decrease towards zero tells us we are smoothly approaching a local minimum in the objective function. From here changing the relative ratios of the three polymers will allow us to create a calibration curve to relate the true MMD to the MMD found by MALDI. This work is current in progress.

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[1] W.E. Wallace, A.J. Kearsley, C.M. Guttman, Analytical Chemistry 76 (2004) 2446

[2] C.M. Guttman, S.J. Wetzel, W.R. Blair, B.M. Fanconi, J.E. Girard, R.J. Goldschmidt, W.E. Wallace, D.L. Vanderhart, Analytical Chemistry 73 (2001) 1252